Interaction between influenza and tuberculosis in South Africa, a country with high prevalence of tuberculosis and HIV.

Dr Sibongile Walaza

A thesis submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the degree of Doctor of Philosophy.

Johannesburg, 4 November 2018

DECLARATION

I, Sibongile Walaza, declare that this thesis is my work. It is being submitted for the degree of Doctor of Philosophy at the University of Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other university.

Signature: 

Sibongile Walaza

Date: 4 November 2018

DEDICATION

In loving memory of my grandmother, Lele Adelaide Walaza.

"Dongo, Langa, Bhelekazi, Nyathi"

ABSTRACT

BACKGROUND

For both influenza disease and tuberculosis disease, the lungs are the main site of pathology and HIV is an important risk factor for severe illness. There are limited published data on the interaction between tuberculosis disease and influenza disease.

OBJECTIVES

We aimed to assess the effect of influenza and tuberculosis coinfections on clinical presentation, progression of illness and disease outcome; and to evaluate the burden of excess mortality associated with influenza infection in patients with tuberculosis disease.

METHODS

A systematic review of published literature on the interaction between influenza and tuberculosis disease was conducted. Using surveillance data, we estimated the burden of tuberculosis disease among patients hospitalized with severe respiratory illness (SRI) and assessed the risk of influenza and tuberculosis coinfection on in-hospital mortality. For SRI patients we included HIV infection status in our models when this information was available. Ecological models were used to estimate excess influenza–associated mortality among individuals with tuberculosis disease compared to non-tuberculosis respiratory deaths.

RESULTS

The systematic review revealed paucity and poor quality of available literature on the interaction between influenza and tuberculosis disease. Experimental animal studies suggested that influenza-tuberculosis coinfection resulted in more severe outcomes than single infection. Whereas the majority

of the analytical studies (4/5) showed no association. Analysis of surveillance data revealed that the prevalence of tuberculosis among patients hospitalized with SRI was 28% (593/2097); 18% (133/729) among patients with symptoms duration ≤14 days. Among patients with symptom duration ≥7 days, patients coinfected with tuberculosis and influenza as compared to patients with tuberculosis only were at increased risk of death (aRRR 5.5, 95% CI 1.2-25.3). In a separate analysis, laboratory-confirmed tuberculosis disease was associated with an increased risk of death in hospitalised patients who tested positive for influenza virus (aOR 4.5, 95% CI 1.5-13.3) and laboratory-confirmed influenza virus infection was associated with an increased risk of death in hospitalised patients who tested positive for tuberculosis (aOR 2.3, 95% CI 1.02-5.2).

In individuals <65 years, the risk of influenza-associated mortality was greater among pulmonary tuberculosis deaths irrespective of HIV status compared to HIV-infected non-tuberculosis respiratory deaths (relative risk (RR): 5.2; 95% CI: 4.6–5.9 and HIV-uninfected (RR: 61.0; CI: 41.4–91.0) non-tuberculosis respiratory deaths.

CONCLUSION

Tuberculosis was common in patients hospitalised with SRI, including among individuals with duration of symptoms ≤14 days. Tuberculosis and influenza coinfection compared to single tuberculosis or influenza infection was associated with increased risk of mortality. These data support recommendations for patients with tuberculosis disease to be prioritised for influenza vaccination.

PUBLICATIONS CONTRIBUTING TO THIS THESIS

The thesis is composed of five articles encompassing a synthesis of available literature as well as ecological (aggregated data) and case-based studies (individual level data) on the association of influenza and tuberculosis disease. The first article is a systematic review that describes the current understanding of the interaction between influenza infection or disease and tuberculosis disease and identifies knowledge gaps. Articles II-IV include analyses of individual level data derived from a surveillance programme for severe respiratory illness (SRI) to address knowledge gaps identified in the systematic review, including: (i) describing the tuberculosis burden and presentation among hospitalised individuals with SRI in South Africa and (ii) describing the presentation and outcome of tuberculosis-influenza coinfection. Article V uses ecological modelling of aggregated data to estimate the excess mortality associated with influenza disease among tuberculosis deaths.

LIST OF ORIGINAL ARTICLES

I. **Sibongile Walaza**, Cheryl Cohen, Stefano Tempia, Jocelyn Moyes, Athermon Nguweneza, Shabir Madhi, Meredith McMorrow, Adam L. Cohen. **Influenza and tuberculosis co-infection: a systematic review. Full draft submitted.**

II. **Sibongile Walaza**, Stefano Tempia, Andries Dreyer, Halima Dawood, Ebrahim Variava, Neil A Martinson MD, Jocelyn Moyes, Adam L. Cohen, Nicole Wolter, Claire von Mollendorf, Anne von Gottberg, Sumayya Haffejee, Florette Treurnicht, Orienka Hellferscee, Nazir Ismail, Cheryl Cohen. **The burden and clinical presentation of pulmonary tuberculosis in adults with severe respiratory illness in a high HIV prevalence setting, 2012-2014. Open Forum Infect Dis. 2017 Aug 7;4(3):ofx116. doi: 10.1093/ofid/ofx116. eCollection 2017 Summer. PMID: 28852676.**

III. **Sibongile Walaza**, Stefano Tempia, Halima Dawood, Ebrahim Variava, Jocelyn Moyes, Adam L. Cohen, Nicole Wolter, Michelle Groome, Claire von Mollendorf, Kathleen Kahn, Marthi Pretorius, Marietjie Venter, Shabir A. Madhi, Cheryl Cohen. **Influenza virus infection is associated with increased risk of death amongst patients hospitalized with confirmed pulmonary tuberculosis in South Africa, 2010-2011. BMC Infect Dis .(2015) Jan 27;15:26. doi: 10.1186/s12879-015-0746-x. PMID: 25623944**

IV. **Sibongile Walaza**, Stefano Tempia, Halima Dawood, Ebrahim Variava, Neil A Martinson, Jocelyn Moyes, Meredith McMorrow, Nicole Wolter, Andries Dreyer, Claire von Mollendorf, Anne von Gottberg, Sumayya Haffejee, Florette Treurnicht, Orienka Hellferscee, Shabir Madhi, Nazir Ismail, Cheryl Cohen. **Interaction of influenza and tuberculosis on mortality among individuals aged ≥ 15 years hospitalised with severe respiratory illness in South Africa, 2010-2016** – Full draft awaiting CDC clearance

