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Ensaio clínico randomizado sobre o impacto dos macrolídeos na mortalidade de pacientes infectados pelo HIV e com pneumonia adquirida na comunidade

Tese apresentada à Faculdade de Medicina da Universidade de São Paulo para obtenção do título de Doutora em Ciências

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Orientadora: Profa. Dra. Anna Sara Shafferman Levin

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Claudia Figueiredo Mello

Ceftriaxone versus ceftriaxone plus a macrolide for community acquired pneumonia in hospitalized patients with HIV/AIDS: a randomized controlled trial

Thesis presented to Faculdade de Medicina FMUSP, Universidade de Sao Paulo to obtain the degree of Doctor of Science

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To Vó Toty and Prof. Tuba, two elegant, intelligent and strong woman who still lives through their teachings.

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NORMATIZAÇÃO

Essa tese está de acordo com as seguintes normas, em vigor no momento desta publicação:

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Universidade de São Paulo. Faculdade de Medicina. Divisão de Biblioteca e Documentação. *Guia de apresentação de dissertações, teses e monografias*. Elaborado por Anneliese Carneiro da Cunha, Maria Julia de A. L. Freddi, Maria F. Crestana, Marinalva de Souza Aragão, Suely Campos Cardoso, Valéria Vilhena. 3a ed. São Paulo: Divisão de Biblioteca e Documentação; 2011.

Abreviaturas dos títulos dos periódicos de acordo com *List of Journals Indexed in Index Medicus*.

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Resumo

Mello CF. Ensaio clínico randomizado sobre o impacto dos macrolídeos na mortalidade de pacientes infectados pelo HIV e com pneumonia adquirida na comunidade [Tese]. São Paulo: Faculdade de Medicina, Universidade de São Paulo; 2017.

O objetivo principal dessa tese foi avaliar se o tratamento com ceftriaxona e um macrolídeo leva a melhores desfechos quando comparada a monoterapia com ceftriaxona em pacientes hospitalizados com HIV/AIDS e pneumonia adquirida na comunidade (PAC). 227 adultos com HIV hospitalizados por uma suspeita de PAC foram randomizados numa proporção 1:1 para receber um dos dois regimes, ceftriaxona mais macrolídeo ou ceftriaxona mais placebo. Houve 2 exclusões após a randomização, um paciente retirou consentimento para uso de seus dados e outro paciente já havia sido incluído previamente no estudo, perfazendo um total de 225 pacientes analisados (112 receberam ceftriaxona mais placebo e 113 receberam ceftriaxona mais macrolídeo). Os pacientes tinham HIV há um longo tempo (período mediano de 10 anos) e a maioria não fazia uso regular de terapia antirretroviral. Somente 32/202 pacientes (16%) tinham carga viral menor que 50 cópias/mL e 146/202 (72%) tinham contagem de linfócitos T CD4+ menor que 200 células/mm³. A frequência do desfecho primário, letalidade durante a internação, não foi estatisticamente distinta entre os regimes estudados: 12/112 (11%) pacientes que receberam ceftriaxona mais placebo e 17/113 (15%) que receberam ceftriaxona mais macrolídeo foram a óbito durante a hospitalização (HR: 1.22, 95% CI: 0.57-2.59). Não foram encontradas diferenças entre os regimes para os desfechos secundários: letalidade em 14 dias (RR: 2.38, 95% CI: 0.87-6.53), uso de drogas vasoativas (OR: 1.18, 95% CI: 0.60-2.29) e ventilação mecânica (OR: 1.24, 95% CI: 0.64-2.40). A etiologia das infecções pulmonares adquiridas na comunidade nesses pacientes com infecção pelo HIV também foi estudada e determinada prospectivamente. Essa investigação também buscou analisar a contribuição de diferentes métodos diagnósticos e o impacto de diferentes abordagens de

investigação microbiológica. Além disso, os achados microbiológicos foram analisados levando em consideração a contagem de linfócitos T CD4+, gravidade da doença e a situação da vacina pneumocócica. 224 pacientes foram submetidos a investigação microbiológica estendida e 143 (64%) tiveram uma etiologia determinada. Por outro lado, a investigação microbiológica de rotina foi capaz de determinar o agente etiológico em 92 (41%) pacientes. Métodos baseados na reação em cadeia da polimerase foram essenciais para o diagnóstico de bactérias atípicas e vírus, além de melhorar a detecção de *Pneumocystis jirovecii*. Entre os 143 pacientes com uma etiologia determinada, *Pneumocystis jirovecii* foi o principal agente, detectado em 52 (36%) casos, seguido pelo *Mycobacterium tuberculosis* responsável por 28 (20%) casos. *Streptococcus pneumoniae* e Rhinovírus foram diagnosticados em 22 (15%) casos cada e Influenza em 15 (10%) casos. Entre as bactérias atípicas, *Mycoplasma pneumoniae* foi responsável por 12 (8%) e *Chlamydophila pneumoniae* por 7 (5%) casos. Infecções mistas ocorreram em 48 casos (34%). *Streptococcus pneumoniae* foi associado com maiores escores de gravidade, sem associação com o estado vacinal. A análise de agentes etiológicos baseada na contagem de linfócitos T CD4+ demonstrou que a etiologia da pneumonia nos pacientes que estavam gravemente imunossuprimidos (CD4+ < 200 células/mm^3) foi similar aos que não estavam. *Pneumocystis jirovecii* foi o único agente mais frequente no primeiro grupo, um achado esperado levando em consideração os critérios diagnósticos empregados.

Descritores: infecções por HIV; pneumonia; ensaio clínico controlado aleatório; infecções comunitárias adquiridas; macrolídeos; mortalidade; ceftriaxona.

Abstract

Mello CF. Ceftriaxone versus ceftriaxone plus a macrolide for community acquired pneumonia in hospitalized patients with HIV/AIDS: a randomized controlled trial [Thesis]. São Paulo: “Faculdade de Medicina, Universidade de São Paulo”; 2017.

The main purpose of this thesis was to evaluate if treatment with ceftriaxone and a macrolide improved patient outcome when compared with monotherapy with ceftriaxone in hospitalized patients with HIV/AIDS with community acquired pneumonia (CAP). 227 adult patients with HIV hospitalized due to suspected CAP were randomized to receive one of two regimens, ceftriaxone plus macrolide or ceftriaxone plus placebo, at a 1:1 proportion. We had 2 exclusions after randomization, one patient who withdrew consent for data inclusion and use and one that had previously been included, leaving a total of 225 patients to analyse (112 received ceftriaxone plus placebo and 113 received ceftriaxone plus macrolide). Patients had prolonged HIV infection, the median period was twelve years, and most of them did not make regular use of antiretroviral therapy. Only 32/202 patients (16%) had viral load below 50 copies/mL and 146/202 (72%) had a CD4+ T cell count below 200 cells/mm^3. The frequency of the primary outcome, in-hospital mortality, was not statistically different between the studied regimens: 12/112 (11%) patients who received ceftriaxone plus placebo and 17/113 (15%) who received ceftriaxone plus macrolide died during hospitalization (HR: 1.22, 95% CI: 0.57-2.59). We did not find differences between the regimens for the secondary outcomes: mortality within 14 days (RR: 2.38, 95% CI: 0.87-6.53), need for vasoactive drug (OR: 1.18, 95% CI: 0.60-2.29) or mechanical ventilation (OR: 1.24, 95% CI: 0.64-2.40). The etiology of community-acquired pulmonary infections in these hospitalized patients with HIV was also studied and determined prospectively. This investigation also aimed to analyze the contribution of different diagnostic methods as well of the impact of different approaches to microbiological evaluation and to evaluate the microbiological findings in relation to the CD4+ T cell count, the severity of disease and

pneumococcal vaccine status. 224 patients underwent the extended microbiological investigation of which 143 (64%) had an etiology determined. On the other hand, the microbiological routine investigation was able to determine the etiological agents in 92 (41%) patients. Polymerase chain reaction-based methods were essential for the diagnosis of atypical bacteria and viruses, besides contributing to ameliorate *Pneumocystis jirovecii* detection. Among the 143 patients with a determined etiology, *Pneumocystis jirovecii* was the main agent, detected in 52 (36%) cases and followed by *Mycobacterium tuberculosis* accounting for 28 (20%) cases. *Streptococcus pneumoniae* and Rhinovirus were diagnosed in 22 (15%) cases each and Influenza in 15 (10%) cases. Among atypical bacteria, *Mycoplasma pneumoniae* was responsible for 12 (8%) and *Chlamydophila pneumoniae* for 7 (5%) cases. Mixed infections occurred in 48 cases (34%). *Streptococcus pneumoniae w*as associated with higher severity scores and not associated with vaccine status. Performing an analysis of causative agents based on CD4+ T cell count, we found that the etiology of pneumonia in those severely immunosuppressed (CD4+<200 cells/mm^3) was similar to those who were not. *Pneumocystis jirovecii* is the only agent more frequent in the former group, an expected finding considering our diagnostic criteria.

Descriptors: pneumonia; HIV infections; randomized controlled trial; community-acquired infections; macrolides; mortality; ceftriaxone.

1. General introduction

Pneumonia is an infection of the lung tissue (1) and community acquired pneumonia (CAP) is the leading cause of death among the infectious diseases worldwide (2).

Every year between 0.5% and 1% of adults in the UK will have CAP (1). CAP, together with influenza, remains the seventh leading cause of death in the United States (3).

In Brazil, pneumonia and influenza were the fifth most frequent cause of death between 2011 and 2015, responsible for 339,539 (6%) of 6,053,351 deaths (4).

Pneumonia burden in patients with HIV/AIDS

Since the first description of HIV/AIDS, the lung has been the most commonly affected organ (5) and over two-thirds of patients with HIV/AIDS may have at least one episode of respiratory disease (6).

Pulmonary infiltrates are a frequent cause of hospital admission, with a described incidence of 18 episodes per 100 admissions-years (7).

The range of HIV-associated respiratory diseases is vast and includes infectious agents (particularly tuberculosis, bacterial pneumonia, *Pneumocystis jirovecii* and various other opportunistic infections) and non-infectious diseases (such as lung cancer, diffuse parenchymal lung disorders and pulmonary vascular disorders) (5,6,8).

Bacterial CAP incidence has decreased after the introduction of antiretroviral therapy, but patients with HIV/AIDS still have a higher risk of acquiring CAP than the general population and have higher mortality rates (8,9).

Aetiological agents of CAP in patients with HIV/AIDS

The epidemiology of HIV-associated pulmonary disease is complex and influenced by various factors, notably the regional prevalence of pathogens, such as tuberculosis, and the accessibility to health care, effective antiretroviral therapy and antimicrobial prophylaxis(10).

The study of the etiological agents of CAP is important to guide empirical therapy, requires constant updating and has a substantial impact on the prognosis of patients (11). However, few studies have systematically investigated the aetiology of pneumonia in patients with HIV/AIDS (5).

Those studies consisted of heterogeneous cohorts and various diagnostic algorithms. A summary of the main ones is presented in Table 1.

In general, bacterial cause of CAP is similar in patients with HIV/AIDS and the general population. *Streptococcus pneumoniae* is the most frequent bacterial agent. Atypical bacteria appear to be infrequent, described as rare in patients with HIV/AIDS according to the results of two studies (12,13).

Opportunistic pathogens such as *P. jirovecii* and *Mycobacterium tuberculosis* have been consistently described (8). *P. jirovecii* pneumonia (PCP) has a greater importance, as it was the most frequent agent in various studies (12,14,15), mainly in patients with CD4+ T cell counts below 200 cells per mm^3 (13,15).

Viral agents had variable importance, as investigation methods and interpretive criteria varied between studies (12,13).

Polymicrobial infections occurred on many occasions (12,13,15) and this hypothesis must be taken into account when dealing with a CAP episode in patients with HIV/AIDS.

In clinical practice, it is difficult to differentiate between bacterial CAP and PCP and to date there is no widely validated score to predict the CAP aetiology in patients with HIV/AIDS. Symptomatology for < 5 days, C-reactive protein level > 22mg/dL and hepatitis C virus co-infection were described as predictors of bacterial CAP, as white blood cell count < 4×10^{12}/L and lactate dehydrogenase level > 598 U/L were predictors of PCP (13).

Table 1. Summary of studies on microbiological findings of community acquired pneumonia (CAP) in patients with HIV/AIDS

Country/ Year/ Author	Patients´ characteristics	Number of patients	Microbiological findings
EUA 1995 Mundy (14)	General population hospitalised with CAP. Sub analysis of patients with HIV/AIDS	180 patients 74% with determined etiological agent	*Pneumocystis jirovecii* was the main agent in patients with HIV/AIDS, followed by *Streptococcus pneumoniae*. Accounting all patients, only 8% had atypical bacteria (*Legionella pneumophila*, M*ycoplasma pneumoniae* or *Chlamydophila pneumoniae*).
EUA 1994-1996 Park (15)	General population hospitalised by CAP with analysis of patients with HIV/AIDS	112 patients 65% with determined etiological agent	*P. jirovecii* was the main agent (1/3), followed by *Mycobacteria tuberculosis*, *S. pneumoniae and M. pneumoniae.* Bacterial pathogens present in 25% of cases, atypical bacteria found in 12%. Mixed infection was demonstrated in 17%. *P. jirovecii* most commonly found in patients with CD4<200/mm^3.
EUA 1994-1996 Rimland (13)	Patients with HIV/AIDS and CAP	230 patients 67% with determined etiological agent	*P. jirovecii* accounted for 35%, bacterial aetiologies were common. Mixed infection was demonstrated in 64 cases. Association between *P. jirovecii* and bacterial was frequent (19 cases). 12 patients had mycobacteria and 4 had fungus as single agents. Atypical bacteria and viruses were rare and always occurred in association with other agents.
Italy 2000-2001 Viale (16)	Patients with HIV/AIDS and CAP	231 patients 49% with determined etiological agent	44 bacterial pneumonia cases (19%), 29 *P. jirovecii* cases (13%), 6 *M. tuberculosis* cases (3%). *S. pneumoniae* was the most frequent isolated bacteria (34 cases), followed by *Staphylococcus aureus* (12 cases) and *Pseudomonas aeruginosa* (9 cases).
Spain 2007-2012 Cilloniz (12)	Patients with HIV/AIDS and CAP	331 patients 69% with determined etiological agent	*S. pneumoniae* was the main agent (30%), followed by *P. jirovecii* (13%). Polymicrobial infection were identified in 17%. Respiratory viruses were identified in 18%. *Haemophilus influenzae* 7%. *Staphylococcus aureus* 6%. Atypical bacteria were rare (1%). Fungi other than *P. jirovecii* and mycobacteria were not analysed.

Overview of studies on CAP treatment

The treatment of CAP is often empiric and different approaches have been studied and compared across the literature. The use or not of a macrolide in hospitalized patients is a major part of this debate (17).

There are three main explanations why a macrolide added to a beta-lactam treatment may have an effect on the outcome of patients with CAP: coverage against atypical bacteria; synergic activity with beta-lactams; and immunomodulatory properties (18).

The immunomodulatory effects of macrolides remain incompletely understood (19) and could influence both the pathogen and the host (18). Macrolides reduce the production of the pneumococcal toxin pneumolysin (20) and appear to inhibit adherence and virulence factors of *P. aeruginosa*, besides affecting biofilm formation (21). It is postulated that the systemic inflammatory response syndrome generated by CAP could be modulated through macrolide effects in various cellular functions, including inflammatory cytokine production, cell proliferation and mucin secretion, reducing the pulmonary parenchyma injury(18,21).

Current studies have heterogeneous target populations, treatment regimens and evaluated outcomes, a summary of selected studies is presented in Table 2.

Even though some of the current evidence suggests a benefit in mortality from macrolide-based antibiotic therapy (22–27) different conclusions about the impact of macrolides on mortality can be drawn from recently published meta-analyses and, apparently, this effect is more pronounced in severely ill patients (28–30).

Two recently published clinical trials showed somewhat conflicting results for moderately severe CAP. One is a pragmatic, cluster-randomized, crossover trial that found that β-lactam monotherapy was not inferior to β-lactam-macrolide combination or fluoroquinolone monotherapy concerning 90-day mortality (31).

The other one was an open-label, multicentre, randomized trial that was unable to demonstrate the non-inferiority of clinical stability at day 7 comparing

empirical treatment with a β-lactam alone compared with a β-lactam-macrolide combination. Patients infected with atypical pathogens or category IV pneumonia severity index (PSI) were less likely to reach clinical stability if they received monotherapy. In this study, severely immunosuppressed patients were excluded (32).

Although patients with HIV/AIDS are at increased risk of acquiring pneumonia when compared to the general population and have higher mortality rates (9), there is a lack of studies in this population. Several studies presented in Table 2 excluded patients with HIV or severely immunosuppressed patients.

A multicentre prospective observational study of patients with pneumococcal bacteraemia, predominantly pneumonia, found that combination antibiotic therapy was associated with significantly lower 14-day mortality in severely ill cases, even when adjusted for HIV infection. In this study, it was not possible to determine which specific components of combination therapy would be most effective and nonmacrolide combinations were also successful in reducing mortality among the severely ill patients (33).

To the best of our knowledge, the only study that compared different treatments of CAP in patients with HIV/AIDS is a retrospective study that showed similar mortality rates between hospitalised patients who received ceftriaxone and those with ceftriaxone plus clarithromycin (34), and there are no clinical trials of antibiotic treatment for CAP in patients with HIV/AIDS.

Table 2. Summary of studies that evaluated the treatment of community acquired pneumonia (CAP)

Author	Country/ Year	**Population**	**Exclusion of patients with HIV/AIDS or severely immunocompromised**	**Methods**	**Main results**
Gleason (35)	USA 1999	>65 years CAP	Yes	Retrospective Multicentre	Smaller 30 days mortality with β-lactam+ macrolide or quinolone vs other regimes
Mufson (22)	USA 1999	Pneumococcal bacteraemia	No	Prospective Multicentre	Smaller mortality with macrolide association to the regimen
Waterer (36)	USA 2001	Pneumococcal bacteraemia	Yes	Retrospective Multicentre	Smaller inhospital mortality with combined therapy vs monotherapy
Martinez (23)	Spain 2003	Pneumococcal bacteraemia	No	Retrospective Single centre	Smaller inhospital mortality with β-lactam+ macrolide vs β-lactam alone
Weiss (24)	Canada 2004	Pneumococcal bacteraemia	No	Retrospective Single centre	Smaller inhospital mortality with cephalosporin + macrolide vs cephalosporin alone
Baddour (33)	International 2004	Pneumococcal bacteraemia	No	Prospective Multicentre	Smaller 14 days mortality in severe patients with combined therapy vs monotherapy
Rodriguez (37)	Spain 2007	CAP with shock	No	Prospective Multicentre	Smaller 28 days mortality in severe patients with combined therapy vs monotherapy
Metersky (25)	USA 2007	Pneumococcal pneumonia	No	Retrospective Multicentre	Smaller 30 days mortality with macrolide association to the regimen
Martín (27)	Europe 2009	CAP with mechanical ventilation	Yes	Prospective Multicentre	Smaller mortality with β-lactam + macrolide vs β-lactam + quinolone
Restrepo (26)	USA 2009	CAP with severe sepsis	Yes	Retrospective Multicentre	Smaller 30 and 60 days mortality in patients that received macrolide vs who did not
Garin (32)	Switzerland 2014	CAP with moderate severity	Yes	Prospective Multicentre	Severely ill patients had delayed clinical stability with β-lactam vs β-lactam+ macrolide
Postma (31)	Netherlands 2015	CAP	No	Prospective Multicentre	β-lactam vs β-lactam + macrolide or quinolone Non- inferior regarding 90-day mortality

Outline of this thesis

The main purpose of this thesis was to evaluate if treatment with ceftriaxone and a macrolide improved patient outcome when compared with monotherapy with ceftriaxone in hospitalized patients with HIV/AIDS with CAP.

Chapter 2, written in Portuguese, is the original research project submitted to appropriate institutional Committee of Ethics in Research.

In chapter 3 we present the randomized clinical trial in which adult patients with HIV hospitalized due to suspected CAP were randomized to receive one of two regimens, ceftriaxone plus macrolide or ceftriaxone plus placebo, at a 1:1 proportion. The primary outcome was in-hospital mortality and the secondary outcomes were mortality within 14 days, need for vasoactive drugs, need for mechanical ventilation, time to clinical stability and length of hospitalization.

The aetiology of community-acquired pulmonary infections in these hospitalised patients with HIV was also studied and determined prospectively, as presented in chapter 4. This investigation also aimed to analyse the contribution of different diagnostic methods, including those PCR-based, as well of the impact of different approaches to microbiological evaluation and to evaluate the microbiological findings in relation to the CD4+ T cell count, the severity of disease and pneumococcal vaccine status.

Chapter 5 brings a critical analysis and recommendations for adults with HIV/AIDS who seek medical care with acute pulmonary community-acquired infections, based on the findings presented on this thesis along with current knowledge on the matter.

Finally, chapter 6 presents the conclusions of this thesis.

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2. Research project: Ensaio clínico randomizado sobre o impacto dos macrolídeos na mortalidade de pacientes infectados pelo HIV e com pneumonia adquirida na comunidade.

Introdução

A pneumonia bacteriana é uma das principais causas de morbidade e mortalidade nos pacientes com vírus da imunodeficiência humana (HIV) (1,2), sua incidência diminuiu após a introdução da terapia antirretroviral altamente ativa (TARV) (3,4), porém estes pacientes ainda apresentam maior risco de adquirir esse tipo de infecção que a população geral (3), além de terem maiores taxas de doença pneumocócica invasiva (5) e mortalidade (6,7).

A etiologia da pneumonia bacteriana adquirida na comunidade (PAC) em pacientes infectados pelo HIV é semelhante à encontrada em imunocompetentes, sendo o principal agente o *Streptococcus pneumoniae*. São comuns as infecções por bactérias gram-negativas (incluindo *Haemophylus influenzae*, *Pseudomonas aeruginosa* e *Legionella pneumophilla, entre outras*) e pelo *Sthaphylococcus aureus* (1–3,6).

São considerados fatores de risco para PAC em pacientes infectados pelo HIV: baixo nível socioeconômico, tabagismo, alcoolismo, uso de drogas intravenosas (IV), comorbidades (incluindo doença cardiovascular, afecção renal ou cirrose hepática), desnutrição, baixas contagens de célula T CD4+ (CD4<200 células/µL), replicação do HIV e ausência de TARV (2).

Tratando-se de prevenção, o papel da vacina antipneumocócica em pacientes infectados pelo HIV ainda não está bem definido, já que os estudos apresentam resultados controversos (8,9). Também a profilaxia com sulfametoxazol-trimetoprim (SMX-TMP) é uma questão em debate, já que parece prevenir a pneumonia bacteriana apenas nos pacientes que não fazem uso de TARV (1,3).

Até o presente momento, foram propostas algumas maneiras de avaliar a gravidade de um episódio de pneumonia em um paciente infectado pelo HIV, porém nenhuma delas foi validada neste grupo de pacientes (2). São regras

interessantes o *Pneumonia Severity Index* (PSI), que tem a capacidade de predizer a mortalidade em pacientes com HIV (10), e o CURB-65, adotado pelo Consenso Brasileiro de PAC por sua simplicidade (11).

Outra estratégia para predizer gravidade é combinar os escores com a avaliação da contagem de CD4, já que pacientes com CD4 menor que 200 células/µL apresentam maior mortalidade. Sendo assim, seriam considerados graves os pacientes com CD4<200 células/µL ou com alta pontuação em escores de gravidade (2,10).

A avaliação da gravidade do paciente é importante para a decisão do tipo de tratamento e o local onde ele deve ser realizado. Na realidade, a escolha do tratamento empírico para pneumonia bacteriana em pacientes infectados pelo HIV é um grande desafio, já que a maioria dos estudos e consensos é voltada para pacientes imunocompetentes.

Além disso, os estudos que tratam da escolha de terapia empírica para pneumonia adquirida na comunidade são heterogêneos quanto aos esquemas comparados, à população incluída no estudo e aos desfechos analisados. Alguns estudos contêm um grupo de pacientes com imunodeficiências, mas nenhum deles avaliou particularmente pacientes infectados pelo HIV (12–14).

Um estudo espanhol, realizado em 2003, avaliou pacientes com bacteremia por pneumococo (sem excluir os pacientes com HIV) e demonstrou menor mortalidade durante a internação no grupo beta-lactâmico mais macrolídeo (p= 0,001) (14).

No estudo citado, os pacientes que pertenciam ao grupo que não recebeu macrolídeo apresentavam comorbidades com mais frequência, particularmente HIV e doenças hematológicas malignas (p= 0,0002). Porém, não há relato do porcentual de pacientes com HIV entre os sujeitos estudados e este grupo de pacientes não foi analisado separadamente (14).

Já o estudo multicêntrico (internacional) e prospectivo realizado em 2004, com o objetivo de avaliar o efeito na mortalidade da combinação de antibióticos em pacientes com bacteremia por pneumococo, especificou o número de pacientes portadores do HIV em cada grupo. No grupo que recebeu terapia combinada 11,4% dos pacientes eram portadores do HIV e no grupo de monoterapia 37,0%, esta diferença foi significativa, com p<0,01 (13).

O estudo fez uma análise por regressão logística e demonstrou que, mesmo após o ajuste para o HIV, a antibioticoterapia combinada manteve associação positiva com a sobrevivência (*Odds ratio* 0,10; SE 1,7; Intervalo de Confiança (IC) 95% 1,1-9,2; p= 0,028). Cabe ressaltar que este estudo não conseguiu especificar quais classes de antibiótico que compunham a terapia combinada eram responsáveis por sua superioridade (13).

Há estudos que mostram diminuição da mortalidade de pacientes tratados em regime hospitalar com a combinação de beta-lactâmicos com macrolídeos quando comparados a outros regimes (14). Outros trabalhos avaliam que há benefício dos regimes que contém macrolídeos quando comparados a regimes que não possuem esta classe de antibiótico (12,15). Há ainda os que demonstram o benefício da terapia combinada sobre a monoterapia (13).

As razões pelas quais o macrolídeo pode levar à melhores resultados em pacientes com PAC são: sinergismo de antimicrobianos, cobertura de germes atípicos e efeito imunomodulador. Dentre estas, a mais impactante é a imunomodulação da resposta inflamatória e interferência na patogenicidade do agente etiológico, já que existem trabalhos que mostram o benefício da terapia com macrolídeos mesmo em pacientes com microorganismos resistentes a esta classe de antibiótico (15,16).

Um estudo americano retrospectivo e multicêntrico, realizado em 2009, incluiu pacientes com PAC e sepse grave e excluiu pacientes infectados pelo HIV. Este estudo mostrou menor mortalidade em 30 e 60 dias com o uso de macrolídeos, inclusive no grupo de pacientes que tinham sepse grave por germe resistente a esta classe de antibiótico (*Hazard ratio* 0,10; IC95% 0,02-0,49; p= 0,005) (15).

Os efeitos imunomoduladores dos macrolídeos ainda não são bem esclarecidos, sendo que há estudos que sugerem interferência na ação dos neutrófilos (16,17) e outro estudo que demonstra diminuição da produção de pneumolisina pelo *Streptococcus pneumoniae* (18).

Um estudo em particular revela que a azitromicina pode ter um efeito bifásico, inicialmente aumentando os efeitos bactericidas dos neutrófilos e posteriormente, quando o microrganismo já foi eliminado, induzindo a apoptose

deste e interrompendo a inflamação, levando a diminuição do dano tecidual e tempo de doença (17).

Há muito se sabe que a infecção pelo HIV leva a uma disfunção qualitativa e quantitativa dos neutrófilos (19,20). A proteção conferida por estas células frente à infecção bacteriana está comprometida nestes pacientes devido às deficiências na quimiotaxia e fagocitose, produção de radicais livres e expressão alterada de moléculas de adesão celular (20). Há também aceleração do processo de apoptose dos neutrófilos, contribuindo para a disfunção e diminuição da população destas células (21).

Um recente estudo retrospectivo brasileiro, realizado em 2010, analisou somente pacientes em regime hospitalar, infectados pelo HIV e com um episódio de PAC para avaliar o impacto da terapia com macrolídeo. Foram comparados dois grupos: um que recebeu monoterapia com beta-lactâmico e outro que recebeu beta-lactâmico e macrolídeo. Não houve diferença quanto ao desfecho primário analisado, mortalidade durante a internação, entre os grupos (22).

Considerando que até o presente momento não há consenso sobre a melhor terapia empírica para pacientes infectados pelo HIV internados por um quadro de PAC, será realizado este estudo prospectivo com o objetivo de avaliar o impacto da terapia com macrolídeo nesta população.

Observação especial sobre este estudo

Este estudo é um estudo randomizado de uso de drogas antimicrobianas para o tratamento de pneumonia em uma população especial de pacientes. Classicamente, os estudos randomizados de antimicrobianos têm sido custeados pela indústria farmacêutica, de acordo com os interesses de comercialização dos produtos. No entanto, em situações ou indicações em que o interesse comercial no uso das drogas não é grande, a indústria não tem interesse em realizar ensaios clínicos. Por isso, restam muitas perguntas não respondidas e há pouquíssimos estudos clínicos de antimicrobianos independentes da indústria. Propomos realizar este estudo independe pela importância que tem o tema. Acreditamos que agências de fomento à pesquisa podem viabilizar estudos que atendam a interesses científicos, porém que não

apresentem interesse comercial direto para a indústria. Observem que optamos em utilizar a azitromicina de referência para que a qualidade do produto não seja objeto de questionamento.

Objetivo

Comparar a eficácia de dois regimes de tratamento para PAC em pacientes hospitalizados infectados pelo HIV:

Regime 1: monoterapia com ceftriaxona;

Regime 2: associação de ceftriaxona com macrolídeo.

O objetivo primário deste estudo é testar se a associação de um macrolídeo a um regime composto por ceftriaxona leva a uma redução na mortalidade. Os objetivos secundários são avaliação do tempo de internação e do tempo até atingir estabilidade clínica.

Métodos

Desenho do estudo

Trata-se de um ensaio clínico randomizado (1:1), com cegamento, de grupos paralelos de adultos infectados pelo HIV hospitalizados por quadro de pneumonia adquirida na comunidade (PAC) em enfermaria ou unidade de terapia intensiva (UTI) no Instituto de Infectologia Emílio Ribas (IIER), que busca testar se há superioridade entre algum dos dois regimes estudados.

População do estudo

Serão avaliados como potenciais pacientes do estudo maiores de 18 anos, infectados pelo HIV, que buscaram atendimento no pronto-socorro do Instituto de Infectologia Emílio Ribas com um quadro pulmonar.

O Instituto de Infectologia Emílio Ribas é um hospital público terciário do Sistema Único de Saúde, referência para o atendimento de moléstias infecciosas. Nos últimos anos, pacientes infectados pelo HIV correspondem a mais de 50% dos pacientes internados na instituição.

Critérios de inclusão

Serão incluídos no estudo os pacientes com diagnóstico de pneumonia, segundo evidências de alterações clínicas, radiológicas e laboratoriais, de acordo com critérios previamente estabelecidos (11):

- Presença de sintomas de doença aguda do trato respiratório inferior (tosse e um ou mais dos seguintes sintomas: expectoração, falta de ar e dor torácica) **E**
- Presença de uma opacidade pulmonar nova detectada por radiografia de tórax ou outro método radiológico.

Critérios de exclusão

Serão excluídos os pacientes:

- Com internação hospitalar por dois ou mais dias nos 90 dias prévios à admissão **OU**
- Provenientes de asilos, abrigos, casas de apoio ou casas de saúde, desde que nestes locais haja assistência à saúde **OU**
- Que receberam antibióticos por via endovenosa, quimioterapia ou tratamento de escaras nos 30 dias anteriores à doença **OU**
- Em tratamento em clínicas de diálise **OU**
- Que tenham outra causa diagnosticada para pneumopatia, como fibrose pulmonar, pneumocistose, tuberculose ou outra doença não bacteriana (estes pacientes, mesmo que incluídos na randomização inicial, serão excluídos da análise) **OU**
- Que tenham recebido o regime determinado na randomização por menos de 48 horas (estes pacientes, incluídos na randomização, serão excluídos da análise) **OU**
- Que estejam grávidas ou amamentando **OU**
- Que já tenham apresentado reação alérgica às medicações do estudo (ceftriaxona e azitromicina) **OU**
- Que já tenham sido randomizados neste estudo em outra internação.

Questões éticas

O estudo será submetido ao Comitê de Ética das instituições envolvidas. Aos pacientes que preencherem os critérios de inclusão e que não tiverem nenhum critério de exclusão será apresentado um termo de consentimento livre e esclarecido (Apêndice A). Isto deverá ser feito pelo pesquisador principal ou por pesquisadores colaboradores do trabalho devidamente cadastrados no projeto de pesquisa submetido ao comitê de ética.

Será mantida a confidencialidade e sigilo sobre a identidade dos pacientes participantes do projeto.

Há o compromisso de tornar público os resultados desta pesquisa, quaisquer que sejam eles.

Randomização

Os pacientes serão randomizados para receber um dos dois regimes de tratamento, na proporção 1:1. A sequência de randomização será criada no programa Excel por um auxiliar da pesquisa que não terá nenhum envolvimento em outras etapas e entregue à diretora da farmácia.

Os pacientes serão alocados para receber um de dois regimes:

- Regime 1: monoterapia com ceftriaxona mais placebo por no mínimo 5 dias,
- Regime 2: associação de ceftriaxona com macrolídeo por no mínimo 5 dias.

A randomização ocorrerá no momento em que for indicado pelo médico assistente um tratamento antibiótico para PAC.

Se houver azitromicina injetável disponível, o médico assistente prescreverá este tratamento da seguinte maneira: ceftriaxona 1g intravenoso (IV) a cada 12 horas e “medicação do protocolo 17/11” IV uma vez ao dia.

Ao receber esta prescrição, o farmacêutico fornecerá a ceftriaxona conforme a prescrição e uma bolsa identificada como “medicação do protocolo 17/11”. O conteúdo da “medicação do protocolo 17/11” dependerá do regime em que o paciente foi alocado:

- Regime 1: bolsa de 500mL de soro fisiológico (SF0,9%) puro.

- Regime 2: bolsa de 500mL de SF0,9% contendo 500mg de azitromicina.

Se não houver azitromicina injetável disponível, o médico assistente prescreverá este tratamento da seguinte maneira: ceftriaxona 1g intravenoso (IV) a cada 12 horas e “medicação do protocolo 17/11” IV a cada 12 horas.

Ao receber esta prescrição, o farmacêutico fornecerá a ceftriaxona conforme a prescrição e uma bolsa identificada como “medicação do protocolo 17/11”. O conteúdo da “medicação do protocolo 17/11” dependerá do regime em que o paciente foi alocado:

- Regime 1: bolsa de 500mL de soro fisiológico (SF0,9%) puro.
- Regime 2: bolsa de 500mL de SF0,9% contendo 500mg de claritromicina.

O farmacêutico fará a alocação de acordo a sequência de randomização gerada pelo programa Excel. A sequência randomizada será afixada na farmácia junto à medicação do estudo.

A equipe que prestará assistência aos pacientes e os pesquisadores não terão acesso a esta lista, sendo assim garantido o cegamento dos pacientes, prestadores de assistência e coletores de dados.

Caso haja algum efeito adverso grave, o médico assistente poderá requisitar a um profissional da farmácia o desmascaramento do regime daquele paciente específico.

Será permitida a associação com agentes antimicrobianos direcionados para agentes como *Pneumocystis jiroveccii,* micobactérias ou fungos, de acordo com a indicação clínica pelo médico que assiste o paciente (exceto fluorquinolona). Não será permitida a administração de macrolídeos ao paciente incluído no estudo enquanto o mesmo estiver recebendo o regime proposto.

Após o esquema original IV, quando necessário usar esquema oral este poderá ser feito com quinolona respiratória ou macrolídeo, segundo decisão do médico que assiste o paciente.

Métodos laboratoriais

Todos os pacientes incluídos no estudo serão submetidos a diversos exames em busca de um diagnóstico etiológico, conforme listado no Apêndice B.

Será realizada coleta de sangue na admissão para realização de hemograma, provas bioquímicas, hemoculturas, prova de aglutinação de látex para antígeno de *Cryptococcus spp.*, pesquisa de antigenemia para citomegalovírus (CMV) e sorologia para *Chlamydophila pneumoniae* e *Mycoplasma pneumoniae*.

As sorologias para *Chlamydophila pneumoniae* e *Mycoplasma pneumoniae*, deverão ser repetidas na fase de convalescença (quatorze dias após a admissão).

Haverá também tentativa de coleta de escarro (simples ou induzido) ou outras amostras respiratórias (aspirado traqueal e lavado broncoalveolar) conforme indicação do médico assistente para realização de coloração de gram, pesquisa direta de *Pneumocystis jirovecii* e de bacilo álcool-ácido resistente (BAAR), culturas, PCR (*Polimerase Chain Reaction)* para pesquisa de *Chlamydophila pneumoniae, Legionella pneumophilla e Mycoplasma pneumoniae* e painel de vírus respiratórios.

Serão processadas em cultura as amostras de escarro que, ao exame direto, tenham menos de 10 células epiteliais e mais de 25 polimorfonucleares, em campo de pequeno aumento (x100).

Pacientes com derrame pleural maior que 5 cm em uma radiografia em decúbito lateral devem ser submetidos a toracocentese e o líquido pleural deverá ser enviado para análise quimiocitológica, pesquisa de BAAR e culturas.

Será coletada também uma amostra de urina para pesquisa de antígenos de *Legionella pneumophila* sorogrupo 1.

Se houver indicação clínica para realização de biópsias, estas serão submetidas a estudos anatomopatológicos e microbiológicos. Os estudos microbiológicos incluem as colorações de Gram, Ziehl-Neelsen e coloração de prata de Gomori, assim como culturas.

Ressaltamos que todas as culturas colhidas serão semeadas em meios adequados para o crescimento de bactérias, fungos e micobactérias.

Outros exames para diagnóstico ou seguimento poderão ser solicitados a critério do médico assistente.

Dados coletados na admissão

As seguintes informações serão obtidas na admissão do paciente pelo médico assistente e complementadas pelos autores do trabalho, com o auxílio da ficha de admissão que consta do Apêndice C:

Características demográficas: idade em anos completos e sexo.

Hábitos:

- Alcoolismo (serão considerados não usuários os pacientes que não consumiram nenhuma dose no último mês, usuários leves os que consumiram até 100 doses no último mês e usuários pesados os que consumiram mais de 100 doses no último mês). Adaptado da referência (23).
- Tabagismo (serão considerados tabagistas os pacientes que fumaram pelo menos 100 cigarros na vida e fumaram pelo menos um cigarro no último mês, ex-tabagistas os que fumaram pelo menos 100 cigarros na vida e não fumaram nenhum cigarro no último mês e não tabagistas os que nunca fumaram ou fumaram menos de 100 cigarros durante a vida). Adaptado da referência (23).
- uso de drogas intravenosas (IV) (serão considerados usuários os pacientes que usaram algum tipo de droga IV no último ano, ex-usuários os que usaram droga IV alguma vez na vida, mas não usaram nenhuma vez durante o último ano e não usuários os que nunca usaram droga IV).
- uso de drogas inalatórias (VI) (serão considerados usuários os pacientes que usaram algum tipo de droga VI no último ano, ex-usuários os que usaram droga VI alguma vez na vida, mas não usaram nenhuma vez durante o último ano e não usuários os que nunca usaram droga VI).

Dados relativos ao HIV

- Tempo desde o diagnóstico
- Uso de TARV (sim ou não, qual esquema atual e há quanto tempo faz uso deste esquema, avaliação sobre aderência: serão considerados não aderentes os pacientes que tiverem deixado de tomar a TARV algum dia no último mês).

- Carga viral (serão considerados resultados obtidos durante a internação ou em até 3 meses antes da admissão).
- Contagem de células CD4+ (serão considerados resultados obtidos durante a internação ou em até 3 meses antes da admissão).
- Recebimento de vacina antipneumocócica, independente da data.
- Uso de profilaxias com antimicrobianos no último mês (sim ou não e quais medicações).
- Doenças oportunistas prévias e data de seus diagnósticos.