V. **Sibongile Walaza**, Cheryl Cohen, Ananta Nanoo, Adam L. Cohen, Jo McAnerney, Claire von Mollendorf, Jocelyn Moyes, Stefano Tempia. **Excess mortality associated with influenza among tuberculosis deaths in South Africa, 1999-2009. PLoS One. 2015 Jun 15;10(6):e0129173. doi: 10.1371/journal.pone.0129173. eCollection 2015. PMID:26076197.**

The publishers have given permission for reprinting of the published manuscripts. My role in each of the articles is included in appendix A

TABLE OF CONTENTS

DECLARATION ii

DEDICATION iii

ABSTRACT iv

PUBLICATIONS CONTRIBUTING TO THIS THESIS vi

TABLE OF CONTENTS viii

ABBREVIATIONS x

1. BACKGROUND 1

1.1. BURDEN, EPIDEMIOLOGY AND MANAGEMENT OF TUBERCULOSIS 1

1.2. BURDEN, EPIDEMIOLOGY AND MANAGEMENT OF INFLUENZA DISEASE 3

1.3. BURDEN, EPIDEMIOLOGY AND MANAGEMENT OF HIV 4

1.4. THE ROLE OF HIV COINFECTION ON THE BURDEN OF INFLUENZA-AND TUBERCULOSIS-ASSOCIATED ILLNESS 5

1.5. INFLUENZA AND TUBERCULOSIS COINFECTION: GAPS IN LITERATURE 6

1.6. POSSIBLE MECHANISMS FOR SEVERE OUTCOMES IN INDIVIDUALS WITH INFLUENZA AND TUBERCULOSIS COINFECTION 8

1.7. STATEMENT OF THE PROBLEM AND JUSTIFICATION FOR THE RESEARCH 11

1.8. AIMS AND OBJECTIVES 12

2. METHODS 14

2.1. STUDY DESIGN 14

2.2. ETHICAL CONSIDERATIONS 27

3. RESULTS AND DISCUSSION 28

3.1. SUMMARY OF PUBLISHED LITERATURE ON INFLUENZA AND TUBERCUOSIS CO-INFECTION- A SYSTEMATIC REVIEW 28

3.2. THE BURDEN AND CLINICAL PRESENTATION OF PULMONARY TUBERCULOSIS IN ADULTS WITH SEVERE RESPIRATORY ILLNESS IN A HIGH HIV PREVALENCE SETTING, 2012-2014 33

3.3. INCREASED RISK OF MORTALITY AMONG PATIENTS ADMITTED WITH SEVERE RESPIRATORY ILLNESS WHO ARE COINFECTED WITH INFLUENZA VIRUS AND *M.TUBERCULOSIS* COMPARED TO PATIENTS WITH SINGLE INFLUENZA OR TUBERCULOSIS INFECTION 38

3.4. EXCESS MORTALITY ASSOCIATED WITH INFLUENZA AMONG TUBERCULOSIS DEATHS IN SOUTH AFRICA, 1999-2009 44

4. CONCLUSION 49

5. ACKNOWLEDGEMENTS 50

6. REFERENCES 51

7. APPENDICES 58

ABBREVIATIONS

AFB: Acid fast bacilli

AIDS: Acquired immune deficiency syndrome

ALRTI: acute lower respiratory tract infection

ARR: adjusted relative risk

ARRR: adjusted relative risk ratio

ART: Antiretroviral therapy

AOR: adjusted odds ratio

AV: adenovirus

CAP: Community acquired pneumonia

CI: Confidence intervals

CDC: Centers for Disease Control and Prevention

EDTA: Ethylenediaminetetraacetic acid

ELISA: enzyme-linked immunosorbent assay

EML; Essential medicines list

HBC: High burden country

H.influenzae: Haemophilus influenzea

HIV: Human immunodeficiency virus

HMPV: human metapneumovirus

ICD 10: International Classification of Diseases, Tenth Revision

ICU: Intensive care unit

ILI: Influenza-like-illness

LRTI: Lower respiratory tract infection

MTB: *Mycobacterium tuberculosis*

NDoH: National Department of Health

NHLS: National Health Laboratory Services

NICD: National institute for Communicable Diseases

NP: Nasopharyngeal

OP: oropharyngeal

PIV: parainfluenza

P. jirovecii: Pneumocystis. Jirovecii

PMTCT: Prevention of mother to child transmission

PCR: polymerase chain reaction

PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses

PTB: Pulmonary tuberculosis

RR: relative risk

rRT-PCR: real time reverse-transcription polymerase chain reaction

RSV: Respiratory syncytial virus

SARI: Severe acute respiratory illness

SCRI: Severe chronic respiratory illness

SRI: severe respiratory illness

S. pneumoniae: Streptococcus. pneumoniae

TB: Tuberculosis

TIV: Trivalent inactivated influenza vaccine

1. BACKGROUND

Influenza and tuberculosis disease are important causes of morbidity and mortality globally. The burden of severe influenza- and tuberculosis-associated illness is greater in Africa compared to other regions, with much of the global burden reported to be in sub-Saharan Africa [1-3]

1.1.BURDEN, EPIDEMIOLOGY AND MANAGEMENT OF TUBERCULOSIS

Tuberculosis continues to be a major cause of mortality and a global health problem. In 2009, tuberculosis was the second most common cause of infectious disease–related deaths globally, after human immunodeficiency virus (HIV) and acquired immune deficiency syndrome (AIDS) [4]. In 2015, tuberculosis superseded HIV and AIDS as the commonest cause of infectious disease-related deaths [3]. The global tuberculosis incidence ranged from 8.6 million cases in 2012 to 10.4 million in 2015, mortality increased from 1.3 million deaths in 2012 to 1.4 million deaths in 2015 [3, 5, 6]. Studies have suggested increased transmission of tuberculosis during winter and an increase in tuberculosis disease notifications towards the end of winter, early spring and summer [7, 8]. A paediatric study from Cape Town, reported an increase in prevalence of positive tuberculosis cultures during the winter and early spring [9].