Presença de comorbidades, de acordo com diagnósticos dados pelos médicos assistentes (doença neoplásica, doença hepática, insuficiência cardíaca, hipertensão arterial sistêmica, acidente vascular cerebral prévio, diabetes, doença renal);

Uso de antimicrobianos em dose terapêutica no último mês (prévios e em uso).

Dados clínicos (confusão mental, definida como qualquer pontuação na escala de coma de Glasqow menor que 15, frequência respiratória, pressão arterial sistólica e diastólica, temperatura, frequência cardíaca);

Características radiológicas (infiltrado unilobar, multilobar ou derrame pleural);

Dados laboratoriais (pH, uréia, sódio, glicose, hematócrito, PaO2, satO2, DHL).

Pontuação nos escores de gravidade CURB-65 e PSI.

Estratificação de gravidade

Serão considerados graves:

- Pacientes com 2 ou mais pontos de acordo com o CURB-65 (Apêndice D, Tabelas 1 e 2) (24) **OU**
- Pacientes com escore IV ou V de acordo com o PSI (Apêndice D, Tabelas 3 e 4)(25) **OU**
- Pacientes com contagem de células CD4 abaixo de 200 (serão consideradas medidas de CD4 realizadas durante a internação ou até três meses antes da data de admissão).

Dados relativos à evolução

Outros diagnósticos feitos durante a internação.

Qual foi o diagnóstico etiológico do quadro pulmonar.

Tempo de uso do regime estabelecido pelo estudo.

Houve troca do regime inicial por caracterização de falha?

Houve troca do regime inicial por antibiótico VO? Quinolona ou azitromicina?

Houve troca do regime inicial por outras causas?

Uso de outros antimicrobianos direcionados para outros agentes, em dose terapêutica. Quais?

Uso de outros antimicrobianos em dose profilática.

Desfechos

O desfecho primário analisado será:

- Mortalidade durante a internação.

Os desfechos secundários serão:

- Mortalidade nos primeiros 14 dias da admissão.
- Duração total da hospitalização.
- Dia da estabilização da temperatura (indica o primeiro dia no qual todas as medidas tomadas foram menores ou iguais a 37,8ºC). Adaptado da referência (26).
- Dia da estabilização da freqüência cardíaca (indica o primeiro dia no qual todas as medidas tomadas foram menores ou iguais a 100bpm). Adaptado da referência (26).
- Dia da estabilização da freqüência respiratória (indica o primeiro dia no qual todas as medidas tomadas foram menores ou iguais a 24ipm). Adaptado da referência (26).
- Dia da estabilização da pressão arterial (indica o primeiro dia no qual todas as medidas de PAS tomadas foram maiores ou iguais a 90mm Hg). Adaptado da referência (26).
- Dia da estabilidade clínica completa (indica o primeiro dia no qual todos sinais vitais estudados - T, FC, FR e PA - são considerados estáveis).
- Duração da internação em unidade de terapia intensiva.

- Tempo de uso de VNI.
- Tempo de uso de VM.
- Tempo de uso de oxigênio suplementar. (cateter de oxigênio ou máscara de Venturi).
- Presença de instabilidade hemodinâmica, definida como necessidade de drogas vasoativas.
- Troca do esquema inicial por caracterização clínica de falha (segundo médico assistente).
- Troca do esquema inicial por outras causas (por exemplo, alergia).

Cálculo da amostra e análise estatística

A amostra calculada foi 87 pacientes para cada regime de tratamento. Foi determinado um erro alfa de 0,05 e um poder de 80%, a relação de pacientes entre os grupos de 1:1, uma estimativa de mortalidade nos pacientes que receberem o Regime 1 de 29% (15) e de 11% nos pacientes que receberem o Regime 2.

Tendo em vista que a randomização ocorrerá no momento da prescrição do tratamento para PAC e supondo que 30% destes pacientes serão excluídos após a randomização somaremos esta perda ao cálculo da amostra. Assim, serão necessários 114 pacientes para cada grupo de tratamento.

Serão feitas tabelas de freqüência das variáveis categóricas e estatísticas descritivas (média, desvio-padrão, valores mínimos e máximos e mediana) das variáveis contínuas. O dia da admissão será considerado o dia 1.

Para comparação das variáveis categóricas entre os grupos serão utilizados os testes Qui-Quadrado ou, quando necessário, o teste exato de Fisher. Para as variáveis contínuas será utilizado o teste de Mann-Whitney. O nível de significância para os testes estatísticos será 5%. Os dois grupos serão comparados quanto a cada um dos desfechos. Será calculado o risco relativo e o intervalo de confiança de 95%.

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3. Manuscript 1: Ceftriaxone versus ceftriaxone plus a macrolide for community acquired pneumonia in hospitalized patients with HIV/AIDS: a randomized controlled trial

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Abstract

Objectives: Evaluate if treatment with ceftriaxone and a macrolide, improved patient outcome when compared with monotherapy with ceftriaxone, in hospitalized patients with HIV/AIDS with community-acquired pneumonia (CAP).
Methods: Adult patients with HIV hospitalized due to suspected CAP were randomized to receive one of two regimens, ceftriaxone plus macrolide or ceftriaxone plus placebo, at a 1:1 proportion (Brazilian Clinical Trials Registry: RBR-8wtq2b). The primary outcome was in-hospital mortality and the secondary outcomes were mortality within 14 days, need for vasoactive drugs, need for mechanical ventilation, time to clinical stability and length of hospitalization.
Results: 227 patients were randomized, 2 were excluded after randomization. A total of 225 patients were analysed (112 receiving ceftriaxone plus placebo and 113 receiving ceftriaxone plus macrolide). The frequency of the primary outcome, in-hospital mortality, was not statistically different between the regimens: 12/112 (11%) patients who received ceftriaxone plus placebo and 17/113 (15%) who received ceftriaxone plus macrolide died during hospitalization (HR: 1.22, 95% CI: 0.57-2.59). We did not find differences between the regimens for any of the secondary outcomes, including mortality within 14 days, which occurred in 5/112 (4%) patients with ceftriaxone plus placebo and in 12/113 (11%) patients with ceftriaxone plus macrolide (RR: 2.38, 95% CI: 0.87-6.53).
Conclusions: Among hospitalized patients with HIV/AIDS with CAP, treatment with ceftriaxone and a macrolide did not improve patient outcomes, when compared with ceftriaxone monotherapy.

Introduction

The treatment of community-acquired pneumonia (CAP) is often empirical and different approaches have been studied and compared across the literature. The use of a macrolide in addition to a β-lactam in hospitalized patients is a major part of this debate (1).

There are three main explanations why macrolide added to a β-lactam treatment may have an effect on the outcome in patients with CAP: coverage against atypical bacteria; synergistic activity with β-lactams; and immunomodulatory properties (2).

Even though some of the current evidence suggests a benefit in mortality from macrolide-based antibiotic therapy (3–8), different conclusions about the impact of macrolides on mortality can be drawn from recently published meta-analyses and, apparently, this effect is more pronounced in severely ill patients (9–11).

Two recently published clinical trials showed somewhat conflicting results for moderately severe CAP. One is a pragmatic, cluster-randomized, crossover trial that found that β-lactam monotherapy was not inferior to β-lactam-macrolide combination or fluoroquinolone monotherapy concerning 90-day mortality (12).

The other one was an open-label, multicentre, randomized trial that was unable to demonstrate no inferiority of clinical stability at day 7 comparing empirical treatment with a β-lactam alone relative to a β-lactam-macrolide combination. Patients infected with atypical pathogens or category IV pneumonia severity index (PSI) were less likely to reach clinical stability if they received monotherapy. In this study, severely immunosuppressed patients were excluded (13).

Current studies have heterogeneous target populations, treatment regimens and evaluated outcomes. The majority excluded patients with HIV or severely immunosuppressed patients. Although patients with HIV/AIDS are at increased risk of acquiring pneumonia when compared to the general population and have higher mortality rates (14), there is a lack of studies in this population.

To the best of our knowledge, the only study that compared different treatments of CAP in patients with HIV/AIDS is a retrospective study that showed similar mortality rates between patients who received ceftriaxone and those with ceftriaxone plus clarithromycin, and there are no clinical trials of antibiotic treatment for CAP in patients with HIV/AIDS (15).

The aim of this study was to evaluate if treatment with ceftriaxone and a macrolide improved patient outcome when compared with monotherapy with ceftriaxone, in hospitalized patients with HIV/AIDS with CAP.

Methods

Trial design and participants

This is a randomized controlled trial of parallel groups (1:1), conducted at Instituto de Infectologia Emílio Ribas, a tertiary teaching infectious disease hospital in São Paulo, Brazil (Brazilian Clinical Trials Registry: RBR-8wtq2b).
The eligibility criteria for participants were: patients 18 years of age or older, who refer HIV infection at admission, with clinically and radiologically suspected CAP who required antibiotic treatment and hospitalization. Patients were eligible irrespective of CAP severity and requirement of ICU admission.

Suspected CAP was defined by the three following criteria: 1 – cough, 2 - dyspnoea or chest pain or sputum production, 3 - lung opacity detected by a radiologic method. These criteria are derived from previously suggested diagnosis approaches (16,17).

The exclusion criteria were: empirical antibiotic treatment directed for CAP other than ceftriaxone, risk factors for healthcare-associated pneumonia (hospitalization for 2 days or more in the preceding 90 days, residence in a nursing home or extended care facility, home infusion therapy, chronic dialysis within 30 days or home wound care) (18), presence of an aetiology established prior to admission that explained all the symptoms, previous inclusion in the trial and pregnancy or breastfeeding.

The attending physicians identified patients who met the inclusion criteria and did not meet any exclusion criteria. The investigators obtained informed consent.

All patients provided written informed consent and the study was approved by the Institutional Committee of Ethics in Research (number 17/11).

Interventions

Physicians prescribed intravenous (IV) ceftriaxone 1g, each 12 hours and, after informed consent, the IV “study medication”. The pharmacy prepared its content according to the allocation: regimen 1 - NaCl 0.9% 500mL (placebo) or regimen 2 - Macrolide diluted in NaCl 0.9% 500mL. Irrespectively of the content, the containers were indistinguishable and were labelled as “17/11 study medication”.

The preferred macrolide was azithromycin 500mg, once a day. When unavailable, clarithromycin 500mg every 12 hours was dispensed. During the period in which only clarithromycin was available, two containers were dispensed per day, ensuring the masking.

The first dose of the assigned regimen was administered within the first 48 hours of hospitalization and was given in-hospital for at least 7 days.

Outcomes

The primary outcome was in-hospital mortality and the secondary outcomes were mortality within 14 days, need for vasoactive drugs, need for mechanical ventilation, time to clinical stability and length of hospitalization.

Patients discharged before day 14 were considered alive for the analyses of the mortality within 14 days if confirmed by review of health records (Brazilian CD4+ T cell count/HIV viral load database and medical records from Instituto de Infectologia Emílio Ribas, Instituto Adolfo Lutz and Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo) of consultations, diagnostic procedures or antiretroviral (ART) dispensation after the discharge.

Patients who left the hospital against medical recommendation where excluded from the analysis of clinical stability and length of hospitalization. Patients who died were counted as maximum value +1.

The first day of admission was considered day 1 and the time to clinical stability was considered as the first day on which all the vital signs were stable or

the discharge day. The stability cut points for vital signs were: heart rate ≤ 100 beats/min; systolic blood pressure > 90mmHg; respiratory rate ≤ 24 breaths/min (19); and axillary temperature ≤ 37.8ºC.

Sample size

Based on a mortality rate of 29% with regimen 1 and 11% with regimen 2 (7), and assuming a two-sided 5% significance level, a power of 80% and a dropout rate of 30%, the calculated sample size was 228 patients (114 per regimen).

Randomization and masking

A collaborator generated a simple randomized sequence using Microsoft Excel version 2013 (Microsoft Corporation, Redmond, WA) in which participants were assigned to receive one of two regimens, at a 1:1 proportion. This list was delivered to the pharmacy. Allocation to the study was done in the pharmacy. Thus, patients, caregivers and those who evaluated outcomes were blinded to the antibiotic treatment regimen.

Unmasking the regimen was only possible in two situations: identification of serious adverse event at the discretion of the attending physician in agreement with the principal investigator, and microbiological findings that required appropriate antibiotic.

Statistical methods

The primary outcome, in-hospital mortality, was compared between regimens using Cox regression. Mortality within 14 days was compared using log-binomial regression and the other dichotomous secondary outcomes were compared with logistic regression. Continuous secondary outcomes, time to clinical stability and length of hospitalization, were compared between regimens using Mann-Whitney test.

Analyses were performed in accordance with the intention-to-treat principle.

We did four *post hoc* subgroup analyses: severely ill patients (CURB-65 score (20) > 2 or PSI (21) > III), patients with an identified bacterial pathogen,

patients with identified atypical bacteria, and patients with CD4+ T cell count > 200 cells/mm^3.

A sensitivity analysis was performed to take into account competing events: we constructed a competing-risks model for in-hospital mortality, treating discharge as a competing event.

The level of significance was set at 0.05 (two-tailed). Analyses were performed using STATA 14.0 (StataCorp. 2007. *Stata Statistical Software: Release 14. College Station*, TX: StataCorp LP).

Follow up

The following data were recorded on admission: sociodemographic characteristics, time since HIV diagnosis, use of ART, comorbidities, drug use, antibiotic use within the last 30 days and pneumococcal vaccination status.
CD4+ T cell counts and HIV viral load were recorded if collected within the last 3 months or during hospitalization.

Subjects of this study were submitted to an extensive microbiological investigation, with details and results described elsewhere (22). CAP caused by atypical organisms was defined by *Chlamydophila pneumoniae*, *Mycoplasma pneumonia* or *Legionella pneumophilla* infection. The results of serology and polymerase chain reaction for atypical organism were not accessible for the clinicians, as they were performed posteriorly for analysis purposes only.

Administration of a macrolide or a fluoroquinolone in therapeutic or prophylactic doses was not allowed while the patient was receiving the study regimens. As indicated by the attending physician, other antimicrobial agents could be associated with the study regimen to ensure proper treatment of other microorganisms, such as fungi or mycobacteria.

The patients were followed until hospital discharge, and the following data were registered: use of other antimicrobial drugs, use of antiretroviral treatment and causes of change or interruption of the initial antibiotic regimen.

Results

Baseline characteristics and clinical data

Patients were assessed for eligibility between September 2012 and July 2014 and 227 were randomized. We had 2 exclusions after randomization, one patient who withdrew consent for data inclusion and use and one that had previously been included (Figure 1), leaving a total of 225 patients to analyse (112 received ceftriaxone plus placebo and 113 received ceftriaxone plus macrolide).

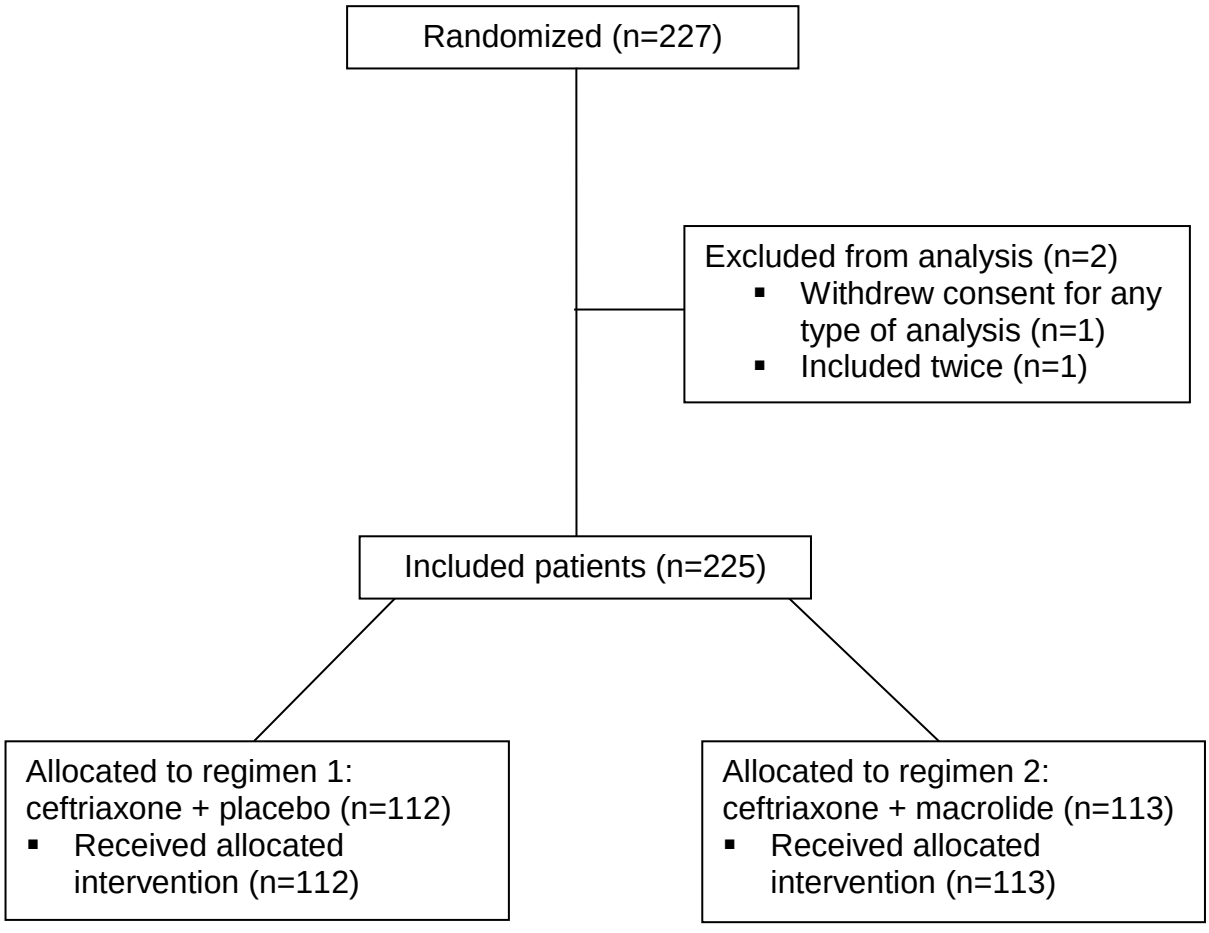


Figure 1 - Inclusion of patients, randomization and analysis of a controlled trial of hospitalized patients infected with HIV/AIDS treated for community-acquired pneumonia

The baseline characteristics of the patients are shown in Table 1. Patients had prolonged HIV infection, the median period was twelve years, and most of them did not make regular use of ART. Only 32/202 patients (16%) had viral load below 50 copies/mL and 146/202 (72%) had a CD4+ T cell count below 200 cells/mm^3.

Regarding the severity of the pneumonia, 16/225 (7%) had a CURB-65 score greater than two and 39/225 (17%) had a PSI higher than three.

Microbiological findings and antimicrobial treatment

A microbiological agent was determined in 144/225 (64%) patients. No important differences were observed between the regimens (Table 2).
Mixed aetiology was found in a large proportion of cases 48/225 (21%), with multiple combinations detailed elsewhere (22).

Among patients who received macrolide treatment as part of the study regimen, 97/113 (86%) patients received azithromycin and 16/113 (14%) patients received clarithromycin. Clarithromycin was given for a 2-month period when azithromycin was unavailable.

Initial antibiotic regimen was interrupted or changed in 35/112 (31%) patients who received the ceftriaxone plus placebo regimen and in 52/113 (46%) patients who received the ceftriaxone plus macrolide regimen, the detailed reasons are listed in the supplementary material (Supplementary Table 1).

The use of additional antimicrobials administered outside the study regimens was similar for both regimens (Table 2). ART was prescribed during hospitalization for 63/112 (56%) patients who received ceftriaxone plus placebo and 68/113 (60%) patients who received ceftriaxone plus macrolide. No serious adverse events were observed during the study.

Outcomes

The frequency of the primary outcome, in-hospital mortality, was not statistically different between the studied regimens: 12/112 (11%) patients who received ceftriaxone plus placebo and 17/113 (15%) who received ceftriaxone plus macrolide died during hospitalization (HR: 1.22, 95% CI: 0.57-2.59) (Table 3).

Table 1 - Baseline characteristics of the patients allocated to the treatment regimens

Characteristics	Ceftriaxone + Placebo N = 112	Ceftriaxone + Macrolide N = 113
Age (years) - mean (SD)	40.0 (12.5)	40.7 (10.6)
Male sex	75 (67%)	80 (71%)
Years of HIV infection - median (range) (n=188[1])	12 (1-30)	11.5 (1-27)
Regular usage of ART	22 (20%)	20 (18%)
Viral load < 50 copies/mL (n=202[1])	19 (19%)	13 (13%)
CD4 T cell count (/mm3) - median (range) (n=202[1])	100.5 (1-1108)	36.5 (1-920)
1-49	40 (39%)	58 (58%)
50-199	30 (30%)	18 (18%)
200-349	10 (10%)	10 (10%)
350-499	10 (10%)	7 (7%)
>499	12 (12%)	7 (7%)
Comorbidities	35 (31%)	33 (29%)
Hypertension	11 (10%)	15 (13%)
Liver disease	11 (10%)	11 (10%)
Neoplastic disease	6 (5%)	4 (3%)
Diabetes mellitus	4 (4%)	5 (4%)
Cardiac insufficiency	4 (4%)	5 (4%)
Renal disease	3 (3%)	2 (2%)
Chronic obstructive pulmonary disease dependent on oxygen	0	3 (3%)
Cerebrovascular disease	1 (1%)	2 (2%)
Drug use (n=224[1])	63 (57%)	69 (61%)
Tobacco (n=223[1])	45 (41%)	47 (42%)
Alcoholism (n=221[1])	36 (33%)	48 (42%)
Inhaled drug (n=224[1])	32 (29%)	36 (32%)
Intravenous drug (n=224[1])	1 (<1%)	1 (<1%)
Antibiotic use in the last 30 days (n=219[1])	56 (50%)	54 (50%)
Prophylactic (n=216[1])	20 (18%)	17 (16%)
Therapeutic (n=215[1])	44 (41%)	43 (40%)
Pneumococcal vaccination (n=162[1])	26 (30%)	24 (32%)
CURB-65 score		
0-1	82 (73%)	80 (71%)
2	20 (18%)	27 (24%)
3-5	10 (9%)	6 (5%)
Pneumonia Severity Index		
I-II	69 (61%)	68 (60%)
III	21 (19%)	28 (25%)
IV-V	22 (20%)	17 (15%)

Data is shown as frequency (%) unless otherwise indicated. ART: antiretroviral therapy. [1] Number of patients for whom data was available.

Table 2 - Microbiological findings and antimicrobial treatments administered outside the study regimens

	Ceftriaxone + Placebo N = 112 (%)	Ceftriaxone + Macrolide N = 113 (%)
Seven most frequent pathogens:		
Fungi	23 (20)	33 (29)
Pneumocystis jirovecii	23 (20)	29 (26)
Bacteria	21 (19)	21 (19)
Streptococcus pneumoniae	11 (10)	11 (10)
Mycoplasma pneumoniae	8 (7)	4 (3)
Clamydophila pneumoniae	2 (2)	5 (4)
Virus	22 (20)	22 (19)
Rhinovirus	10 (9)	12 (11)
Influenza	7 (6)	8 (7)
Mycobacteria	21 (19)	14 (12)
Mycobacterium tuberculosis	15 (13)	14 (12)
Seven most frequent additional antimicrobials:		
Trimethoprim-Sulfamethoxazole	50 (45)	62 (55)
Fluconazole	38 (34)	48 (43)
Rifampicin + Isoniazid + Pyrazinamide + Ethambutol	21 (19)	21 (19)
Acyclovir	15 (13)	16 (14)
Vancomycin	11 (10)	19 (17)
Clindamycin	10 (9)	11 (10)
Piperacillin-Tazobactam	10 (9)	11 (10)

Data is shown as frequency (%)

Table 3 - Primary and secondary outcomes according to treatment regimen

Outcome	Ceftriaxone + Placebo N = 112	Ceftriaxone + Macrolide N = 113	Relative Risk (95% confidence interval)	p-value
Primary outcome:				
In-hospital mortality	12 (11)	17 (15)	1.22 (0.57-2.59)[1]	0.61
Secondary outcomes:				
Mortality within 14 days	5 (4)	12 (11)	2.38 (0.87-6.53)[2]	0.09
Use of vasoactive drug	20 (18)	23 (20)	1.18 (0.60-2.29)[3]	0.63
Use of mechanical ventilation	20 (18)	24 (21)	1.24 (0.64-2.40)[3]	0.52
Days to reach clinical stability, *median (range)*	5 (1-44)	6 (1-44)	N/A	0.80[4]
≤7 days	64 (57.1)	63 (55.8)	0.98 (0.76-1.23)	0.83
Days of hospitalization, *median, (range)*	15 (2-114)	18 (2-114)	N/A	0.31[4]
≤10 days	32 (28.6)	29 (25.7)	0.90 (0.58-1.38)[2]	0.62
≤20 days	68 (60.7)	68 (53.1)	0.87 (0.70-1.10)[2]	0.25

N/A: not applicable. Data is shown as frequency (%) unless otherwise indicated. [1] Hazard ratio calculated with Cox regression. [2] Risk ratio calculated with log-binomial regression. [3] Odds ratio calculated with logistic regression. [4] Mann-Whitney test.

We did not find differences between the regimens for the secondary outcomes: mortality within 14 days (RR: 2.38, 95% CI: 0.87-6.53), need for vasoactive drug (OR: 1.18, 95% CI: 0.60-2.29) or mechanical ventilation (OR: 1.24, 95% CI: 0.64-2.40) (Table 3).

The median time until clinical stability was 5 days for those who received ceftriaxone monotherapy and 6 days for those who received ceftriaxone plus macrolide and the median length of hospitalization was 15 days for those who received ceftriaxone monotherapy and 18 days for those who received ceftriaxone plus macrolide (Table 3).

In the sensitivity analysis, a competing-risk model for in-hospital mortality, treating discharge as a competing event, there was no significant difference in the cumulative incidence function curves between groups (Supplementary Figure 1, subhazard ratio: 1.49, 95% CI: 0.71-3.10, p=0.29).

There was no statistically significant difference for in-hospital mortality between the regimens in all four subgroup *post hoc* analyses: severely ill patients, patients with an identified bacterial pathogen, patients with identified atypical bacteria, and patients with CD4+ T cell count > 200 cells/mm^3 (Supplementary Table 2).

Discussion

Patients with HIV/AIDS treated with ceftriaxone and a macrolide did not have better outcomes than patients treated with ceftriaxone alone. Double therapy, with ceftriaxone and a macrolide did not improve the outcomes in any of the studied subgroups: patients with an identified bacterial pathogen, patients with identified atypical bacteria, and patients with CD4+ T cell count > 200 cells/mm^3.

The sensitivity analysis, treating discharge as an in-hospital mortality competing event, strengthened our confidence in the conclusion that the frequency of the primary outcome, in-hospital mortality, was not statistically different between the regimens.

The low CD4+ T cell counts of our cohort reflected their impaired immunologic status. Although patients in the combination arm tended to have

lower CD4+ T cell counts, the randomization ensures that allocation of patients to treatments is left purely to chance (23). Moreover, the proportion of patients with CD4+ T cell counts < 200/mm³ is similar between the groups and this is the threshold considered as a prognostic factor for HIV infected patients with CAP (14).

The immunomodulatory effects of macrolides remain incompletely understood (24) and could influence both the pathogen and the host (2), being postulated that the systemic inflammatory response syndrome generated by CAP could be modulated through macrolide effects (2,25). Notwithstanding, not all patients suffer from an excessive inflammatory response during pneumonia and we speculate that for our group of patients, the immunomodulatory effects of macrolides are unpredictable and may range from reducing inflammation to worsening the inflammation due to immune reconstitution.

Atypical bacteria occurred in a substantial proportion of our population (19/225, 8%). This finding would suggest that coverage against atypical agents could be beneficial. However, we failed to demonstrated a clinical benefit considering the entire cohort and in the subgroup of patients with proven atypical infection, although the subanalysis are underpowered (Table 3 and Supplementary Table 2).

The ability to detect differences between the regimens may have been reduced due to the small number of observations and due to the low proportion of severe outcomes expected for *Mycoplasma pneumoniae* and *Chlamydophila pneumoniae* infections. CAP caused by atypical organisms tend to present mild-to-moderate severity, with low in-hospital mortality (around 5%) and leads to very low rates of use of mechanical ventilation and septic shock (<1%). Furthermore, the length of hospitalization is usually short (median of 3 days) (26). *L. pneumophila*, which is most frequently responsible for severe cases (27), was investigated in the majority of patients and all were negative. This pathogen seems to have a lower incidence in South America than globally (28).

In our study, we resorted to an extended microbiological investigation and found a high frequency of non-bacterial (111/225, 49%) and mixed diagnoses (48/225, 21%) (22). However, there is no accurate method to differentiate between bacterial CAP and other causative agents (for example, virus or

Pneumocystis jirovecii) in patients with HIV/AIDS and studies of empiric CAP treatment have to deal with this difficulty (29).

We performed a subgroup analysis of patients with an identified bacterial pathogen that did not indicate a benefit of combination therapy, even though the sample size was limited (Supplementary Table 2).

This is a single centre study, conducted in “Instituto de Infectologia Emílio Ribas”, in the metropolitan region of São Paulo (approximately 20 million inhabitants). Not all patients who met the criteria for inclusion were enrolled in the trial as we used convenience sampling and data on number of screened patients or number of patients excluded by each criteria were not recorded. While this could limit the external validity of our results, this is attenuated by the fact that it was performed in a hospital that is a reference for the entire state and we have no reason to believe that the group of patients who were not included would have been substantially different from the studied patients.

Some severe cases could have been excluded (neutropenic patients, for example) if this was the reason why the attending physician decided to start empirical antibiotic treatment with something different from ceftriaxone, but the low CD4+ T cell counts of our cohort indicates that severe immunosuppressed patients were not likely to be excluded.

The overall mortality rate of our study (13%) was lower than the study used for sample size calculation (24%) and hence we could not rule out a type II error. On the other hand, we found a slightly higher mortality with the ceftriaxone plus macrolide regimen.

Finally, the macrolide or placebo was initiated within 48 hours of admission and this could have influenced the lack of an effect founded in this study. It is possible that a more prompt start of macrolide therapy could have improved efficacy. The initial antibiotic regimen was discontinued or changed in 39% of the subjects, which reflects real life challenges when dealing with a CAP episode in patients with HIV/AIDS.

In conclusion, among hospitalized HIV/AIDS patients with CAP treatment with ceftriaxone plus macrolide was not superior to ceftriaxone monotherapy in spite of a non-negligible prevalence of atypical bacteria.

Supplementary Table 1 - Reasons for changing or discontinuing the initial antimicrobial regimen

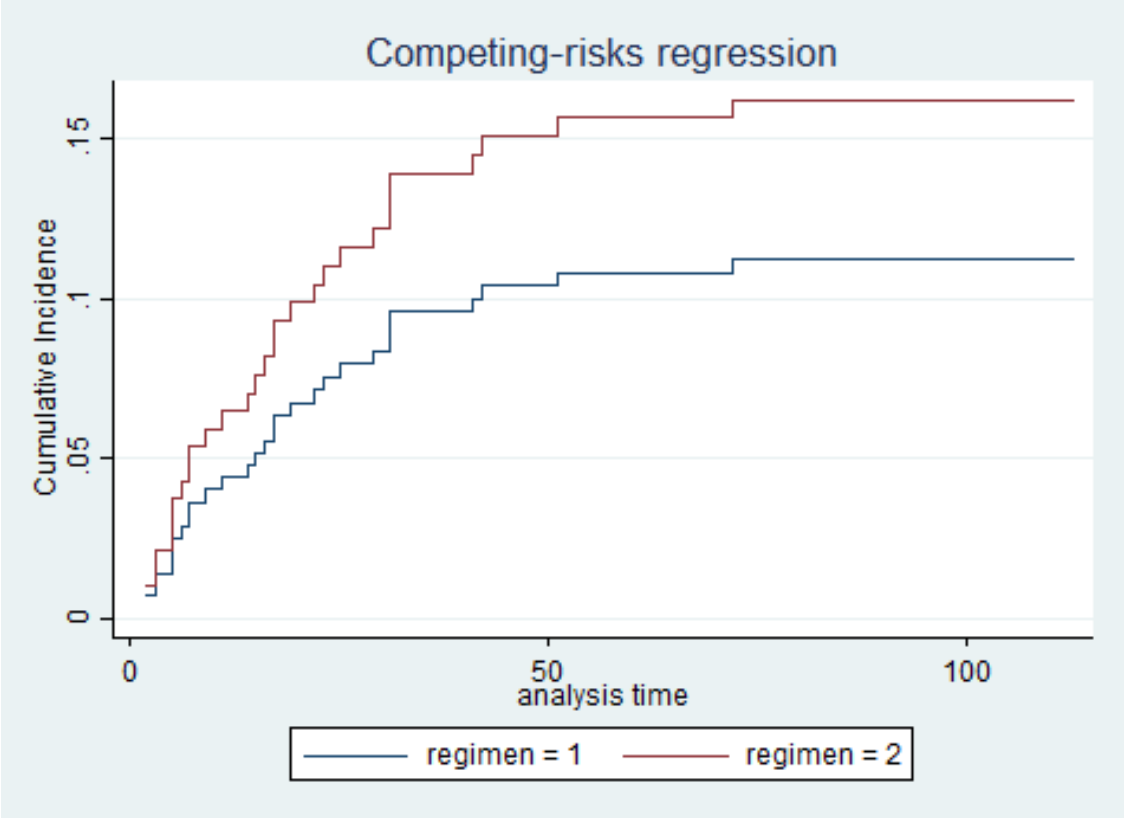
	Ceftriaxone + Placebo N = 112	**Ceftriaxone + Macrolide N = 113**
Clinical failure	3 (3)	8 (7)
Hospital-acquired pneumonia or pneumonia associated with mechanical ventilation	5 (4)	6 (5)
Azithromycin prophylaxis given by mistake	4 (4)	7 (6)
Refused the study medication	5 (4)	4 (3)
Hospital discharge against medical recommendation	4 (4)	5 (4)
Administration or prescription error	4 (4)	5 (4)
Others[1]	3 (3)	5 (4)
Exclusion of pneumonia diagnosis	2 (2)	5 (4)
Hospital discharge in less than 7 days	3 (3)	5 (4)
Attending physician wanted to treat atypical bacteria empirically	2 (2)	2 (2)

Data is shown as frequency (%). [1] Others: attending physician attributed the clinical worsening to the study regimen; switch to target treatment when a causative agent was identified; empirical broadening of antimicrobial spectrum to target *Pseudomonas aeruginosa* or resistant bacteria; empirical coverage of atypical mycobacteria.

Supplementary Table 2 - In-hospital mortality in sub-groups according to treatment regimens

	Ceftriaxone + Placebo	Ceftriaxone + Macrolide	RR (95% CI)	p-value
Severely ill patients (N= 43)	4/23 (17%)	6/20 (30%)	1.52 (0.41-5.53)[1]	0.53
Patients with identified bacteria pathogen (N= 42)	1/21 (5%)	4/21 (19%)	3.79 (0.42-33.95)[1]	0.23
Patients with identified atypical bacteria (N= 19)	1/10 (10%)	2/9 (22%)	1.82 (0.63-20.37)[1]	0.62
Patients with CD4+ > 200 cells/mm^3 (N= 56)	2/32 (6%)	2/24 (8%)	1.48 (0.21-10.53)[1]	0.69

Data is shown as frequency (%). RR: relative risk, 95% CI: 95% confidence interval. [1]Hazard ratio calculated with Cox regression.



Regimen 1 = ceftriaxone + macrolide, Regimen 2 = ceftriaxone + placebo, analysis time is measured in days. Subhazard ratio: 1.49, 95% CI: 0.71-3.10, p=0.29.

Supplementary Figure 1 - Cumulative incidence function of a competing-risk model for in-hospital mortality, treating discharge as a competing event

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4. Manuscript 2: Prospective etiological investigation of community-acquired pulmonary infections in hospitalized people living with HIV.

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Abstract

The study of the etiological agents of community-acquired pulmonary infections is important to guide empirical therapy, requires constant updating and has a substantial impact on the prognosis of patients. The objective of this study is to determine prospectively the etiology of community-acquired pulmonary infections in hospitalized adults living with HIV. Patients were submitted to an extended microbiological investigation that included molecular methods. The microbiological findings were evaluated according to severity of the disease and pneumococcal vaccine status. 224 patients underwent the extended microbiological investigation of which 143 (64%) had an etiology determined. Among the 143 patients with a determined etiology, *Pneumocystis jirovecii* was the main agent, detected in 52 (36%) cases and followed by *Mycobacterium tuberculosis* accounting for 28 (20%) cases. *Streptococcus pneumoniae* and Rhinovirus were diagnosed in 22 (15%) cases each and Influenza in 15 (10%) cases. Among atypical bacteria, *Mycoplasma pneumoniae* was responsible for 12 (8%) and *Chlamydophila pneumoniae* for 7 (5%) cases. Mixed infections occurred in 48 cases (34%). *Streptococcus pneumoniae w*as associated with higher severity scores and not associated with vaccine status. By using extended diagnostics, a microbiological agent could be determined in the majority of patients living with HIV affected by community-acquired pulmonary infections. Our findings can guide clinicians in the choice of empirical therapy for hospitalized pulmonary disease.

Introduction

Pneumonia is a major cause of morbidity and mortality in people living with the human immunodeficiency virus (PLHIV). Its incidence has decreased after the introduction of highly active antiretroviral therapy (HAART), but these patients still have a higher risk of acquiring this type of infection than the general population and have higher mortality rates (1).

The epidemiology of HIV-associated pulmonary disease is complex and influenced by various factors, notably the regional prevalence of pathogens, such as tuberculosis, and the accessibility to health care, mainly the access to effective antiretroviral therapy and antimicrobial prophylaxis (2).

The study of the etiological agents of community-acquired pneumonia (CAP) is important to guide empirical therapy, requires constant updating and has a substantial impact on the prognosis of patients (3). However, few studies have systematically investigated the etiology of pneumonia in PLHIV and there is no consensus on a diagnostic algorithm for these patients (4).

A recently studied algorithm among non-HIV patients showed that, by supplementing traditional diagnostic methods with new polymerase chain reaction (PCR)-based methods, a high microbial yield is achieved among adults admitted to a general hospital due to CAP. This study also showed that mixed infections are frequent in this setting (5).

The main purpose of this study was to determine prospectively the etiology of community-acquired pulmonary infections in hospitalized adults living with HIV. This study also aimed to analyze the contribution of different diagnostic methods, including those PCR-based, as well of the impact of different approaches to microbiological evaluation and to evaluate the microbiological findings in relation to the CD4+ T cell count, the severity of disease and pneumococcal vaccine status.

Methods

This is a sub analysis of a clinical trial that evaluated the treatment of CAP in 228 patients with HIV (Brazilian Clinical Trials Registry: RBR-8wtq2b) carried out at “Instituto de Infectologia Emílio Ribas”, a tertiary teaching infectious diseases hospital in São Paulo, Brazil.

The eligibility criteria for participants were: patients 18 years of age or older with clinically and radiologically suspected CAP (cough and dyspnea or chest pain or sputum production and lung opacity detected by a radiologic method) who required antibiotic treatment (denoting an clinical diagnosis of bacterial pneumonia) and hospitalization, as decided by the attending physician. Patients were excluded if they fulfilled the following criteria: risk factors for healthcare-associated pneumonia as defined by the American Thoracic Society (6), an etiology established prior to admission that justified all the symptoms, previously included, pregnancy or breastfeeding (based on the exclusion criteria of the clinical trial).

All patients provided written informed consent. The study occurred from September 2012 through July 2014 and was approved by the Institutional Committee of Ethics in Research (number 17/11).