Tuberculosis in South Africa

South Africa is one of the 22 high burden countries (HBC) for tuberculosis which accounted for approximately 80% of world's tuberculosis cases in 2014 [6] and is one of the 30 HBC in the recently revised list of countries which account for 87%-92% of global tuberculosis burden[3]. The tuberculosis incidence in South Africa ranged from an estimated 390 000 cases in 2011 to 454 000 cases in 2015 [3, 10]. In 2011, South Africa had the 3rd highest incidence of tuberculosis globally, after India and China[10]. Although the proportion of overall deaths due to tuberculosis disease (excluding HIV disease

resulting in tuberculosis) showed a downward trend between 2011 and 2013 in South Africa, tuberculosis remained the leading underlying natural cause of death during these years accounting for 10.7%, 9.9% and 8.8% of deaths in 2011, 2012 and 2013 respectively. [11]. In South Africa, annual tuberculosis mortality, excluding tuberculosis mortality among HIV-infected individuals, ranged from 31 000 in 2012 to 25 000 in 2015 [3, 5]. Tuberculosis deaths among HIV-infected individuals ranged from 88 000 in 2012 to 73 000 in 2015 [3, 5].

Diagnosis and management of tuberculosis in South Africa

The South African guidelines for tuberculosis management advise that patients presenting with persistent cough of >2 weeks in HIV-uninfected individuals or any duration in HIV-infected individuals, fever for > 2 weeks, night sweats (drenching) or unexplained weight loss (>1.5 kg in a month for adults or loss of >5% of the highest recorded weight in the last 3 months for children) should be investigated for tuberculosis. Additional criteria for testing among children include exposure to a culture or smear positive tuberculosis case or indication of tuberculosis infection (Montoux positive test). Recommendations for testing include at least one sputum (or; lavage/ gastric washing or lymph node fine needle aspirate in children) tested for bacteriological confirmation of tuberculosis disease. Xpert® MTB/RIF(Cepheid, Sunnyvale, California, USA) (when available) or smear microscopy are recommended as the first line tests for diagnosis of tuberculosis. An additional smear is collected for all Xpert® MTB/RIF positive cases for bacteriological monitoring of response to treatment and contact tracing. Tuberculosis culture is recommended for HIV-infected individuals who test negative on Xpert® MTB/RIF, as well as susceptibility testing of other drugs for GeneXpert rifampicin resistant cases and diagnosis of paucibacilliary tuberculosis in tuberculosis suspects with two smear negative results [12-14]. A list and description of bacteriological tests used for confirmation of tuberculosis disease are included in Table 2. Treatment for tuberculosis disease is made available in the public health sector in South Africa and it is initiated based on bacterial confirmation of tuberculosis and/or clinical diagnosis of tuberculosis (i.e., chest X-ray changes consistent with

tuberculosis, history or clinical picture suggestive of tuberculosis or histological or biochemical tests suggestive of tuberculosis) [12-14]. Clinical picture suggestive of pulmonary tuberculosis disease included persistent cough of ≥2 weeks, fever of ≥2 weeks, drenching night sweats and unexplained loss (>1.5kg in a month).

1.2. BURDEN, EPIDEMIOLOGY AND MANAGEMENT OF INFLUENZA DISEASE

Influenza virus infection causes substantial annual morbidity and mortality worldwide and in South Africa [1, 15, 16]. Globally, it is estimated that seasonal influenza disease epidemics result in three to five million cases of severe illness, and ~ 250 000-500 000 deaths each year [16]. A number of risk factors have been described for severe outcomes among patients with influenza disease; these including extremes of age, prematurity, pregnancy, malnutrition, obesity and chronic underlying diseases, including pulmonary disease, cardiovascular diseases, diabetes and immune-suppression [2, 17-29].

Influenza in South Africa

In South Africa, an estimated 47 000 episodes of influenza-associated severe acute respiratory illness occur annually of which 22 481 result in hospitalization[30]. In addition, it is estimated that 11 800 (95%CI 5672-15732) deaths occur annually due to seasonal influenza disease, of which 63% (7447, 95%CI 2067-10945) occur out-of-hospital.[31] In South African public facilities, approximately 6% of patients hospitalised with lower respiratory tract infection (LRTI) and 15% of outpatients with influenza-like illness (ILI) test positive for influenza virus on polymerase chain reaction (PCR) annually [32]. Data from the syndromic surveillance for respiratory illness has also shown that influenza viruses when detected among outpatients with ILI, and inpatients with severe acute respiratory illness (SARI – symptoms duration ≤10 days) and severe chronic respiratory illness (SCRI – symptoms duration >10 days) are likely attributable to illness. The attributable fraction of influenza virus

detection to illness was 92.6% (95% CI: 89.3%-94.8%), 87.4% (95% CI: 81.3%-91.5%) and 86.2% (95% CI: 77.7%-91.5%) amongst ILI, SARI and SCRI cases respectively [33].

Influenza seasonality, prevention and management in South Africa

The South African influenza season falls in the winter months with average onset of the season being the first week of June (range week 17-week 28) [34]. The average duration of the influenza season is 13 weeks (range 7-25 weeks) [35]. In response to the 2009 influenza virus pandemic, the South African National Department of Health (NDoH) initiated its first national influenza vaccination campaign in 2010 and subsequently yearly vaccination campaigns were conducted targeting high risk groups (i.e., pregnant women, HIV-infected individuals, individuals ≥65 years, individuals with chronic underlying conditions and children 6 months-≤5 years). The number of vaccines allocated for high risk groups is far less than the number of individuals in these groups in South Africa (±1 million doses vs ± 20 million individuals in risk groups)[36]. The NDoH has set itself a target of reaching 15% of high risk groups by 2020 including 21 861 of the estimated 145 742 HIV-uninfected individuals with tuberculosis[36]. Guidelines for influenza vaccination are published annually [37, 38]. According to influenza treatment guidelines, patients admitted with severe respiratory illness during the influenza season should be started on influenza antivirals. The guidelines recommend that decisions should be based on clinical judgment taking into consideration the patient's disease severity and progression, age, underlying medical conditions and likelihood of progressing to severe influenza disease [37, 38]. Testing for influenza virus is not conducted as part of standard of care. Influenza antiviral treatment, Oseltamivir, although not listed in the Department of Health's Essential Medicines List (EML) under guidelines for influenza treatment, is available in public sector hospitals; however, use is limited in practice.