Data collected on admission included demographic, clinical characteristics and pneumococcal vaccine status as reported by the patient. HIV viral load and CD4+ T cell count were registered if collected within three months before admission or during hospitalization.

Severity was evaluated using two scoring systems: Pneumonia Severity Index (PSI) (7) and CURB-65 (8), as recommended by the American and British Thoracic Societies, respectively. The scores are able to stratify patients according to their risk of mortality. PSI is based on clinical, laboratory and radiologic criteria while CURB-65 is remarkable due to its simplicity, with only five criteria.

Our Institute´s CAP protocol states that all patients should have blood samples collected for bacterial (two samples), fungal and mycobacterial cultures. Sputum should be collected for direct examination for *Pneumocystis jirovecii* and acid-alcohol resistant bacilli and cultured for fungi and mycobacteria.

Nasopharyngeal swabs were collected as indicated by the attending physician and tested for influenza A viruses (including H1N1) by PCR. Pleural effusion, tracheobronchial aspirates, bronchoalveolar lavage fluid and biopsies were collected if clinically indicated.

For analytic purposes, this approach was considered the routine investigation. All patients in this study also had at their disposal a wider microbiological investigation, considered here as the extended investigation (the extended investigation included also included the tests available in the routine investigation).

Blood samples were collected for serology for *Chlamydophila pneumoniae* and *Mycoplasma pneumoniae*. The first 50 patients had blood samples tested for *Streptococcus pneumoniae* and *Haemophilus influenzae* by PCR.

For this study, sputum was also cultured for bacteria and urinary antigen test for *Legionella pneumophila* serogroup 1 was performed.

Non-quantitative PCR methods were also used to investigate *Chlamydophila pneumoniae*, *Legionella pneumophila*, *Mycoplasma pneumoniae*, *P. jirovecii* and adenovirus in respiratory samples. *Legionella pneumophila* was only investigated in respiratory samples of the first 100 patients enrolled.

A hundred consecutive patients who had nasopharyngeal swabs available were tested by real time (RT)-PCR for the following agents: parainfluenza viruses 1-3, respiratory syncytial virus, influenza viruses A and B, human coronaviruses CoV NL63, HKU1, OC43 and229E, enterovirus, rhinovirus, adenovirus, bocavirus, human metapneumovirus, *C. pneumoniae, Bordetella pertussis* and *M. pneumoniae* (9)

Due to operational reasons, including difficulties in obtaining biological samples and scarcity of tests, not all the available microbiological analyses were performed for every included patient.

The diagnostic criteria are outlined in the Supplemental Digital Content 1.

Categorical variables were compared using the Chi-squared test or Fisher´s exact test, the level of significance was set at p=0.05 (two-tailed). Analyses were performed using STATA 10.1®.

Results

Enrolment

228 patients were consecutively enrolled. Four patients were excluded after this stage: one withdrew consent, one had no pneumonia (mistaken inclusion), one revealed an exclusion criterion after inclusion and one had been previously included. Thus, 224 cases were included in the analyses.

Patients' characteristics

The mean age of the 224 patients was 40.3 years, with a standard deviation of 11.6 years, 154 (69%) were males, comorbidities were referred by approximately one third of the patients, wherein liver disease and hypertension were the two most frequent.

Approximately one third of the patients who knew about their vaccination status referred anti-pneumococcal vaccination.

The majority of patients never used, abandoned or referred irregular use of HAART. The CD4+ T cell count was available for 90% of the patients, whereas 73% of cases were under 200 cells/mm^3.

Regarding severity of pneumonia, 63 (28%) patients had a CURB-65 score greater than one and 88 (39%) had PSI above three.

The detailed baseline characteristics of the 224 patients are shown in the Supplementary Table 1.

Microbiological findings

The microbiological routine investigation was able to determine the etiological agents in 92 (41%) patients (Table 1). Based on this investigation, the main etiological agent was *Mycobacterium tuberculosis* accounting for 28 cases (30% of those with an etiology determined); followed by *Streptococcus pneumoniae*, with 21 (23%) cases; influenza, 13 (14%) cases; and *Pneumocystis jirovecii*, 11 (12%) cases.

On the other hand, when including the extended microbiological investigation a microbiological agent was determined in 143 (64%) patients (Table 1). Among the 143 patients with microbiological findings, *Pneumocystis*

Table 1 - Findings of microbiological investigation in 224 cases of community-acquired pulmonary infections in hospitalized patients living with HIV

Etiology	Routine investigation N(%)	Routine + extended investigation N(%)
Fungi	**17 (8)**	**58 (26)**
Pneumocystis jirovecii	11	52
Histoplasma spp.	5	5
Cryptococcus spp.	1	1
Bacteria	**27 (12)**	**48 (21)**
Streptococcus pneumoniae	21	22
Mycoplasma pneumoniae	0	12
Chlamydophila pneumoniae	0	7
Staphylococcus aureus	4	4
Proteus spp.	1	1
Rhodococcus spp.	1	1
Bordetella pertussis	0	1
Virus	**14 (6)**	**48 (21)**
Rhinovirus	0	22
Influenza A non H1N1	9	7
Influenza A H3N2	0	2
Adenovirus	0	4
Influenza A H1N1	4	4
Coronavirus	0	2
Influenza B	0	2
Metapneumovirus	0	2
Bocavirus	0	1
Cytomegalovirus	1	1
Enterovirus	0	1
Mycobacteria	**35 (16)**	**35 (16)**
Mycobacterium tuberculosis	28	28
Mycobacterium avium complex	4	4
Mycobacteria	2	2
Nonchromogenic slowly growing Mycobacteria	1	1
Non-infectious causes	**13 (6)**	**13 (6)**
Pulmonary thromboembolism	4	4
Neoplastic diseases (except Kaposi´s sarcoma)	4	4
Kaposi´s sarcoma	5	5
Mixed etiology	**13 (6)**	**48 (21)**
Non-identified etiology	**132 (59)**	**81 (36)**

jirovecii was the main agent, responsible for 52 (36%) cases. *Mycobacterium tuberculosis* was the cause of 28 (20%) cases, the same number as in the routine investigation. *Streptococcus pneumoniae* and Rhinovirus were diagnosed in 22 (15%) cases each, followed by Influenza in 15 (10%) cases. Atypical bacteria were also diagnosed: *Mycoplasma pneumoniae* was responsible for 12 (8%) and *Chlamydophila pneumoniae* for 7 (5%) cases.

Mixed etiology was found in a large proportion of cases (34%) by the extended microbiological investigation, the multiple combinations are detailed in the Supplementary Table 2 and the most frequent of which were: *Mycoplasma pneumoniae* + *Pneumocystis jirovecii, Pneumocystis jirovecii* + Rhinovirus, *Pneumocystis jirovecii* + *Mycobacterium tuberculosis* and *Streptococcus pneumoniae* + Rhinovirus.

The contribution of the different methods to the etiological diagnosis of the seven most frequent agents are shown in Table 2, PCR-based methods were essential for the diagnosis of atypical bacteria and viruses, besides contributing to ameliorate *Pneumocystis jirovecii* detection.

Sputum cultures for bacteria were collected for 120 patients (54%), but in many cases this occurred after the beginning of antibiotic therapy, which hampers the interpretation of results difficult (the detailed results are presented in the Supplemental Digital Content 2). The sputum cultures were used to corroborate diagnoses made by other methods and to provide antibiotic susceptibilities, but were not considered sufficient for a definitive diagnosis.

Performing an analysis of causative agents based on CD4+ T cell count, we found that the etiology of pneumonia in those severely immunosuppressed (CD4+ T cell count<200 cells/mm^3) was similar to those who were not. *Pneumocystis jirovecii* is the only agent more frequent in the former group, an expected finding taking into account our diagnostic criteria (the detailed analysis is available in the Supplementary Table 3).

Frequencies of the seven most common agents were compared between patients admitted during the summer and winter (as shown in the Supplementary Table 4). Due to the limited amount of included patients we were not able to fully consider seasonal variation but we found that *Mycoplasma pneumonia* was detected exclusively during the summer season (p=0.01).

Table 2 - Contribution of different methods to the etiological diagnosis of the seven most frequent pathogens causing community-acquired pulmonary infections in hospitalized patients living with HIV

	Pathogen	*Pneumocystis jirovecii*	*Mycobacterium tuberculosis*	Rhinovirus	*Streptococcus pneumoniae*	Influenza	*Mycoplasma pneumoniae*	*Chlamydophila pneumoniae*
	No. (%) of patients with positive findings (n=224)	52 (23%)	28 (12%)	22 (10%)	22 (10%)	15 (7%)	12 (5%)	7 (3%)
Blood	Culture[1] (n=223)	…	5	…	21	…	…	…
Blood	Serology[2] (n=180)	…	…	…	…	…	7	7
Blood	PCR[2] (n=54)	…	…	…	3	…	…	…
Sputum	Direct visualization[1] (n=121)	5	…	…	…	…	…	…
Sputum	PCR[2] (n=141)	38	…	…	…	…	5	0
Sputum	Culture for mycobacteria[1] (n=179)	…	20	…	…	…	…	…
Nasopharyngeal	PCR for influenza A[1] (n=206)	…	…	…	…	13	…	…
Nasopharyngeal	PCR for multiple agents[2] (n=94)	…	…	22	…	4	1	1
Endotracheal	Direct visualization[1] (n=16)	2	…	…	…	…	…	…
Endotracheal	PCR[2] (n=14)	8	…	…	…	…	0	0
Endotracheal	Culture for mycobacteria[1] (n=31)	…	3	…	…	…	…	…
Bronchoalveolar	Direct visualization[1] (n=22)	4	…	…	…	…	…	…
Bronchoalveolar	PCR[2] (n=14)	7	…	…	…	…	0	0
Bronchoalveolar	Culture for mycobacteria[1] (n=30)	...	5	…	…	…	…	…
Lung	Biopsy[1] (n= 19)	2	…	…	…	…	…	…
Pleural	Culture for mycobacteria[1] (n=10)	...	4	...	…	...	…	…

[1] Methods performed in routine microbiological investigation, [2] methods performed in extended microbiological investigation, n: number of cases in which each test was performed, PCR: Polymerase chain reaction.

In relation to severity of disease, bacteria were most frequent among patients with higher scores, notably *Streptococcus pneumoniae*, which was associated with severe cases as stratified by CURB-65 and PSI (Figure 1). *Streptococcus pneumoniae* infection frequency between individuals that referred pneumococcal vaccination when compared to individuals who denied having been vaccinated was not statistically different (6% *versus* 12%, p=0.23).

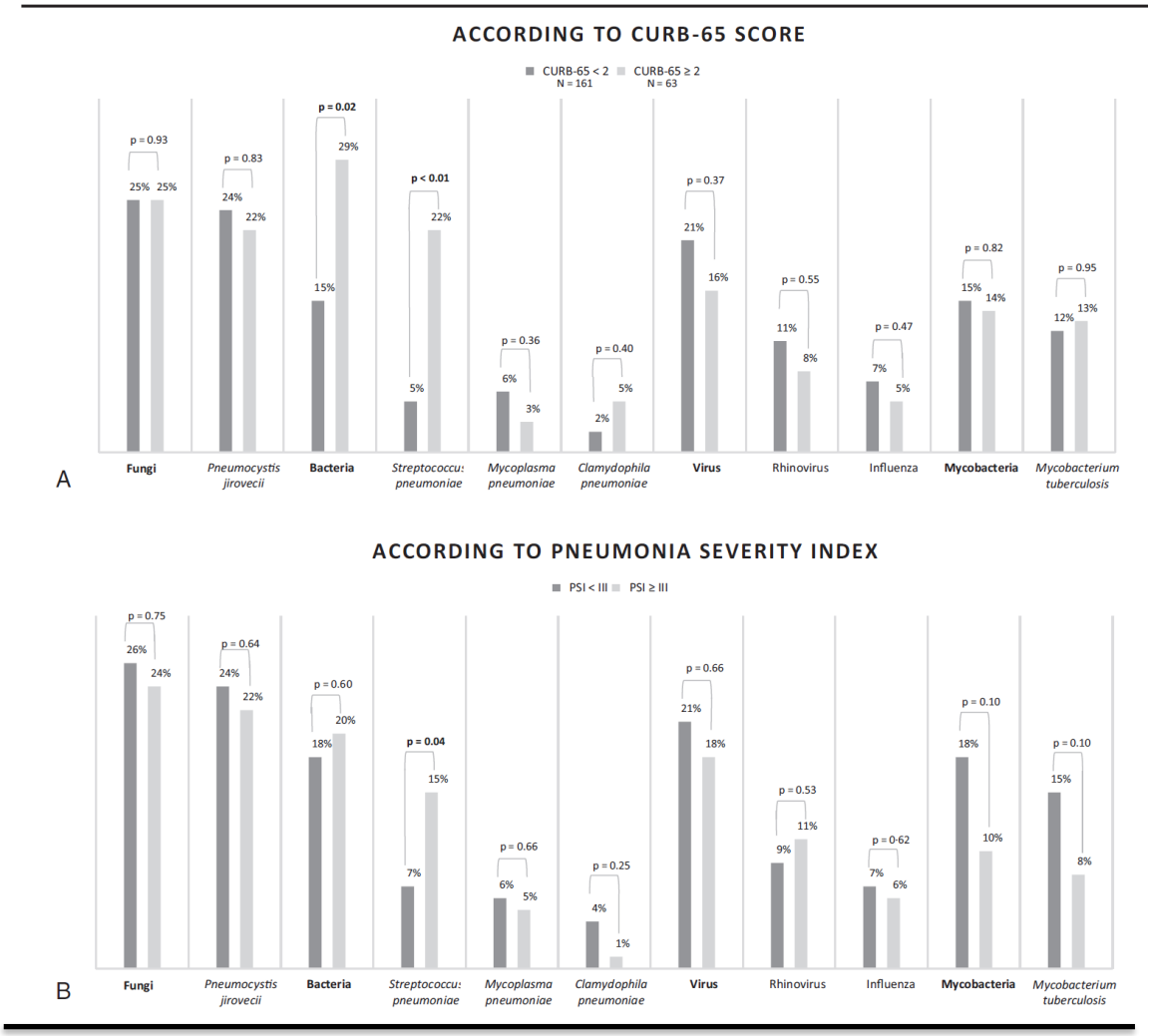

Figure 1 - Microbiological findings in relation to severity of community-acquired pulmonary infections in hospitalized patients living with HIV[1]

[1] Analyses restricted to the seven most common microbiological agents.

Discussion

This study resorted to an extended microbiological investigation that included molecular methods, therefore an etiological diagnosis was found in a high proportion of cases (64%). Our 224 patients represent one of the largest cohorts of community-acquired pulmonary infections in adults living with HIV and is the largest cohort in South America. The most frequently identified agents in this study were *Pneumocystis jirovecii*, *Mycobacterium tuberculosis* and *Streptococcus pneumoniae*.

Although all patients included in the study had a clinical diagnosis of bacterial pneumonia on admission, bacterial disease was only confirmed microbiologically in 21% of them. There are recent studies that propose predictors and scores that could help the clinicians distinguish between bacterial pneumonia and tuberculosis or *Pneumocystis jirovecii* pneumonia (PCP) in PLHIV, but their results are based on retrospective analyses thus their accuracy is not completely reliable (10,11). It is difficult to predict the etiology of a pulmonary infiltrate in PLHIV based on clinical findings.

In our study, we found that bacteria were more frequent among patients with higher severity scores and *S. pneumoniae* was more common in patients with severe disease. This finding could be due to the fact that bacterial infections tend to produce more pronounced alterations of vital signs. It is noteworthy that no severity score is validated for PLHIV and that a specific mortality risk score in this population must be further investigated (1)

An elevated rate of mixed diagnosis (34%) was observed due to our extended investigation. This finding highlights the complexity involved in the choice of the empiric treatment for these patients and the need to perform extensive microbiological diagnosis. Mixed etiology had already been described as relatively common in PLHIV (around 11%) (10,12) and in the general population with CAP (35%) (5). A combination of viruses and bacteria was the most frequently found in those studies, however our study stands out by encountering a large variety of different combinations.

As expected, since tuberculosis is endemic in Brazil, we found a higher proportion of cases (20%) than in non-endemic countries, such as the United

States (4.3%) (12) and Spain (8.5%) (10). A small Chilean study also found a lower frequency of tuberculosis (5%) but a higher percentage of *Mycobacterium avium* complex infection (12%) (13). The regional prevalence of specific diseases, such as tuberculosis, can guide clinicians on the different possible diagnoses for hospitalized PLHIV affected by pulmonary disease. In high prevalence settings, tuberculosis should be always investigated.

Another interesting finding of our study is that, as we systematically investigated the atypical bacteria (*M. pneumoniae, C. pneumoniae* and *L. pneumophila*), we founded high rates of atypical bacterial infections (13%) in comparison with previous studies (<3%) (10,12,13), although we did not find any cases of *L. pneumophila*. Our finding of high rates of atypical bacterial infections may support atypical coverage in the empirical treatment of these patients.

In Brazil, for PLHIV, the use of 23-valent polysaccharide vaccine is recommended. In this study, approximately one third of the patients who knew about their vaccination status referred anti-pneumococcal vaccination. The frequency of *Streptococcus pneumoniae* infection was similar for vaccinated and non-vaccinated individuals. This finding is in agreement with a systematic review that concluded that clinical evidence provides only moderate support for recommendation of pneumococcal polysaccharide vaccination in PLHIV (14), however the number of confirmed pneumococcal pneumonias in our study was small and this may have limited the statistical power to detect differences.

The time between the diagnosis of the HIV infection and admission in our study was long (median: 8.9 years) and the rates of regular use of HAART and of viral suppression were low (less than 20%), as well as the CD4+ T cell counts (73% had CD4+<200 cells/mm^3). Thus, our population were late presenters and presented poor adherence to HAART, as described previously (10,15) in CAP cohorts of PLHIV and that appears to be the general profile of PLHIV who require hospitalization. The immunosuppression of these patients probably contributed to the high proportion of mixed infections and to the difficulty in differentiating clinical and radiological features of the various etiological agents.

In our study, we performed an extensive laboratory investigation, using a variety of molecular methods. Following our institutional routine investigation, we would have been capable of establishing the etiology in 41% of cases, which was

increased to 64% with our extended investigation. This was particularly important for PCP and viral infections.

Molecular methods can improve the diagnosis of viral respiratory infections in hospitalized patients with lower respiratory tract infections (5,16) but bring us the challenge of how to interpret these findings since is difficult to define the virus as the causative agent of pneumonia (17). A recent review suggests that the persistence of positive PCR for virus is infrequent (≤5%) in asymptomatic subjects among the general population (18). This indicates that the finding of viral agents in symptomatic patients reflects the presence of viruses that often contribute to the disease, but further studies in symptomatic and asymptomatic PLHIV are needed to clarify this.

When considering the diagnosis of PCP, the difficulty lies in distinguishing colonization from infection (19), to date there is no validated method described. In our study, we used CD4+ T cell counts and clinical criteria to define PCP infection and in only four cases the diagnosis of PCP pneumonia was excluded by these criteria.

Our study has limitations. First, not all patients who met the criteria for inclusion were enrolled in the study as we used convenience sampling. Second, the specimen collection was not complete for all enrolled patients. These issues are inherent to all trials enrolling patients with CAP. We have no reason to believe that the group of patients who were not included would have been substantially different from the group of patients that we studied. Selection bias is possible but unlikely.

As in patients living with HIV mixed infections are very common we relied on at least two CAP definition criteria and the clinical judgment of the attending physician for the identification of possible bacterial CAP, expressed by the administration to treatment directed for bacteria. We believe this definition is valid since it reflects that real clinical situation and it is difficult to differentiate between bacterial and non-bacterial causes of community-acquired pulmonary infections in PLHIV.

Our study is a single center study, limiting its external validation, but this is attenuated by the fact that “Instituto de Infectologia Emílio Ribas” is the reference hospital for the metropolitan region of São Paulo (approximately 20

million inhabitants) and PLHIV comprise approximately 70% of the hospitalized patients.

In conclusion, resorting to an extended microbiological evaluation, this study was capable of defining the etiological diagnosis of a high proportion of cases of community-acquired pulmonary infections in hospitalized patients living with HIV. The main agents were *Pneumocystis jirovecii*, *Mycobacterium tuberculosis* and *Streptococcus pneumoniae*. Mixed infections were very frequent. Prospective studies of the etiological agents of community-acquired pulmonary infections in different settings and populations are important to guide clinical practices.

Supplemental Digital Content 1. Diagnostic criteria.

Bacteria

The definitive etiological diagnosis of a bacterial infection was made by identification of a microorganism by culture of a sterile site (blood or pleural fluid) or a respiratory sample. Endotracheal aspirate cultures were considered positive if the number of colonies were ≥10^6 ufc/ml and for bronchoalveolar lavage cultures if the number of colonies were ≥ 10^4 ufc/mL. The agents identified by these methods were considered the causative agents if they were not usual colonizers of the upper airways, such as coagulase negative staphylococci, and if there were no other agent more likely identified in blood cultures or by molecular tests.

Positive IgM in blood sample (semi-quantitative IgG and IgM, RIDASCREEN®, *R-Biopharm AG, Darmstat*, Germany) or presence of positive polymerase chain reaction (PCR) in a respiratory sample or nasopharyngeal swab (*Film Array, BioFireDiagnostics, Salt Lake City*, UT) was considered confirmatory for *Chlamydophila pneumoniae* and *Mycoplasma pneumonia* infection.

A positive PCR in nasopharyngeal swab was confirmatory for *Bordetella pertussis* infection (semi-quantitative IgG and IgM, RIDASCREEN®, *R-Biopharm AG, Darmstat*, Germany). A positive PCR in blood confirmed *S. pneumoniae* and *H. influenza*e infection.

Legionella pneumophilla infection was defined by the presence of a positive PCR in a respiratory sample or a positive urinary antigen (SAS™ *Legionella Test, SA Scientific, San Antonio*, TX).

Fungi

The definitive diagnosis of a fungal infection was made by identification of a microorganism by culture of a sterile site (blood or pleural fluid) or a positive culture in respiratory sample for one of these agents: *Cryptococcus neoformans, Histoplasma capsulatum, Coccidioides immitis* or *Blastomyces dermatitidis* or through the characterization of the agent in histopathology.

Direct visualization or a positive PCR in a respiratory sample for *Pneumocystis jirovecii* was considered confirmatory, unless the patient had documented CD4+

T cell count over 200 cells/mm³ and did not receive therapy directed against this agent.

Probable diagnosis of *Histoplasma capsulatum* was considered in the presence of a positive serology plus a compatible clinical condition plus treatment initiated by the attending physician.

Mycobacteria

Mycobacterium tuberculosis was confirmed by culture in any sample (blood, pleural fluid or respiratory sample).

A positive smear or a histopathologic finding, without a positive culture, was considered confirmatory for a mycobacterial infection without species identification.

Identification in blood culture or in two cultured respiratory samples was confirmatory for non-mycobacterial species.

Virus

Positive PCR in a respiratory sample or in a nasopharyngeal swab confirmed adenovirus infection. Positive PCR in nasopharyngeal swab was considered confirmatory of parainfluenza viruses 1-3, respiratory syncytial virus, influenza viruses A and B, human coronaviruses CoV NL63, HKU1, OC43 and229E, enterovirus, rhinovirus, adenovirus, bocavirus, human metapneumovirus infection (*Film Array, BioFireDiagnostics, Salt Lake City*, UT). Cytomegalovirus and herpes virus 8 were characterized based on histopathological studies.

Non-infectious causes

Pulmonary thromboembolism was diagnosed based on computed tomography. Suggestive lesions observed in bronchoscopy were defined as Kaposi´s Sarcoma.

Supplementary Table 1 - Baseline characteristics of 224 patients living with HIV admitted to the hospital with community-acquired pulmonary infections (September 2012-July 2014)

Age (years) mean (SD)	40.3 (11.6)
Male sex	154 (69%)
Comorbidities	67 (30%)
Hypertension	26 (12%)
Liver disease	22 (10%)
Neoplastic disease	10 (4%)
Diabetes mellitus	9 (4%)
Cardiac insufficiency	9 (4%)
Renal disease	5 (2%)
Chronic obstructive pulmonary disease dependent on oxygen	3 (1%)
Cerebrovascular disease	2 (<1%)
Drug use (n=223[1])	131 (59%)
Tobacco use (n=222[1])	91 (41%)
Alcoholism (n=220[1])	83 (38%)
Inhaled drug use (n=223[1])	67 (30%)
Intravenous drug use (n=223[1])	2 (<1%)
Antibiotic use in the last 30 days (n=218[1])	109 (50%)
Prophylactic dose (n=215[1])	37 (17%)
Therapeutic dose (n=214[1])	86 (40%)
Pneumococcal vaccination (n=161[1])	49 (30%)
Years of HIV infection median (range) (n=187[1])	12 (1-30)
Regular usage of HAART	42 (19%)
Viral load < 50 copies/ml (n=202[1])	32 (16%)
CD4 T cell count (/mm^3) median (range) (n=202[1])	57.5 (1-1108)
1-49	98 (49%)
50-199	48 (24%)
200-349	20 (10%)
350-499	17 (8%)
>499	19 (9%)
CURB-65	
0-1	161 (72%)
2	47 (21%)
3-5	16 (7%)
Pneumonia Severity Index	
I-II	136 (61%)
III	49 (22%)
IV-V	39 (17%)

[1] Data not available for all patients; SD: standard deviation; HAART: highly active antiretroviral therapy; results are shown as N (%) if not otherwise specified.

Supplementary Table 2 - Frequency of mixed etiology findings observed during routine plus extended microbiological investigation of community-acquired pulmonary infections in hospitalized patients living with HIV

Etiology	**N**
Mycoplasma pneumoniae + *Pneumocystis jirovecii*	4
Pneumocystis jirovecii + Rhinovirus	3
Pneumocystis jirovecii + *Mycobacterium tuberculosis*	3
Streptococcus pneumoniae + Rhinovirus	3
Adenovirus + *Pneumocystis jirovecii*	2
Chlamydophila pneumoniae + *Pneumocystis jirovecii*	2
Histoplasma spp. + *Pneumocystis jirovecii* + Rhinovirus	2
Mycoplasma pneumoniae + *Streptococcus pneumoniae*	2
Adenovirus + *M. avium* or *M. colombiensis* + *Pneumocystis jirovecii*	1
Adenovirus + *Pneumocystis jirovecii* + Pulmonary thromboembolism	1
Bordetella pertussis + *Streptococcus pneumoniae* + *Staphylococcus aureus* + Rhinovirus	1
Chlamydophila pneumoniae + *Streptococcus pneumoniae*	1
Chlamydophila pneumoniae + *Streptococcus pneumoniae* + *Mycobacterium tuberculosis*	1
Cytomegalovirus + Rhinovirus + Kaposi´s sarcoma	1
Coronavirus + *Pneumocystis jirovecii* + Rhinovirus	1
Coronavirus OC43 + Rhinovirus	1
Histoplasma spp. + *Mycobacterium tuberculosis*	1
Influenza A H1N1 + Pulmonary thromboembolism	1
Influenza A non H1N1 + Neoplastic disease	1
Influenza A non H1N1 + *Pneumocystis jirovecii*	1
Influenza B + *Mycoplasma pneumoniae*	1
Influenza B + Rhinovirus	1
Mycobacterium avium complex+ *Mycoplasma pneumoniae*	1
Mycobacterium avium complex + *Pneumocystis jirovecii*	1
Mycobacterium avium complex + *Mycobacterium tuberculosis*	1
Nonchromogenic slowly growing Mycobacteria + *Pneumocystis jirovecii* + Kaposi´s sarcoma	1
Mycobacteria + Rhinovirus	1
Pneumocystis jirovecii + Mycobacteria	1
Pneumocystis jirovecii + *Streptococcus pneumoniae*	1
Pneumocystis jirovecii + Rhinovirus + *Mycobacterium tuberculosis*	1
Pneumocystis jirovecii + Kaposi´s sarcoma	1
Pneumocystis jirovecii + Pulmonary thromboembolism	1
Kaposi´s sarcoma + *Mycobacterium tuberculosis*	1
Staphylococcus aureus + *Mycobacterium tuberculosis*	1
Mycobacterium tuberculosis + Rhinovirus	1
Total	**48**

Supplemental Digital Content 4. Detailed results of sputum cultures for bacteria.

Sputum cultures for bacteria were collected for 120 patients (54%) and resulted positive in 47 cases. Out of the 47 positive sputum cultures, only 15 cases had potential causative bacteria (1 case of *Escherichia coli*, 2 cases of *Klebsiella pneumoniae*, 3 cases of *Pseudomonas aeruginosa*, 1 case of *Rhodococcus* sp., 6 cases of *Staphylococcus aureus* and 2 cases of *S. pneumoniae*).
An additional 10 patients would have had a possible etiology diagnosed using sputum cultures (1 case of *Escherichia coli*, 2 cases of *Klebsiella pneumoniae*, 3 cases of *Pseudomonas aeruginosa* and 4 cases of *Staphylococcus aureus*), most of which (8 cases) would have been associated with other etiology findings.

Supplementary Table 3 - Microbiological findings of community-acquired pulmonary infections in relation to season in hospitalized patients living with HIV[1]

Etiology	Summer N = 51 n (%)	Winter N = 52 n (%)	P
Fungi	**13 (25)**	**9 (17)**	0.31
Pneumocystis jirovecii	13 (25)	8 (15)	0.20
Bacteria	**13 (25)**	**9 (17)**	0.31
Streptococcus pneumoniae	4 (8)	4 (8)	1[2]
Mycoplasma pneumoniae	6 (12)	0	**0.01[2]**
Clamydophila pneumoniae	0	4 (8)	0.12[2]
Virus	**6 (12)**	**10 (19)**	0.30
Rhinovirus	4 (8)	8 (15)	0.23
Influenza	1 (2)	1 (2)	1[2]
Mycobacteria	**9 (18)**	**9 (17)**	0.96
Mycobacterium tuberculosis	7 (14)	9 (17)	0.62

[1] Only the seven most frequent pathogens are presented. [2] Fisher´s exact test.

Supplementary Table 4 - Microbiological findings of community-acquired pulmonary infections in relation to CD4+ T cell count in hospitalized patients living with HIV[1]

Etiology	CD4+ < 200 N = 146 n (%)	CD4+ ≥ 200 N = 56 n (%)	P
Fungi	**49 (34)**	**2 (4)**	**<0.01**
Pneumocystis jirovecii	46 (31)	2 (4)	**<0.01**
Bacteria	**25 (17)**	**15 (27)**	0.12
Streptococcus pneumoniae	12 (8)	8 (14)	0.20
Mycoplasma pneumoniae	7 (5)	5 (9)	0.27
Clamydophila pneumoniae	6 (4)	1 (2)	0.68[2]
Virus	**25 (17)**	**10 (18)**	0.90
Rhinovirus	15 (10)	3 (5)	0.27
Influenza	5 (3)	5 (9)	0.14[2]
Mycobacteria	**27 (18)**	**6 (11)**	0.18
Mycobacterium tuberculosis	22 (15)	6 (11)	0.42

[1] Only the seven most frequent pathogens are presented. [2] Fisher´s exact test.

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5. Critical analysis and recommendations

Introduction

Currently, there are no specific guidelines for community-acquired pneumonia management in patients with HIV/AIDS, although there are some publications addressing this issue (1–3).

The development of our study and its findings brought some important insights to the field.

In this section, we propose recommendations for the diagnosis and management of community-acquired pneumonia in patients with HIV/AIDS that could be used as basis for institutional guidelines.

Diagnosis

The suspicion of a pulmonary infection in adults (≥18 years) with HIV/AIDS comes from suggestive signs and symptoms (cough, dyspnoea, chest pain or sputum production), that can be accompanied by constitutional complaints (such as fever, malaise and inappetence) and alteration on physical examination (localised bronchial breathing or crackles, dullness on percussion, decreased chest expansion).

We consider that acute presentation is the one with symptoms lasting up to 7 days. Acute presentation includes acute worsening of previous symptoms.

The confirmation of a pulmonary infiltrate must be made by the detection of a new lung opacity by a radiologic method, preferably a chest radiography.

It is important to assess if there is another aetiology established prior to admission that may explain all the symptoms, such as carcinoma, Kaposi´s sarcoma, pulmonary embolism, pulmonary oedema and acute exacerbations of chronic obstructive pulmonary disease.

Severity assessment and point of care decisions

The most validated severity scores for community acquired pneumonia (CAP), CURB-65 (4) and Pneumonia Severity Index (PSI) (5), were not extensively studied in patients with HIV/AIDS. Findings of an observational study showed that PSI IV or V predicted mortality accurately (RR 15.2, 95% CI 3.2-71.7, p=0.001) but also that mortality was higher in patients with CD4+ T cell counts < 200 cells/µL (p=0.022) (6).

Some authors suggest (2) that patients with CD4+ T cell counts < 200 cells/µL must be hospitalised.

A post hoc analysis of our database (7) showed that CURB-65, PSI and quick SOFA (qSOFA) (8) presented a high negative predictive value (around 90%) for in-hospital mortality. In this analysis, low median CD4+ T cell counts were associated with in-hospital mortality (p=0.01).

qSOFA is easily done and independent of laboratory analyses, so we propose that patients should be treated as outpatients if they do no present any of the criteria: altered mental status; respiratory rate > 22 per minute; or systolic blood pressure < 100 mm Hg.

Patients with qSOFA>1 must be assessed for evidence of organ dysfunction using the SOFA score and, if they have more than one criteria, hospitalised and treated as septic patients.

Patients without sepsis criteria must be hospitalised if they present CD4+ T cell counts < 200 cells/mm^3 (referred or collected in the last 6 months) or PSI > III.

Patients hospitalised for reasons unrelated to severity should be treated as outpatients regarding investigation and antimicrobial use.

We define severely ill patients as those: in intensive care units; receiving vasoactive drugs; and/or under mechanical ventilation.

Aetiological predictors and microbiological evaluation

Although all patients included in our study had a clinical diagnosis of bacterial pneumonia on admission, bacterial disease was only confirmed microbiologically in 21%, with an elevated rate of mixed diagnoses (34%). Our findings highlighted the complexity of the etiologic spectrum and the importance of mixed infection in this population (9).

A recent study of CAP in HIV-infected patients found that time with symptoms ≤ 5 days (OR 2.6; 95% CI 1.5–4.4); C-reactive protein level ≥ 22 mg/dL (OR 4.3; 95% CI 2.3–8.2) and hepatitis C virus co-infection (OR 2.3; 95% CI 1.4–3.9) were predictors of bacterial CAP. On the other hand, white blood cell count ≤ 4 $x10^{12}$/L (OR 3.7; 95% CI 1.2–11.5); lactate dehydrogenase (LDH) level ≥ 598 U/L (OR 12.9; 95% CI 4.2–39.7); and multilobar infiltration (OR 5.8; 95% CI 1.9–19.5) were predictors of *Pneumocystis jirovecii* (10).

A retrospective study in patients with HIV compared patients with a positive smear for tuberculosis and patients with bacterial CAP. It demonstrated that sudden onset of signs (OR=8.48; 95%CI 2.50-28.74); a delay in the evolution of symptoms of less than 15 days (OR=3.70; 95%CI 1.11-12.35); chest pain (OR=2.81; 95%CI 1.10-7.18); radiological alveolar shadowing (OR=12.98; 95%CI 4.66-36.12); and high leukocytosis (OR=3.52; CI 95% 1.19-10.44) were associated with bacterial pneumonia (11).

It is still difficult to predict the aetiology of a pulmonary infiltrate in patients with HIV/AIDS based on clinical findings and more studies on this subject are necessary to increase the reliability of criteria.

Since patients could have overlapped infections, the routine microbiological evaluation must consider more than bacterial agents. We recommend that microbiological evaluation be performed on every hospitalised patient with HIV/AIDS.

Results of microbiological evaluation can change the antibiotic management for an individual patient and be an important contribution for the management of an initial antibiotic failure. Narrowing of antibiotic therapy based on microbiological tests can decrease toxicity and costs, but can be difficult in cases where mixed diagnosis is suspected or for cases that could benefit from

double therapy. The microbiological findings of a specific population have epidemiologic implications, being the base for empirical antibiotic recommendations (12).

Besides determining prospectively the etiology of CAP in people living with HIV/AIDS, we also analyzed the contribution of different diagnostic methods. We demonstrated the benefit of broadening the investigation in order to achieve an increased yield: our Institute´s microbiological routine investigation was able to determine the etiological agents in 41% of the cases but, when including the extended microbiological investigation, a microbiological agent was determined in 64% of the cases (9).

All patients should have pretreatment blood samples collected for bacterial culture (two samples), as the identification of a microorganism by this method provides definitive diagnosis of bacterial infection.

The first 50 patients in our study had blood samples tested for *Streptococcus pneumoniae* and *Haemophilus influenzae* by polymerase chain reaction-based methods (PCR). We do not recommend routine investigation using PCR for these agents since it did not increase substantially the yield: 21 of 22 *S. pneumoniae* infected patients were bacteremic, only one had confirmation through PCR and we did not have any *H. influenzae* identification (9).

A properly collected and read Gram stain of a sputum sample can provide a simple, readily available, rapid and inexpensive test result. A prospective study of bacteremic patients showed good sensitivity and specificity for diagnosing staphylococcal pneumonia and Gram-negative bacilli pneumonia, which can help clinician to broaden the initial antimicrobial therapy. Good accuracy was reported for pneumococcal and *H. influenzae* diagnosis also (13).

Yet, in our study, in many cases this occurred after the beginning of antibiotic therapy, which makes the interpretation of results difficult. Thus, the sputum cultures were used to corroborate diagnoses made by other methods and to provide antibiotic susceptibilities, but were not considered sufficient for a definitive diagnosis.

We suggest that a greater effort be made to collect pre-treatment sputum samples of hospitalised patients with productive cough. Good-quality specimens

(>25 polymorphonuclears and <10 epithelial cells per low field power) should also be cultured (14).

With systematically investigation of the atypical bacteria (*Mycoplasma pneumoniae, Chlamydophila pneumoniae* and *Legionella. pneumophila*), we found high rates of atypical bacterial infections (13%) (9) in comparison with previous studies (<3%) (10,15,16).The diagnosis of atypical bacteria remains a challenging task, even more in severely immunocompromised patients, making this condition often undiagnosed in patients with HIV/AIDS (17).

The serology test used as confirmatory in our study was IgM in blood sample (semi-quantitative IgG and IgM, RIDASCREEN®, *R-Biopharm AG, Darmstat*, Germany) (9). It is usually found after the first contact with the pathogen, with a >90% sensitivity and sensibility according to the manufacturer (18,19).

A negative result does not rule out *C. pneumoniae* and *M. pneumonia* infection. If within 3 weeks, no other causative agent is found and there is still a clinical suspicion, the test should be repeated on a second sample. With reinfection, it is usually the case that no IgM antibodies can be found, whereas IgG titres increase very rapidly (18,19).

The respiratory samples in our study were also submitted to non-quantitative PCR methods to investigate *C. pneumoniae*, *L. pneumophila* and *M. pneumoniae*. A hundred consecutive patients who had nasopharyngeal swabs available were tested by real time (RT)-PCR for the following bacteria: *C. pneumoniae, Bordetella pertussis* and *M. pneumoniae.*

Serology and PCR methods in respiratory samples were important for the diagnosis of atypical bacteria (Table 2, (9)), but in settings with budget constraints, these tests can be reserved for severely ill patients.

At the attending physician's discretion, endotracheal aspirate or bronchoalveolar lavage can also be collected. Endotracheal aspirate cultures were considered positive if the number of colonies was $\geq 10^6$ ufc/ml and for bronchoalveolar lavage cultures if the number of colonies was $\geq 10^4$ ufc/mL.

In our study, the urinary antigen test for *L. pneumophila* serogroup 1 was performed for all patients, yet, we did not find any cases of *L. pneumophila*. We

do not recommend doing this test routinely, as we prefer to give preference to PCR methods that can detect all *L. pneumophila* serogroups.