1.3.BURDEN, EPIDEMIOLOGY AND MANAGEMENT OF HIV

HIV causes significant morbidity and mortality globally and is a risk factor for both tuberculosis and influenza-associated morbidity and mortality[3, 39]. In 2016 there were approximately 36.7 million

people living with HIV globally, with an estimated 70% of these living in middle-income countries. An estimated one million individuals died from HIV in 2016 [39]. In 2015 there were an estimated 2.1 million new HIV infections worldwide [40]. Globally approximately 19 million people living with HIV were receiving antiretroviral therapy (ART) in 2016 [39].

HIV in South Africa

In South Africa, the community HIV prevalence is high[39]. In 2010 about a third of women accessing antenatal care in public health facilities tested positive for HIV [41]. From 2011 to 2013, HIV moved from being the 7th to being the 3rd leading underlying natural cause of death in South Africa accounting for 3.4%, 3.9% and 5.1% of deaths in 2011, 2012 and 2013 respectively. [42]. An estimated 12.7% of the general population and 22.3% of women of reproductive age were HIV-infected in 2016[43].

HIV prevention and management in South Africa

Access to ART has increased over the years since its roll-out in the public sector in 2004, with an estimated 3.4 million (48%) individuals living with HIV having access to antiretroviral therapy in 2015 [44, 45]. In recent years, prevention of mother-to-child transmission (PMTCT) programs have managed to reduce the risk for transmission of HIV to infants to <2 % (9.6% in 2008, 2.8% in 2011 and 1.5% in 2016) [39, 44, 46].

1.4.THE ROLE OF HIV COINFECTION ON THE BURDEN OF INFLUENZA- AND TUBERCULOSIS-ASSOCIATED ILLNESS.

HIV-infected individuals are 20 times more likely to develop tuberculosis disease as compared to HIV-uninfected individuals [47]. Globally of the 8.6 million incident cases of tuberculosis in 2012, approximately 1.1 million (13%) were coinfected with HIV. In South Africa for the same year 66% (330 000/530 000) of the incident cases of tuberculosis were HIV coinfected [48]. In recent years, there has been a downward trend in the proportion of HIV-infected individuals among tuberculosis incident

cases: 85% in 2011 (211 800/390 000), 66% (330 000/530 000) in 2012 and 57% (258 000/454 000) in 2015. [3, 5, 10].

Individuals with HIV also experience elevated risk of influenza-associated hospitalisation and mortality [18, 19, 21, 22, 49-53]. Studies from the United States of America reported an elevated risk of influenza-associated mortality in patients with AIDS prior to introduction of ART with downward trend after introduction of ART [19, 50, 53]. In South Africa the influenza-associated excess mortality showed a downward trend after widespread introduction of ART in public health programmes, but high incidences of influenza-associated mortality remained[18, 21, 22, 52]. Surveillance data from South Africa found that compared to HIV-uninfected individuals, HIV–infected individuals had 4-8 times higher risk of influenza-associated hospitalisation (age adjusted relative risk (aRR) 4.2 (95%CI 3.6-4.8) in 2009, aRR 7.5 (95%CI 6.4-8.8) in 2010 and aRR 5.5 (4.7-6.3) in 2011)[52], and 20 times greater estimated rates of mortality, RR 20.4 (15.0-27.4)[18]. In addition, the data showed a differential risk of influenza-associated hospitalization among HIV-infected patients, those with severe immunosuppression (CD4 <200/mm^3) were at increased risk of influenza-associated hospitalisation compared with individuals with mild immunosuppression (CD4 $\geq$ 200mm^3) (aOR 3.0, 95%CI 1.1-8.7)[24]. Ecological data showed that HIV-infected compared to HIV-uninfected individuals were at increased risk of influenza-associated death, aRR 11.5 (95%CI 9.6-12.6) and 7.9 (95% CI: 7.1-8.9) in children <5 years and individuals $\geq$ 5 years respectively [21, 22]

1.5.INFLUENZA AND TUBERCULOSIS COINFECTION: GAPS IN LITERATURE

While chronic lung disease is a known risk factor for severe outcome following influenza virus infection, there are few published data focusing on the association between influenza disease and tuberculosis disease and the contribution of such coinfections to influenza- and tuberculosis-associated morbidity and mortality. In addition, an increase in mortality associated with influenza virus circulation

has been described for a number of syndromes or among individuals with underlying medical conditions including respiratory illness and pneumonia and influenza (P&I), cerebrovascular disease, and diabetes [54-58]; however little data is available on excess mortality due to influenza disease among patients with pulmonary tuberculosis (PTB). As far back as in the 1900s, the scientific community has debated whether patients with active tuberculosis disease were more likely to contract influenza virus infection than persons without tuberculosis disease [59] and whether influenza viral infection aggravated the course of tuberculosis disease using statistics collected from clinical practice or vital statistics [60]. Ecological studies and mathematical modelling of aggregated data suggested an increase in frequency of influenza disease or severe influenza-associated disease in individuals with tuberculosis disease during influenza pandemics as compared to individuals without tuberculosis disease; however, causal relationships could not be determined [61-64]. Some studies suggested that influenza pandemics may have been responsible for the decline in tuberculosis mortality in subsequent years due to selective mortality [65]. More recently case series and individual level data from the 2009 influenza pandemic have been published. Underlying tuberculosis disease was described in 10% of cases who died during the 2009 influenza pandemic in South Africa [66]. This prevalence was greater than the population prevalence (1% in 2006)[66]. Another study from India identified PTB as a risk factor for infection with pandemic influenza A(H1N1) virus. Of the influenza cases, 8.8% had tuberculosis disease compared to 0.4% ($p<0.001$) tuberculosis prevalence in general population[67]. Whereas, in South Korea, in a case series of patients positive for influenza A(H1N1)pdm09 virus, less than 1% (7/12196,0.06%) had newly diagnosed tuberculosis disease and none of the cases with concurrent influenza and tuberculosis disease died[68].