The use of molecular methods allowed the identification of several viruses among the patients in our study. However, there are no specific treatments for most of these agents and the only impact is perceived in generation of epidemiological data. For this reason, we do not recommend routinely viral testing, except for influenza.

PCR test for influenza A viruses (including H1N1) in nasopharyngeal swabs is the most widely available method in our midst and should be collected as indicated by the attending physician, especially in autumn and winter or if the patient presents upper respiratory tract symptoms, such as nasal congestion or sore throat.

The most frequently identified agent in our study was *P. jirovecii*, in spite of the clinical diagnosis of bacterial pneumonia on admission. The use of the PCR method contributed to ameliorate *P. jirovecii* detection. *P. jirovecii* was the only agent more frequent in those severely immunosuppressed (CD4+ T cell count<200 cells/mm^3), an expected finding (9).

Direct visualization in a respiratory sample for *P. jirovecii* should be attempted for every hospitalised patient. Although PCR methods for *P. jirovecii* are not widely available, we recommend that this test be made for at least the severely ill patients.

We recommend investigation of fungi other than *P. jirovecii* in severely ill patients. Those patients must have at least a blood sample cultured for fungi. *Histoplasma capsulatum* and *Paracoccidioides brasiliensis* serologies can be collected according to the local epidemiologic data.

Respiratory samples can also be cultured for fungi and definitive diagnosis is made with isolation of one of these agents: *Cryptococcus neoformans; Histoplasma capsulatum; Coccidioides immitis;* or *Blastomyces dermatitidis.*

A *post-hoc* analysis of our clinical trial showed that the prevalence of cryptococcal antigenemia was considerable (3.5%), especially in patients with lymphocyte T CD4+ counts < 100 cells/mm3 (4.9%). Four of the seven patients with positive cryptococcal latex agglutination test were classified as

asymptomatic cryptococcal infection and three had invasive cryptococcal disease, one of them with pulmonary disease confirmed by histopathology (20).

The results of this analysis suggest that HIV-infected patients hospitalized with CAP, especially those with severe immunosuppression, should be routinely tested for the presence of CrAg, because they may present asymptomatic cryptococcal infection or invasive cryptococcal disease and the prompt antifungal therapy has been shown to reduce mortality and morbidity rates (20).

As expected, considering that tuberculosis is endemic in Brazil, we found a high proportion of cases (20%) with this diagnosis. We recommend that an investigation for mycobacteria be performed in all severely ill patients and in those with cough for more than 3 weeks.

The recommended tests made in our study are direct examination for acid-alcohol resistant bacilli and culture for mycobacteria in sputum (2 samples); and culture for mycobacteria of a blood sample.

Currently, we have available a molecular test for tuberculosis, Xpert MTB RIF (Cepheid, Sunnyvale, CA, USA), that also tests for resistance to the drug rifampicin. The first collected sputum sample should be also tested by this method.

All patients with pleural effusion must be submitted to a pleural puncture to differentiate between transudate and exudate. Samples must be cultured for bacteria, fungi and mycobacteria. Lymphocytic predominance and high adenosine deaminase level are suggestive of tuberculosis (21).

Patients could undergo bronchoscopy if clinically indicated, a complementary examination specially recommended in severely ill patients.

Bronchoscopy is useful in the diagnosis of tuberculosis in suspect cases with a "negative" sputum smear. Both bronchoalveolar lavage and transbronchial biopsy should be performed whenever possible, aiming towards a higher yield of bronchoscopy in the diagnosis of tuberculosis. Transbronchial biopsy establishes a faster diagnosis for HIV-infected patients if associated with acid-fast stains (22).

Biopsies can be collected in selected cases and can stablish an aetiological diagnosis through the characterization of the agent.

In our study, we diagnosed patients with cytomegalovirus and herpes virus 8 based on histological studies (9).

Treatment

The selection of appropriate empiric therapy for an episode of CAP in patients with HIV/AIDS is still a challenge. In these cases, a broader spectrum of possible aetiological agents must be considered and even a polymicrobial infection cannot be ruled out.

Some authors recommend empiric therapy with a β-lactam+macrolide combination or fluoroquinolone monotherapy for hospitalized patients, based on most common bacterial pathogens, which are similar to those of the general population (1,2).

As we found a considerable proportion of patients with demonstrated infection by atypical bacteria (8%) but failed to demonstrate a clinical benefit with macrolide use, some important questions are raised.

We speculate that patients with HIV/AIDS could have altered immunomodulatory effects of macrolides, ranging from reducing inflammation to worsening the inflammation due to immune reconstitution. Besides that, not all patients suffer from an excessive inflammatory response during pneumonia (9).

The identification of a subgroup of patients that would benefit from macrolide use is still pending. Apparently, the benefit of macrolides in the general population with CAP is more pronounced in severely ill patients (23–25).

Some authors suggest that the addition of macrolides for empirical treatment of any hospitalised patient with CAP should be reconsidered, due to the findings of recent prospective studies, reports of resistance and possible increased risks of cardiac effects (26).

Considering that, we recommend β -lactams as the standard therapy for hospitalised HIV/AIDS patients with CAP, while adding a macrolide only for severely ill patients with CD4+ T cell counts over 200 cells/mm^3.

The macrolide of choice in our study was azithromycin because it has some advantages over clarithromycin, such as fewer drug interactions, longer half-life and fewer reports of injection site phlebitis.

Regarding the choice of a β -lactam, the decision to use ceftriaxone in our study was based on IV drug availability in our Institute.

Penicillin G, another IV administrated drug, goes through periods of shortage in national market, so we tend to prioritize its use for syphilis treatment.

As *Streptococcus pneumoniae* penicillin resistance is not a major concern in our region, we suggest that other IV available aminopenicillins, such as amoxicillin or ampicillin be preferred over ceftriaxone.

A study performed in our Institute demonstrated that more than 90% of non-meningeal isolates of *S. pneumoniae* were susceptible to penicillin (27), a similar finding to Brazilian national data (28).

In our study, we had the antimicrobial resistance profile of 18/22 *S. pneumoniae* isolates and all were susceptible to penicillin.

We recommend that fluoroquinolones be reserved as alternative therapy, because we are in a setting with high prevalence of tuberculosis and its use is associated with delayed treatment and resistance (29).

Patients presenting with pneumonia acquired in the community but who have been hospitalised and treated with antimicrobial treatment in the last 3 to 6 months may qualify as nosocomial pneumonia and receive treatment accordingly (30).

Our findings brought also concerns about nonbacterial and mixed infections, as they were frequently found in our population: *P. jirovecii* (23%) and *Mycobacterium tuberculosis* (12%) were among the 7 most frequently found agents, in spite of the clinical diagnosis of CAP on hospital admission. We also observed a high rate of mixed diagnosis (21%) (9).

We suggest that severely ill patients with unknown CD4+ T cell counts or CD4<200 receive *P. jirovecii* treatment, as this was the most frequently agent in our population.

M. tuberculosis and influenza treatment can be associated to CAP treatment at the attending physician discretion.

Treatment subsequently must be modified according to etiologic findings (1,2). Narrowing the antimicrobial therapy may avoid resistance and adverse effects.

In our study, the initial antibiotic regimen was discontinued or changed in 39% of the subjects, which reflects real life challenges when dealing with CAP in patients with HIV/AIDS (31).

Future perspectives

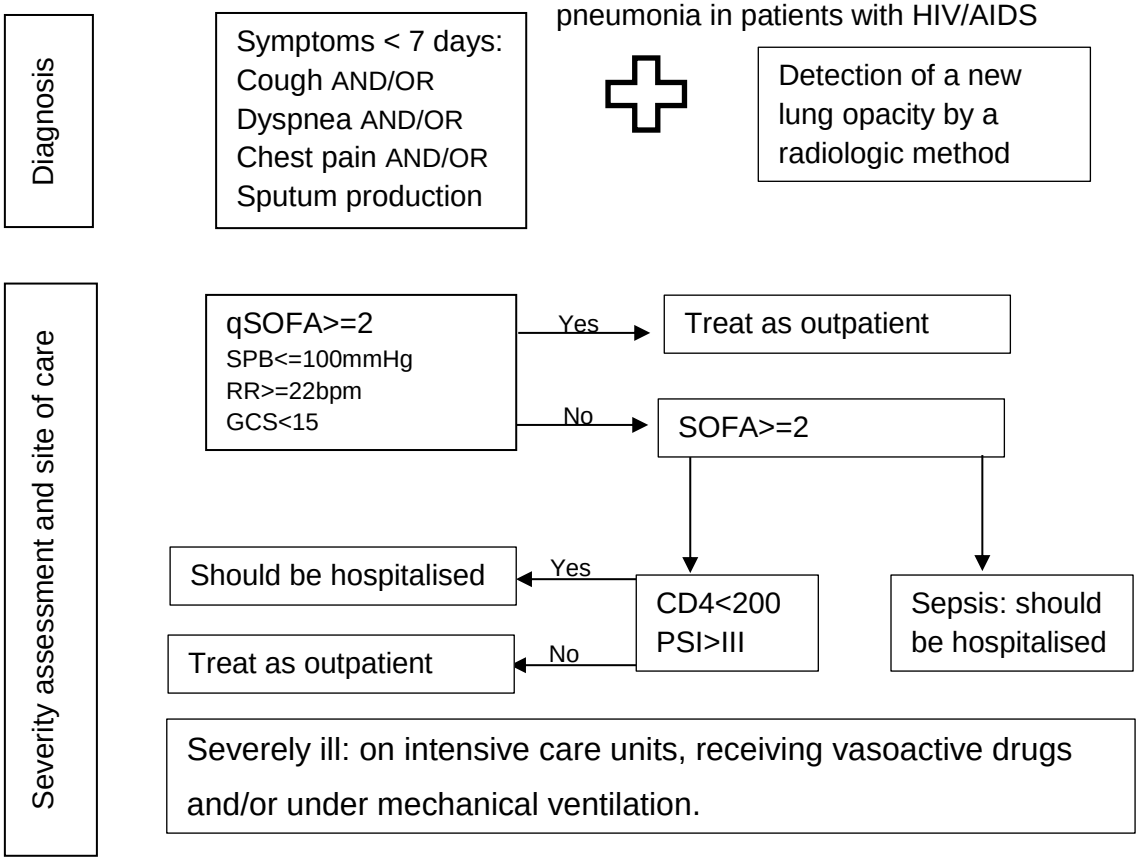
The knowledge about CAP in patients with HIV/AIDS still has substantial gaps that warrant future research.

Locally adapted guidelines should be implemented to improve care and clinical outcomes (12).

We recommend the development of empirical treatment guidelines, based on the local prevalence of pathogens, and the implementation of antimicrobial stewardship programmes.

Local compliance to protocols, aetiological findings, rates of antimicrobial administration according to the protocol, duration of treatment and outcomes must be constantly evaluated and reported to ensure quality.

Figure 1 – Recommendations for diagnosis and management of community-acquired pneumonia in patients with HIV/AIDS

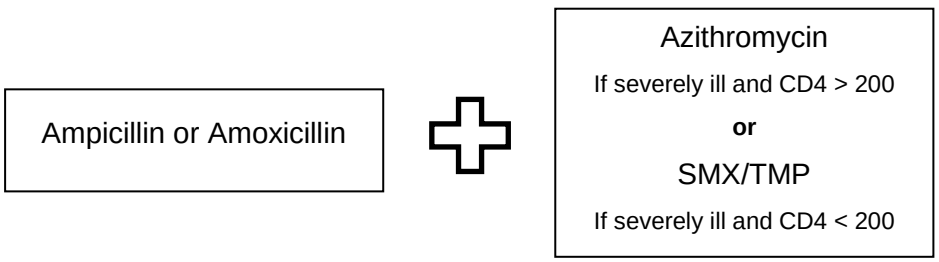


Microbiological evaluation

Specimen	Agent	Test
Blood	Bacteria	Culture Serology for *Chlamydophila pneumoniae* and *Mycoplasma pneumoniae*[1]
	Fungi	Serum cryptococcal antigen test Culture[1] Serology for *Histoplasma capsulatum* and *Paracoccidioides brasiliensis*[1,2]
	Mycobacteria	Culture[1]
Respiratory samples Sputum Endotracheal aspirate[2] Bronchoalveolar lavage[2]	Bacteria	Gram stain Culture PCR for *Chlamydophila pneumoniae, Legionella pneumophilla* and *Mycoplasma pneumoniae*[1]
	Fungi	Grocott-Gomori methenamine silver stain PCR for *Pneumocystis jirovecii*[1] Culture[1]
	Mycobacteria	Direct examination for acid-alcohol resistant bacilli[1,2] Xpert MTB RIF[1,2] Culture[1,2]
Nasopharyngeal swab	Virus	PCR test for influenza A viruses (including H1N1)[2]

[1] Severely ill patients [2] Selected cases PCR: Polymerase chain reaction

Treatment



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6. Conclusion

Among hospitalized HIV/AIDS patients with CAP treatment with ceftriaxone plus macrolide was not superior to ceftriaxone monotherapy in spite of a non-negligible prevalence of atypical bacteria.

Double therapy, with ceftriaxone and a macrolide did not improve the outcomes in any of the studied subgroups: patients with an identified bacterial pathogen, patients with identified atypical bacteria, and patients with CD4+ T cell count > 200 cells/mm³.

Resorting to an extended microbiological evaluation, this study was capable of defining the etiological diagnosis of a high proportion (64%) of cases of community-acquired pulmonary infections in hospitalized patients with HIV/AIDS.

The most frequently identified agents in this study were *Pneumocystis jirovecii*, *Mycobacterium tuberculosis, Streptococcus pneumoniae* and Rhinovirus. Mixed infections were very frequent.

Apêndice A – Termo de consentimento livre e esclarecido

(primeira versão)

São Paulo, 24 de março de 2011.

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Você está sendo convidado a participar do projeto de pesquisa "**Protocolo 17/11.**" Esta pesquisa pretende descobrir se um tipo de antibiótico chamado azitromicina pode melhorar a evolução dos casos de pneumonia em pacientes com HIV.

Todos os pacientes deste estudo receberão um antibiótico "pela veia" que se chama ceftriaxona, que já tem sua eficácia comprovada com relação ao tratamento da pneumonia. Nesta pesquisa alguns pacientes vão receber também um antibiótico da classe macrolídeo e outros vão receber um placebo (um líquido que não contém medicamento), ambos "pela veia". Depois os dados dos pacientes serão comparados para descobrir se a medicação "extra" ajudou o paciente.

Para podermos avaliar isto, o paciente que desejar participar do estudo terá que permanecer internado e receber a medicação do protocolo por pelo menos 5 dias.

Quando o paciente decide participar da pesquisa o médico pede para a farmácia a "medicação do protocolo". O fato de o paciente receber ou não a medicação já terá sido determinada de forma aleatória (sorteio) e só os farmacêuticos tem acesso a lista que determina o que cada paciente vai receber.

Nem os pesquisadores, nem os médicos que estão cuidando do paciente saberão normalmente se é o antibiótico ou o placebo que será dado para cada paciente. Em caso de suspeita de efeito colateral grave o médico terá como descobrir isso, para dar o melhor tratamento possível ao efeito colateral.

Alias, caso haja efeito colateral o paciente será devidamente tratado no próprio Instituto de Infectologia Emílio Ribas. Alguns efeitos colaterais da medicação de ação comprovada (ceftriaxona) são: reação alérgica, diarréia e aumento das células do sangue chamadas plaquetas e eosinófilos. Alguns efeitos colaterais da medicação pesquisada (azitromicina) são: reação alérgica, diarréia, náusea, dor abdominal e falta de apetite. Essas duas medicações por serem administradas pela veia podem causar irritação no local.

Como qualquer paciente com um quadro de pneumonia, os participantes deste estudo serão submetidos a exames buscando a causa exata da sua doença. Entre estes exames está a coleta de sangue por veia periférica que pode levar a dor, edema (inchaço) ou manchas escuras no local (hematomas). Também será colhido material de secreção nasal, que pode trazer algum desconforto dentro do nariz. Serão coletados também urina e escarro.

O sangue coletado servirá para fazer um hemograma (para ver como estão as células de defesa), provas bioquímicas (para ver como está seu rim e fígado, entre outras coisas) e vários outros testes para tentar detectar qual é o "bicho" que está causando sua doença no pulmão (sorologias, culturas, provas de aglutinação, antigenemia).

As amostras respiratórias (como o escarro) também vão servir para tentar descobrir o "bicho" que está causando sua doença no pulmão, através de pesquisa direta, culturas e PCRs. Na urina vai ser feito um teste para tentar detectar uma bactéria específica, a *Legionella pneumophilla*.

Como qualquer paciente com um quadro de pneumonia você corre o risco de precisar ser intubado para que um aparelho o ajude a respirar. Neste caso, podemos coletar outros tipos de amostras respiratórias através do tubo.

Alguns pacientes também precisarão de um exame que se chama broncoscopia (parecido com a endoscopia) e outros pacientes (que descobrem "líquido no pulmão") precisam de um exame que chama punção pleural. Nestes exames também há a possibilidade de coletar amostra respiratória.

Mesmo com todos os esforços das equipes envolvidas em seu tratamento, há o risco de morte, já que a pneumonia é uma doença grave e sua gravidade é ainda maior porque você tem o vírus do HIV.

Os pesquisadores garantem que de nenhuma forma a identidade dos sujeitos pesquisados será revelada durante a pesquisa ou durante a divulgação dos resultados. Os resultados desta pesquisa serão divulgados, mesmo que não sejam os previstos pelos pesquisadores.

O único benefício relacionado à participação neste trabalho será de aumentar o conhecimento científico nesta área. Se você escolher participar desta pesquisa, não terá que gastar nenhum dinheiro.

A decisão de participar ou não da pesquisa é sua! Qualquer que seja sua vontade isto não trará nenhum prejuízo em sua relação com o hospital e seu atendimento não sofrerá modificações. A qualquer momento você pode optar por sair desta pesquisa. Isso não trará nenhuma perda de benefício ou direito adquirido na Instituição.

Mesmo que você decida não participar ou sair da pesquisa, você receberá tratamento para seu quadro. As opções de tratamento serão apresentadas para você pelo médico assistente.

Você receberá uma cópia deste termo onde consta o telefone e o endereço institucional do pesquisador principal e do comitê de ética e pesquisa (CEP), podendo tirar suas dúvidas sobre o projeto e sua participação, agora ou a qualquer momento.

Eu, _____, declaro ter sido informado e concordo em participar, como voluntário, do projeto de pesquisa acima descrito.

_____ Nome do responsável:
Assinatura do paciente ou seu responsável legal Data:

_____ Nome legível:
Assinatura do pesquisador ou colaborador responsável por obter o consentimento Data:

Pesquisador Principal: Claudia Figueiredo Mello. Telefone: 30612521 (ramal 2521 do Emílio Ribas) ou 30697066 (GCIH do HC).
Telefone do comitê de ética e pesquisa (CEP): 38961406
Endereço: Av. Dr. Arnaldo, 165 – Cerqueira César – São Paulo- SP CEP 01246-900

(segunda versão)

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Você está sendo convidado a participar do projeto de pesquisa **"Ensaio clínico randomizado sobre o impacto dos macrolídeos na mortalidade de pacientes com HIV e pneumonia"**. Esta pesquisa pretende descobrir se uma classe de antibióticos chamada macrolídeos pode melhorar a evolução dos casos de pneumonia em pacientes com HIV.

Todos os pacientes deste estudo receberão um antibiótico "pela veia" que se chama ceftriaxona, que já tem sua eficácia comprovada com relação ao tratamento da pneumonia. Nesta pesquisa alguns pacientes vão receber também o antibiótico azitromicina ou o antibiótico claritromicina (da classe macrolídeos) e outros vão receber um placebo (um líquido que não contém medicamento), ambos "pela veia". Depois os dados dos pacientes serão comparados para descobrir se a medicação "extra" ajudou o paciente.

Para podermos avaliar isto, o paciente que desejar participar do estudo terá que permanecer internado e receber a medicação do protocolo por pelo menos 7 dias.

Quando o paciente decide participar da pesquisa o médico pede para a farmácia a "medicação do protocolo". O fato de o paciente receber ou não a medicação já terá sido determinada de forma aleatória (sorteio) e só os farmacêuticos tem acesso a lista que determina o que cada paciente vai receber.

Nem os pesquisadores, nem os médicos que estão cuidando do paciente saberão normalmente se é o antibiótico ou o placebo que será dado para cada paciente. Em caso de suspeita de efeito colateral grave o médico terá como descobrir isso, para dar o melhor tratamento possível ao efeito colateral.

Aliás, caso haja efeito colateral o paciente será devidamente tratado no próprio Instituto de Infectologia Emílio Ribas. Alguns efeitos colaterais da medicação de ação comprovada (ceftriaxona) são: reação alérgica, diarréia e aumento das células do sangue chamadas plaquetas e eosinófilos. Alguns efeitos colaterais da medicação pesquisada (macrolídeos) são: reação alérgica, diarréia, náusea, dor abdominal e falta de apetite. Essas duas medicações por serem administradas pela veia podem causar irritação no local.

Como qualquer paciente com um quadro de pneumonia, os participantes deste estudo serão submetidos a exames buscando a causa exata da sua doença. Entre estes exames está a coleta de sangue por veia periférica que pode levar a dor, edema (inchaço) ou "manchas escuras no local" (hematomas). Também será colhido material de secreção nasal, que pode trazer algum desconforto dentro do nariz. Serão coletados também urina e escarro.

O sangue coletado servirá para fazer um hemograma (para ver como estão as células de defesa), provas bioquímicas (para ver como está seu rim e fígado, entre outras coisas) e vários outros testes para tentar detectar qual é o "bicho" que está causando sua doença no pulmão (sorologias, culturas, provas de aglutinação, antigenemia).

As amostras respiratórias (como o escarro) também vão servir para tentar descobrir o "bicho" que está causando sua doença no pulmão, através de pesquisa direta, culturas e PCRs. Na urina também vamos tentar descobrir um "bicho".

Como qualquer paciente com um quadro de pneumonia você corre o risco de precisar ser intubado para que um aparelho o ajude a respirar. Durante a intubação, algumas complicações podem ocorrer como incapacidade de intubação, levando a falta

de oxigênio no sangue e morte, indução do vômito, levando conteúdo indevido ao pulmão, luxação da mandíbula, arrancamento de dentes e "ar acumulado entre o pulmão e as costelas" (pneumotórax). Caso o paciente precise ser intubado, podemos coletar outros tipos de amostras respiratórias através do tubo.

Alguns pacientes também precisarão de um exame que se chama broncoscopia (parecido com a endoscopia) e outros pacientes (que descobrem "líquido no pulmão") precisam de um exame que chama punção pleural. Nestes exames também há a possibilidade de coletar amostra respiratória.

O exame broncoscopia pode ter algumas complicações, como sangramento junto com a tosse após o exames, incômodo no tórax, febre, sensação de falta de ar e pneumotórax. A punção pleural também pode levar a um pneumotórax e poderá haver incômodo e/ou aparecimento de um hematoma no local.

Mesmo com todos os esforços das equipes envolvidas em seu tratamento, há o risco de morte, já que a pneumonia é uma doença grave e sua gravidade é ainda maior porque você tem o vírus do HIV.

Os pesquisadores garantem que de nenhuma forma a identidade dos sujeitos pesquisados será revelada durante a pesquisa ou durante a divulgação dos resultados. Os resultados desta pesquisa serão divulgados, mesmo que não sejam os previstos pelos pesquisadores.

O único benefício relacionado à participação neste trabalho será de aumentar o conhecimento científico nesta área. Se você escolher participar desta pesquisa, não terá que gastar nenhum dinheiro.

A decisão de participar ou não da pesquisa é sua! Qualquer que seja sua vontade isto não trará nenhum prejuízo em sua relação com o hospital e seu atendimento não sofrerá modificações. A qualquer momento você pode optar por sair desta pesquisa. Isso não trará nenhuma perda de benefício ou direito adquirido na Instituição.

Mesmo que você decida não participar ou sair da pesquisa, você receberá tratamento para seu quadro. As opções de tratamento serão apresentadas para você pelo médico assistente.

Você receberá uma cópia deste termo onde consta o telefone e o endereço institucional do pesquisador principal e do comitê de ética e pesquisa (CEP), podendo tirar suas dúvidas sobre o projeto e sua participação, agora ou a qualquer momento.

Eu, _____, declaro ter sido informado e concordo em participar, como voluntário, do projeto de pesquisa acima descrito.

_____ Nome do responsável:

Assinatura do paciente ou seu responsável legal Data:

_____ Nome legível:

Assinatura do pesquisador ou colaborador Data:

responsável por obter o consentimento

Pesquisador Principal: Claudia Figueiredo Mello. Telefone: 30612521 (ramal 2521 do Emílio Ribas) ou 30697066 (GCIH do HC).

Telefone do comitê de ética e pesquisa (CEP): 38961406

Endereço: Av. Dr. Arnaldo, 165 – Cerqueira César – São Paulo- SP CEP 01246-900

Anexo II - Exames realizados para determinação do agente etiológico.

Apêndice B – Exames complementares previstos no projeto de pesquisa original

Espécime	**Agente**	**Teste**
Sangue	Bactérias	Cultura Sorologias para *Chlamydophila pneumoniae* e *Mycoplasma pneumoniae*.
	Fungos	Cultura Prova de aglutinação de látex para antígeno de *Cryptococcus neoformans*
	Micobactérias	Cultura
	Vírus	Antigenemia de CMV
Amostra respiratória - Escarro, simples ou induzido - Aspirado traqueal ou - Lavado broncoalveolar	Bactérias	Cultura PCR para *Chlamydophila pneumoniae, Legionella pneumophilla* e *Mycoplasma pneumoniae*. Painel de vírus respiratórios
	Fungos	Pesquisa de *Pneumocystis jirovecii* Cultura
	Micobactérias	Pesquisa direta de bacilo álcool-ácido resistente Cultura
Urina	Bactérias	Pesquisa de antígeno de *Legionella pneumophila* sorogrupo 1
Líquido pleural	Bactérias	Cultura
	Fungos	Cultura
	Micobactérias	Pesquisa direta de bacilo álcool-ácido resistente Cultura

Apêndice C – **FICHA DE ADMISSÃO PROTOCOLO 17/11**

DATA DA ADMISSÃO __/__/____ HORA DA ADMISSÃO _____

NOME DO PACIENTE:_____ RH:_____

IDADE:_____ SEXO: M( ) F( ) COMPORTAMENTO DE RISCO:_____

HISTÓRIA CLÍNICA

QUEIXA PRINCIPAL: _____

HPMA:_____
_____
_____
_____
_____

☐ **tosse** ☐ dor torácica
☐ expectoração ☐ falta de ar

- Preenche critérios clínicos de inclusão? ☐ sim ☐ não
(tosse e um ou mais dos seguintes sintomas: expectoração, falta de ar e dor torácica)

	Sim	Não
Ficou internado por dois ou mais dias nos 90 dias prévios à admissão?		
É proveniente de algum local onde há assistência à saúde (por exemplo: asilos, abrigos, casas de apoio ou casas de saúde)?		
Recebeu antibióticos por via endovenosa, quimioterapia ou tratamento de escaras nos últimos 30 dias?		
Está em tratamento em clínicas de diálise?		
Tem outra causa diagnosticada para pneumopatia (como fibrose pulmonar, pneumocistose, tuberculose ou outra doença não bacteriana)?		
Está grávida ou amamentando?		

A resposta afirmativa a qualquer destas perguntas exclui o paciente do estudo no momento da admissão.

ANTECEDENTES PESSOAIS:_____
_____

☐ doença hepática
☐ insuficiência cardíaca
☐ doença neoplásica
☐ doença renal
☐ hipertensão arterial sistêmica
☐ acidente vascular cerebral prévio
☐ diabetes

ANTECEDENTES FAMILIARES: _____
_____

HISTÓRIA EPIDEMIOLÓGICA: _____
_____

DADOS RELATIVOS AO HIV: Tempo desde o diagnóstico: ____ ☐ dias ☐ meses ☐ anos

- Faz uso de TARV? ☐ sim☐ não

- Qual esquema atual?

☐ Abacavir	☐ Efavirenz	☐ Lamivudina	☐ Saquinavir
☐ Amprenavir	☐ Enfuvirtida	☐ Lopinavir/ritonavir	☐ Tenofovir
☐ Atazanavir	☐ Estavudina	☐ Nevirapina	☐ Zidovudina+Lamivudina
☐ Darunavir	☐ Fosamprenavir	☐ Raltegravir	☐ Zidovudina
☐ Didanosina	☐ Indinavir	☐ Ritonavir	☐ Outro:

- Há quanto tempo faz uso deste esquema? ___ ☐ dias ☐meses ☐ anos

- Deixou de tomar a TARV algum dia no último mês? : ☐ sim ☐ não

- CD4: _____ CARGA VIRAL: _____ DATA: __/__/____

- Recebeu vacina antipneumocócica?☐ sim ☐ não

- Uso de profilaxias com antimicrobianos no último mês?☐ sim ☐ não Quais?_____

- Doenças oportunistas prévias e data de seus diagnósticos:_____

_____

HÁBITOS:

Alcoolismo:

☐ Não usuário (o paciente não consumiu nenhuma dose no último mês)
☐ Usuário leve (o paciente consumiu até 100 doses no último mês)
☐ Usuário pesado (o paciente consumiu mais de 100 doses no último mês)

Tabagismo:

☐ Tabagista (o paciente fumou pelo menos 100 cigarros na vida e fumou pelo menos um cigarro no último mês)
☐ Ex-tabagista (o paciente fumou pelo menos 100 cigarros na vida e não fumaram nenhum cigarro no último mês)
☐ Não tabagista (o paciente nunca fumou ou fumou menos de 100 cigarros durante a vida)

Uso de drogas intravenosas (IV)

☐ Usuário (o paciente usou algum tipo de droga IV no último ano)
☐ Ex-usuário (o paciente usou droga IV alguma vez na vida, mas não usou nenhuma vez durante o último ano)
☐ Não usuário (o paciente que nunca usou droga IV).

U**so de drogas inalatórias (VI)**

☐ Usuário (o paciente usou algum tipo de droga VI no último ano)
☐ Ex-usuário (o paciente usou droga VI alguma vez na vida, mas não usou nenhuma vez durante o último ano)
☐ Não usuário (o paciente que nunca usou droga VI)

MEDICAMENTOS EM USO:_____
Fez uso de antimicrobianos em dose terapêutica no último mês?☐Sim☐não

Quais antimicrobianos?_____Fez uso no dia da admissão? Sim☐ não☐

REAÇÕES MEDICAMENTOSAS: _____

- Já apresentou reação alérgica a ceftriaxona ou azitromicina? : ☐ sim (excluído) ☐ não

EXAME FÍSICO

APARÊNCIA: ( )BEG ( )REG ( )MEG PESO:____kg ALTURA:____cm TEMP:____ºC

PULSO: ____bpm PA:____/____ FC:____bpm FR:____rpm

OXIMETRIA:____% FiO2:____% E. C. GLASGOW:____ (AO____MRM____MRV____)

AVALIAÇÃO GERAL:_____

_____

PELE E ANEXOS: _____

MUCOSAS:_____LINFONODOS:_____

AP. CARDIOVASCULAR:_____

AP. RESPIRATÓRIO:_____

_____

AP. DIGESTIVO:_____

NEUROLÓGICO:_____

_____

AP. GENITO-URINÁRIO:_____

MEMBROS:_____

HD:_____

_____

EXAMES SOLICITADOS:_____

_____

CARACTERÍSTICAS RADIOLÓGICAS:

Infiltrado	
Consolidação	
Unilobar	
Multilobar	
Derrame pleural	

DADOS LABORATORIAIS:

Na		Glicose	
Ur		Hematócrito	
pH		PaO2	
DHL		satO2	

- Presença de opacidade pulmonar nova? ☐ Sim(incluído) ☐ Não (excluído)

CURB 65 _____ PSI _____

- Assinou o termo de consentimento? ☐ Sim(incluído) ☐ Não (excluído)

CONDUTA:_____

_____

- Prescreveu a medicação do protocolo? ☐ Sim: Número de randomização:___**R** ☐ Não

INTERCORRÊNCIAS_____

PRECAUÇÕES:_____

_____

ASS. PESQUISADOR(ES) RESPONSÁVEL(IS) PELO PREENCHIMENTO

Apêndice D – Cálculo dos escores de gravidade de pneumonia

Tabela 1 - Escore de pontos segundo o sistema CURB-65[1]

C: Confusão mental	+1
U: Uréia > 50 mg/dL	+1
R: Frequência respiratória > 29 ciclos/min	+1
B: Pressão arterial sistólica < 90 mmHg ou diastólica < 61 mmHg	+1
65: Idade maior ou igual a 65 anos	+1

[1] Adaptado da referência Lim WS, Baudouin S V, George RC, Hill AT, Jamieson C, Le Jeune I, et al. BTS guidelines for the management of community acquired pneumonia in adults: update 2009. Thorax. 2009;64(Suppl 3):iii1-iii55.

Tabela 2 - Estratificação dos pacientes com pneumonia adquirida na comunidade por classes de risco, segundo o sistema CURB-65[1]

Pontuação	Mortalidade, %	Local de tratamento
0 ou 1	Mortalidade baixa, 1,5%	Provável candidato ao tratamento ambulatorial
2	Mortalidade intermediária, 9,2%	Considerar tratamento hospitalar
3 ou mais	Mortalidade alta, 22%	Tratamento hospitalar como PAC grave. Escore 4 ou 5: avaliar internação em UTI.

PAC: pneumonia adquirida na comunidade; UTI: unidade de terapia intensiva. [1] Adaptado da referência Fine, MJ Auble, TE Yealy D et al. A prediction rule to identify low-risk patients with community-acquired pneumonia. N Engl J Med. 1997;336(4):243–50.

Apêndice D – Cálculo dos escores de gravidade de pneumonia (continuação)

Tabela 3 - Escore de pontos segundo a presença de fatores demográficos, clínicos e laboratoriais, segundo o *Pneumonia Severity Index*.[a]

Fatores demográficos		**Achados laboratoriais e radiológicos**	
Idade		Ph < 7,35	+30
Homens	1 ponto/ano de idade	Uréia > 65 mg/dL	+20
Mulheres	Idade -10	Sódio < 130 mEq/L	+20
Procedentes de asilos	Idade +10	Glicose > 250 mg/dL	+10
		Hematócrito < 30%	+10
		PO_2 < 60 mmHg	+10
		Derrame pleural	+10
Comorbidades		**Exame físico**	
Neoplasia	+30	Alteração do estado mental	+20
Doença hepática	+10	FR > 30 ciclos/min	+20
ICC	+10	PA sistólica < 90 mmHg	+20
Doença cerebrovascular	+10	Temperatura < 35°C ou > 40°C	+15
Doença renal	+10	Pulso >124 bpm	+10

ICC: insuficiência cardíaca congestiva; PO_2: pressão arterial de oxigênio; FR: freqüência respiratória e PA: pressão arterial. [1] Adaptado da referência Fine, MJ Auble, TE Yealy D et al. A prediction rule to identify low-risk patients with community-acquired pneumonia. N Engl J Med. 1997;336(4):243–50.

Tabela 4 - Estratificação dos pacientes com pneumonia adquirida na comunidade por classes de risco, segundo o *Pneumonia Severity Index*[1]

Classe	Pontos	Mortalidade, %	Local sugerido de tratamento
I	-	0,1	Ambulatório
II	<71	0,6	Ambulatório
III	71-90	2,8	Ambulatório ou internação breve
IV	91-130	8,2	Internação
V	>130	29,2	Internação

[1] Adaptado da referência Fine, MJ Auble, TE Yealy D et al. A prediction rule to identify low-risk patients with community-acquired pneumonia. N Engl J Med. 1997;336(4):243–50.

Apêndice E – Aprovação e ciência dos Comitês de Ética em Pesquisa

MEDICINA
USP
COMITÊ DE ÉTICA EM PESQUISA

CIÊNCIA

O Comitê de Ética em Pesquisa da Faculdade de Medicina da Universidade de São Paulo, em **23.04.14** tomou ciência do Projeto nº **103/14** intitulado "**Ensaio clínico randomizado sobre o impacto dos macrolídeos na mortalidade de pacientes infectados pelo HIV e com pneumonia adquirida na comunidade**", vinculado à pós-graduação do Departamento de Moléstias Infecciosas e Parasitárias , que não envolverá atividade prática e/ou experimental no âmbito da Faculdade de Medicina ou Instituto do Câncer do Estado de São Paulo e que conta com prévia aprovação do Comitê de Ética em Pesquisa do Instituto de Infectologia Emilio Ribas.

Pesquisador(a) Responsável: Anna Sara S. Levin
Pesquisador(a) Executante : Claudia Figueiredo de Mello

CEP-FMUSP, 23 de abril de 2014

Prof. Dr.Roger Chammas
Coordenador
Comitê de Ética em Pesquisa

Comitê de Ética em Pesquisa
Faculdade de Medicina da Universidade de São Paulo

Apêndice E – Aprovação e ciência dos Comitês de Ética em Pesquisa (continuação)

COORDENADORIA DOS SERVIÇOS DE SAÚDE
INSTITUTO DE INFECTOLOGIA "EMILIO RIBAS"
COMITÊ DE ÉTICA EM PESQUISA
Av. Dr. Arnaldo, 165 - Cerqueira César - São Paulo - SP
CEP: 01246-900 – TEL: 3896-1406
E-mail: comitedeetica-iier@ig.com.br

PARECER

PROTOCOLO DE PESQUISA N.º 17/2011
PARECER N.o ***209/2011*** *Data:* **25/7/2011**

Título da Pesquisa: "Ensaio Clínico randomizado sobre o impacto dos macrolídeos na mortalidade de pacientes com HIV e pneumonia"

Investigador Principal: Marinella Della Negra

CONSIDERAÇÕES: O Comitê de Ética em Pesquisa avaliou a reposta da pendência do parecer 207/2011 e aprova o projeto, a última versão do TCLE encaminhado ao CEP em 08 de julho de 2011 e a última versão da Ficha de Admissão no Projeto enviada ao CEP na data de hoje.

(X) APROVADO
( ) APROVADO COM RECOMENDAÇÕES
( ) REPROVADO
( ) COM PENDÊNCIAS- OBS.: a ausência de resposta em 60 dias, acarretará em arquivamento do processo por falta de interesse do pesquisador.

TEMÁTICA ESPECIAL ☐ SIM ☐ NÃO
CONEP ☐ SIM ☐ NÃO
SVS (SECRETARIA DE VIGILÂNCIA SANITÁRIA) ☐ SIM ☐ NÃO

Anna Christina Nunes D'Ambrosio
Coordenadora do Comitê de Ética em Pesquisas - I.I.E.R

Apêndice E – Aprovação e ciência dos Comitês de Ética em Pesquisa (continuação)

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PARECER

PROTOCOLO DE PESQUISA N.º 17/11
PARECER N.o 263/2012 *Data:* 21/11/2012

Título da Pesquisa: "Ensaio clínico randomizado sobre o impacto dos macrolídeos na mortalidade de pacientes com HIV e Pneumonia"

Investigador Responsável: Dra. Marinella Della Negra

CONSIDERAÇÕES: O Comitê de Ética em Pesquisa toma ciência da modificação na duração dos regimes terapêuticos do estudo e Aprova a nova versão do TCLE que incorpora essa modificação.

(X) APROVADO
( ) REPROVADO
( ) COM PENDÊNCIAS- OBS.: a ausência de resposta em 60 dias, acarretará em arquivamento do processo por falta de interesse do pesquisador.

TEMÁTICA ESPECIAL ☐ SIM ☐ NÃO
CONEP ☐ SIM ☐ NÃO
SVS (SECRETARIA DE VIGILÂNCIA SANITÁRIA) ☐ SIM ☐ NÃO

Dra. Anna Christina Nunes D'Ambrosio
Coordenadora do Comitê de Ética em Pesquisas - I.I.E.R.

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Laura

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