Studies from murine models have also suggested that coinfection with *Mycobacterium tuberculosis* and influenza virus may alter the progression of the tuberculosis infection and outcome [69-71]. A summary of the results of a systematic review on the interaction between laboratory-confirmed

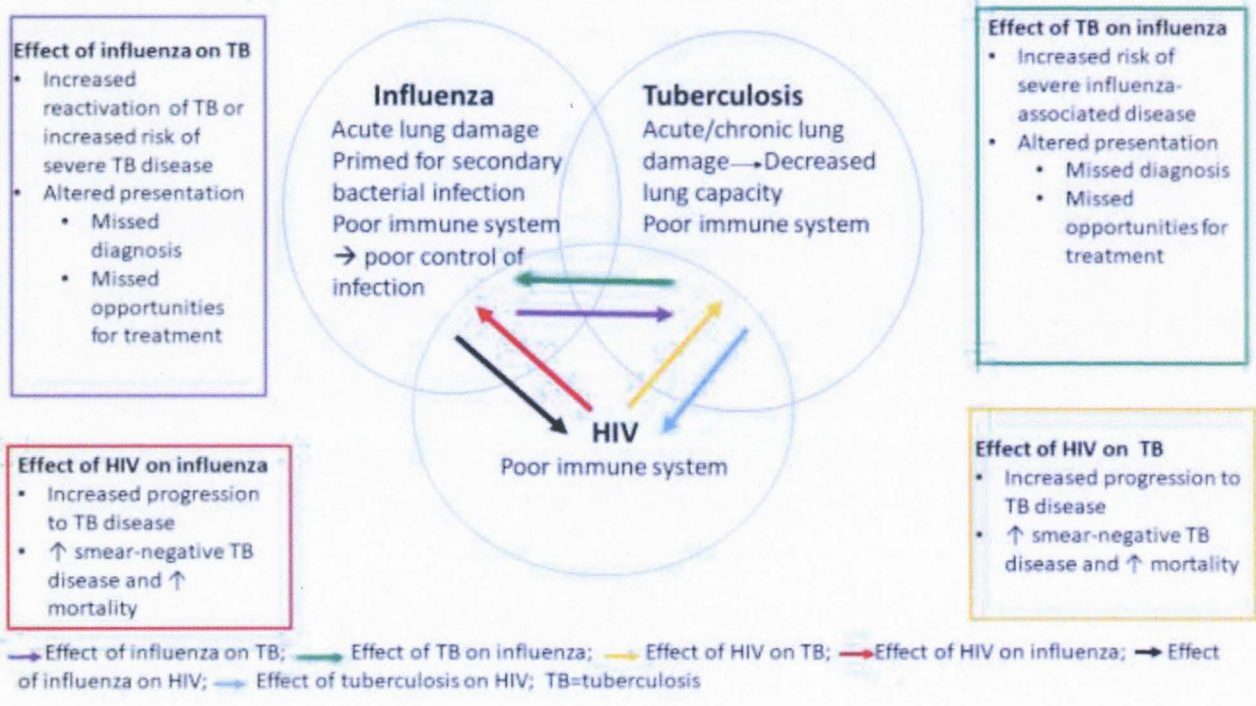
influenza illness and tuberculosis disease conducted as part of this study is presented in the results section of this report.

1.6.POSSIBLE MECHANISMS FOR SEVERE OUTCOMES IN INDIVIDUALS WITH INFLUENZA AND TUBERCULOSIS COINFECTION

Both influenza and tuberculosis have been reported to interact with other pathogens, especially bacterial pathogens resulting in increased morbidity and mortality[72-74]. A systematic review in 2016 reported *Streptococcus pneumoniae* and *staphylococcus aureus* as the most common bacterial coinfections in patients with influenza, accounting for 35% (95% CI, 14%–56%) and 28% (95% CI, 16%–40%) of infections, respectively [73]. Bacterial infection was associated with increased influenza-associated mortality during the 1918 influenza pandemic[74, 75]. A study from the Philippines reported a high prevalence of bacterial coinfection among patients hospitalized with tuberculosis, 49.6%, *H. influenzae* (11%) being the most common pathogen detected followed by *S. pneumoniae* (4%). In this study, bacterial coinfection was significantly associated with an increased risk of 2-week mortality among tuberculosis –pcr positive patients aRR(1.67, 95%CI 1.03-2.7).[72]. A case study from South Africa, reported dual infection with *S. pneumoniae* and tuberculosis in 9 HIV-infected patients hospitalised for community acquired pneumonia[76] Probe vaccine studies from South Africa also suggested an interaction between *S. pneumoniae* and influenza[77] and between *S. pneumoniae* and tuberculosis [78]. In one study, PCV-vaccinated children were less likely to be hospitalized for influenza-associated pneumonia[77]. A vaccine probe study in children suggested that pulmonary tuberculosis was associated with superimposed pneumococcal pneumonia, culture confirmed cases were 43% (95% CI, 9.7%-65,1%) less likely among PCV vaccinees as compared to placebo recipients.[78].

The lungs are the site of pathology for both influenza disease and tuberculosis disease. There are different possible mechanisms by which tuberculosis and influenza disease may interact potentially resulting in more severe clinical outcomes (Figure1). Tuberculosis disease may lead to reduced lung capacity, resulting in impaired ability to cope with viral infections like influenza and potentially increasing the severity of influenza-associated illness. In addition, influenza infection affects antibacterial host defenses by either increasing production of pro-inflammatory cytokines, resulting in cell damage [79] or through impairing pro-inflammatory chemokine responses to infection [80, 81], which could increase susceptibility to tuberculosis. Immunosuppression induced by influenza virus through production of glucocorticoids [82] and immunosuppressive cytokines [79] or T-cell immunity suppression [83] could trigger reactivation of latent *M. tuberculosis*. Lastly, influenza disease may prime those with tuberculosis disease for super-infection by other non-tuberculosis bacteria, e.g *Streptococcus pneumoniae*, leading to pneumonia and the subsequent secondary pneumonia that occurs as a complication of influenza disease could be exacerbated by active tuberculosis disease resulting in severe outcomes. As described earlier, HIV interacts with both tuberculosis and influenza disease. For the purpose of this PhD, I only focussed on the effect of influenza infection or disease on tuberculosis disease and the effect of tuberculosis disease on influenza illness, addressing HIV as a possible confounder. This includes discussion of the possible effect of HIV on tuberculosis disease and on influenza illness although this was not the main focus. I have not discussed the possible effect of influenza illness or tuberculosis infection or disease on HIV (Figure1).

Figure 1: Conceptual Framework showing the possible mechanism of interaction between influenza, tuberculosis and HIV and the possible impact of tuberculosis influenza coinfection on patient presentation and outcome in a setting with high HIV prevalence



1.7. STATEMENT OF THE PROBLEM AND JUSTIFICATION FOR THE RESEARCH

The disproportionately elevated burden of severe influenza disease in sub-Saharan Africa may have a number of contributing factors including elevated prevalence of coinfection and comorbidities, and limited access to good quality care[1, 2, 18, 84, 85]. There are gaps in understanding the interactions of influenza virus infection with conditions that are prevalent in Africa, such as tuberculosis. Influenza-associated-mortality in patients presenting with SARI has been described [1, 2, 15, 18, 20, 21, 23]. However, tuberculosis disease in this group of patients is not well described. There are a few published data on the association between tuberculosis disease and influenza illness. However, these are mostly descriptive and limited to the 2009 influenza pandemic. Influenza virus infection may increase the risk of death among patients with tuberculosis. In turn, tuberculosis, a chronic lung infection may cause lung damage thus compromising lung capacity and possibly impairing the ability to cope with influenza virus infection and potentially increasing the severity of influenza–associated illness. Because influenza illness is considered to be an acute infection it is possible that clinicians do not consider it as a diagnosis in patients with chronic respiratory symptoms and it may be missed in patients presenting with tuberculosis or suspected tuberculosis disease during the influenza season. Tuberculosis, which is considered to be a chronic condition, may also be overlooked in patients presenting with acute respiratory illness.

Understanding the interaction of influenza and tuberculosis coinfection, including the excess mortality associated with influenza infection among tuberculosis patients may provide supporting evidence for prioritising individuals with tuberculosis disease for influenza prevention and treatment interventions such as influenza vaccination and use of antivirals during the influenza season. Countries with influenza vaccination policies recommend influenza vaccination for patients at increased risk of severe

influenza-associated disease and advise influenza antiviral treatment for patients admitted with respiratory illness during the influenza season [86, 87]. However, both of these interventions are costly and may not be feasible or accessible to countries with a high burden of tuberculosis and influenza disease and countries with limited resources. Data on influenza–tuberculosis interaction from countries with high burden may assist policy makers with decision-making regarding prioritisation. Quantifying the excess mortality due to influenza illness among tuberculosis deaths may provide further evidence to support prioritising patients with tuberculosis disease for influenza vaccination and influenza antivirals. This is important for countries that are considering implementing influenza vaccine policy, especially countries with high burden of influenza disease, tuberculosis disease and HIV and with limited resources where the number targeted for vaccination is more than the available vaccine doses. In addition, a systematic review of published literature on the association between influenza illness and tuberculosis disease will identify the gaps in knowledge and direct future research in the field.

1.8. AIMS AND OBJECTIVES

This thesis aimed to assess the effect of influenza and tuberculosis coinfections on clinical presentation, progression of illness and disease outcome; and to evaluate the burden of excess mortality associated with influenza virus infection in patients with tuberculosis disease with the intent to guide policy makers in making decisions about providing influenza vaccination and /or to motivate for prioritisation of patients with tuberculosis disease for influenza vaccination.

Objectives:

1. To summarise the published literature on the interaction between influenza and tuberculosis disease and identify gaps (Paper I- submitted).

2. To describe the incidence of and factors associated with acute and chronic presentation of laboratory-confirmed pulmonary tuberculosis disease overall and by HIV serostatus among patients hospitalized with SRI in South Africa (paper II-Published).
3. To compare the epidemiological and clinical characteristics of patients admitted with laboratory-confirmed tuberculosis, influenza, and tuberculosis-influenza coinfection among HIV–infected and-uninfected patients hospitalized with severe respiratory illness in South Africa (paper III -published and paper IV – submitted).
4. To estimate the mortality burden associated with influenza infection among HIV-infected and HIV-uninfected individuals with pulmonary tuberculosis (PTB) and to estimate the risk of influenza-associated mortality among PTB deaths compared to non-tuberculosis respiratory deaths in South Africa (Paper V- published).

2. METHODS

2.1.STUDY DESIGN

This thesis consists of three separate components to address the different objectives; the first component consists of a systematic review of the literature to address objective 1. The second component includes analysis of data from a prospective surveillance study to address objectives 2 and 3; the last component includes analysis of time-series data from vital statistics and influenza virological surveillance to address objective 4. The choice of study design was influenced by the nature of the research question. A summary of the project objectives is outlined in Figure 2 and Table 1. Descriptions of study methods and statistical analyses are discussed in detail within the methods section of each paper (appendix C) however a brief description of methods which are relevant to this PhD, is provided for each paper in Table1 below.

Figure 2: Overview of study objectives and public health relevance

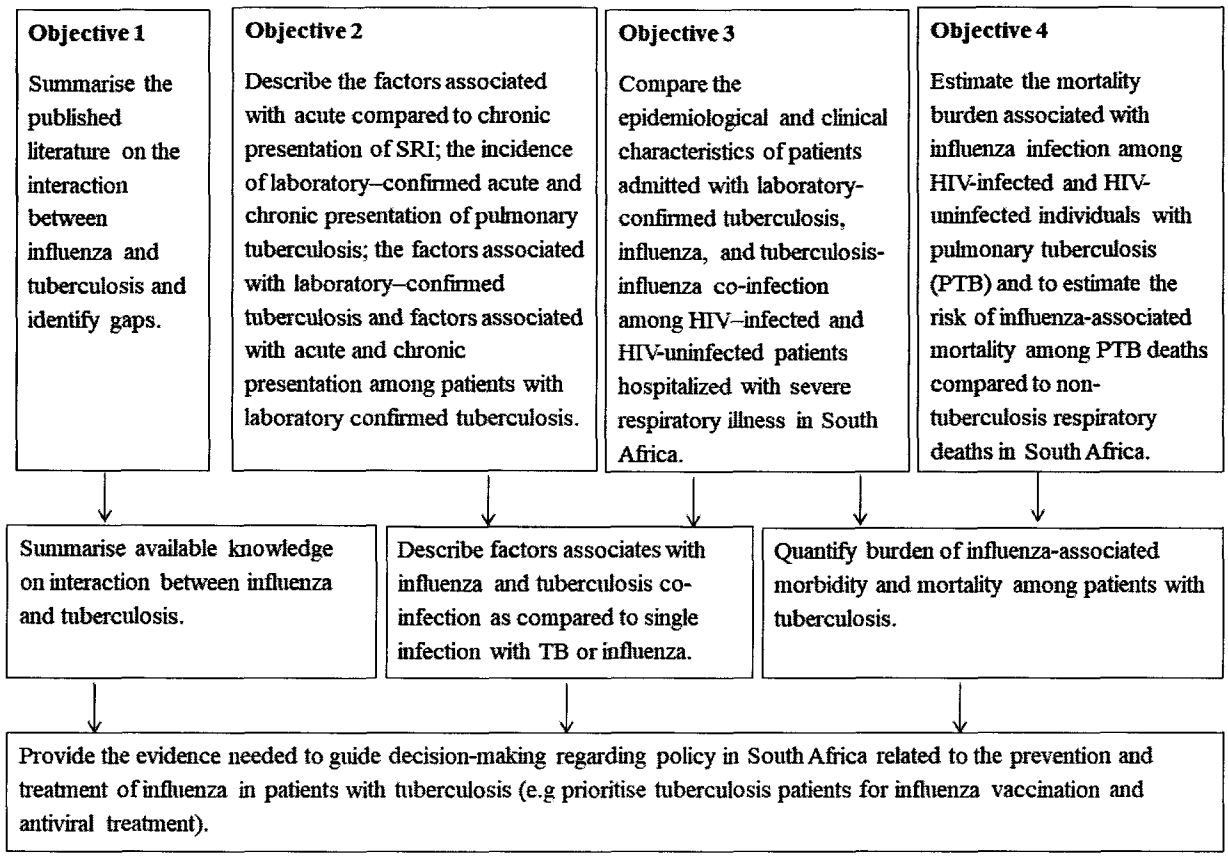


Table 1: Papers included in the PhD submission, objectives and a summary of methodologies

Manuscript Number	Title	Objectives	Study Design	Population (where applicable)	Summary of methodology used to achieve objectives
I	Influenza and tuberculosis coinfection	Summarise the published literature on the interaction between influenza and tuberculosis disease and identify gaps	Systematic review		Conducted a systematic review, followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) for reporting results. Newcastle-Ottawa Scale was used to rate the quality of included cohort or case control studies. Data synthesis consisted of reporting the key findings of the different studies.
II	Burden of tuberculosis in patients with severe respiratory Illness (SRI)	Among HIV-infected and uninfected persons aged ≥15 years hospitalized with SRI in South Africa during 2012–2014, we aimed to describe: (1) factors associated with laboratory-confirmed pulmonary tuberculosis among patients tested for tuberculosis; and (2) factors associated with acute and chronic presentation among patients with laboratory-confirmed tuberculosis disease.	Prospective hospital based surveillance	Individuals ≥ 15 years admitted with SRI at Edendale and Tshepong Hospital, 2012-2014	We calculated estimated incidences of acute and chronic tuberculosis-associated SRI hospitalization/100,000 population for Pietermaritzburg and Klerksdorp for 2013. We used data from sentinel surveillance combined with information from a healthcare utilisation survey at two sites. We implemented multivariable logistic regression model to assess factors associated with acute and chronic presentation among patients with laboratory-confirmed tuberculosis. Predictors: Demographic characteristics, underlying conditions, clinical presentation, viral and bacterial infection, in-hospital outcome Outcome: Duration of symptoms
III	Influenza virus infection is associated with increased risk of death among patients hospitalized with confirmed pulmonary tuberculosis in South Africa, 2010-2011	Describe the epidemiological and clinical characteristics of patients admitted with laboratory-confirmed tuberculosis, influenza, and tuberculosis-influenza coinfection among HIV–infected and -uninfected patients hospitalized with severe respiratory illness in South Africa	Prospective hospital-based surveillance	Individuals all ages hospitalized with SRI (acute and chronic) at 4 sites from June 2010 through December 2011	We implemented multinomial regression model to assess for factors associated with tuberculosis single infection compared to influenza single infection or tuberculosis-influenza coinfection. Predictors- Demographic characteristics, underlying conditions, clinical presentation, viral and bacterial infection, in- hospital management, in-hospital outcome Outcome: Influenza infection, tuberculosis infection and influenza-tuberculosis coinfection
IV	Interaction of influenza and tuberculosis and the impact on mortality among individuals aged ≥ 15 years hospitalised with severe respiratory illness in South Africa, 2010-2016	Among hospitalised patients with severe respiratory illness, determine whether influenza-tuberculosis coinfection was associated with increased mortality among any patients that tested influenza-positive and any patients that tested tuberculosis-positive separately.	Prospective hospital- based surveillance	Patients aged ≥15 years admitted with clinician diagnosis of LRTI or suspected tuberculosis at hospitals (SRI) at two sites- June 2010- May 2016	We implemented multivariable logistic models to assess the association between tuberculosis and influenza coinfection and mortality in two different populations: a. All individuals testing influenza virus positive (who also were tested for tuberculosis) Exposure: tuberculosis Outcome: in-hospital mortality b. All individuals testing tuberculosis positive (who also were tested for influenza) Exposure: influenza infection Outcome: in-hospital mortality
V	Excess mortality associated with influenza among tuberculosis deaths	Estimate the mortality burden associated with influenza infection among HIV-infected	Ecological study	Country-wide tuberculosis-associated deaths from vital statistic data	We modelled the excess influenza-associated mortality by applying Poisson regression models to monthly PTB and non-tuberculosis respiratory deaths from

	in South Africa, 1999-2009	and -uninfected individuals with pulmonary tuberculosis (PTB) and to estimate the risk of influenza-associated mortality among PTB patients compared to non-tuberculosis respiratory mortality in South Africa			vital statistics data, using laboratory-confirmed influenza as a covariate.

Systematic review- Objective I

We conducted a systematic review of published scientific literature from January 1900 to February 2015 to evaluate the prevalence, presentation and outcome of influenza-tuberculosis coinfection. For our search we included terms for influenza ("influenza" or "flu") and for tuberculosis ("tuberculosis" or "TB"). The Medline, CINAHL, Cochrane, Embase, PsycINFO, Web of Science, CAB Abstracts and Global Health databases were searched. The review was restricted to published abstracts and articles from January1900 to February 2015 that reported data on the association (burden of disease, transmission and severity) between laboratory-confirmed influenza disease and clinically diagnosed or laboratory-confirmed tuberculosis disease. Articles that included seasonal or pandemic influenza were also included. The search was restricted to articles published in English, French, Italian, German, Russian, Finnish, Japanese and Portuguese. Animal experimental studies, observational and analytic studies in humans were included. Studies that reported data on the association between laboratory-confirmed influenza disease and clinically diagnosed or laboratory-confirmed tuberculosis disease were included as part of the search. Due to scarcity of published data, descriptive studies, including studies without comparison groups were included. We followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines in reporting results[88]. Data synthesis consisted of reporting the key findings of the different studies. We did not implement a meta-analysis because the studies included had limited data on summary statistics. Because we only

included papers with laboratory-confirmed influenza disease, we excluded a number of reports, especially for the period covering the earlier influenza pandemics, when influenza disease was clinically diagnosed[63, 65, 89-91]. We used the Newcastle-Ottawa Scale to rate the quality of the included papers [92].

Surveillance for severe respiratory illness (objectives II and III)

The data for paper II, III and IV was obtained from an existing umbrella project run by the National Institute for Communicable Disease (NICD) entitled establishing surveillance for SARI in South Africa with a number of specific surveillance enhancements made to allow us to address our specific objectives. The aim of the umbrella protocol was to describe trends in numbers of SARI cases at sentinel surveillance sites and determine the relative contribution of influenza virus and other respiratory viruses (human metapneumovirus (HMPV), respiratory syncytial virus (RSV), parainfluenza virus 1, 2 and 3 (PIV1, 2, 3), and adenovirus (AV)) and *Streptococcus pneumoniae* to this disease presentation in a setting with a high prevalence of HIV. This prospective, hospital-based sentinel surveillance was initiated for SARI in 2009 at four hospitals in three provinces (Chris Hani Baragwanath Hospital in Gauteng Province, Mapulaneng and Matikwana Hospitals in Mpumalanga and Edendale Hospital in KwaZulu-Natal) and was expanded in 2010 to include patients enrolled from Klerksdorp-Tshepong Hospital Complex (KTHC) in North West. Persons hospitalised at the surveillance hospitals with acute respiratory illness (≤7days symptoms) who met predefined inclusion criteria (Table 3) had clinical data and specimens obtained for aetiology testing. During the 2010 influenza season, the SARI surveillance programme was enhanced at selected sentinel sites (KTHC and Edendale Hospitals) to expand the case definition for enrolment to include patients with duration of respiratory symptoms for >7 days and patients with suspected or confirmed tuberculosis i.e. any patient with severe respiratory illness (SRI). For the period from June 2010 to end May 2012, tuberculosis testing was not systematically performed, and was conducted as part of clinical management at the discretion of the attending clinician. In June 2012, an addendum to the SARI

protocol was implemented to include (i) active testing for tuberculosis of all patients meeting the criteria for SRI at the two enhanced surveillance sites (KTHC and Edendale Hospital) and (ii) the collection of an additional induced sputum specimen, and testing of this specimen for tuberculosis and additional respiratory pathogens (*P. jirovecii* and causes of atypical bacterial pneumonia).

Data collection

Data on demographics, clinical presentation, previous medical history, antiretroviral therapy for HIV-infected individuals, inpatient investigations, clinical management and in-hospital outcome were collected through structured interviews and record review. Data was collected on a standardised questionnaire. Hospital and intensive care unit (ICU) admission and collection of specimens for tuberculosis testing (before June 2012), CD4+ T-cell counts and bacterial culture were performed at the discretion of the attending-physician discretion.

Laboratory Methods

Respiratory specimens, including oropharyngeal and nasopharyngeal swabs for patients ≥5 years of age or nasopharyngeal aspirates for children <5 years of age, were collected and placed in a 4ml universal transport medium and transported at 4-8°C to NICD within 72 hours of collection. Respiratory samples were tested by multiplex reverse-transcription real-time polymerase chain reaction (PCR) for influenza virus and 8 other respiratory viruses [93]. The influenza A positive specimens were further subtyped to H1 and H3 using the CDC real-time RTPCR (rRT-PCR) protocol for detection and characterization of influenza virus [94]. From May 2009, whole blood samples were collected in EDTA-containing vacutainer tubes within 24 hours of hospital admission for the detection of *S. pneumoniae* and HIV infection. Whole blood specimens were tested for *S. pneumoniae lytA* gene by a quantitative real-time PCR[95].

Testing for tuberculosis

Testing for *Mycobacterium. tuberculosis* changed between 2009 and 2016. From February 2009 to May 2012, the source of tuberculosis status for all sites was from site testing only which was conducted based on clinician's discretion. From June 2012, systematic testing for *M tuberculosis* which was conducted as part of surveillance was initiated for KTHC and Edendale hospital. This included surveillance officers ensuring that enrolled patients from these sites had a sample tested for *M. tuberculosis* at the site (either as part of clinical management or as part of surveillance if sample was not requested by clinician) and an additional sample was collected and sent to NICD for testing. Microbiological investigation for *M. tuberculosis* at the hospital site laboratory was performed by smear microscopy, culture and/or PCR (Xpert® MTB/RIF). Specimens for tuberculosis testing were examined by light microscopy for the presence of acid fast bacilli (AFB). Tuberculosis culture was performed using the BACTEC MGIT automated culture system (Becton Dickinson, Franklin Lakes, New Jersey). Tuberculosis PCR was performed using the Xpert® MTB/RIF system (Cepheid, Sunnyvale, California, USA). The Xpert® MTB/RIF is an automated sample processing and nucleic acid amplification test for detection of *M. tuberculosis* and is able to detect resistance to rifampicin. At NICD, tuberculosis was identified by microscopy and culture. Smear microscopy of sputum samples was performed using fluorescent auramine-O staining for AFB. Culture was performed using liquid media with the Bactec MGIT 960 system (Becton Dickinson, New Jersey, USA). Positive cultures were identified as *M. tuberculosis* complex using Ziehl-Neelsen staining and MPT64 antigen testing (Becton Dickinson, New Jersey, USA)[96]. A list of and description of different of bacteriologic tests used for confirmation of tuberculosis is included in Table 2. For objectives 2 and 3 a case was considered tested for tuberculosis if it had at least one of microscopy, culture or PCR test done for *M. tuberculosis*. A laboratory-confirmed tuberculosis case was defined as an individual with a positive result for *M. tuberculosis* on microscopy, PCR or culture (or a combination of these) at either the local/site hospital or the NICD TB Laboratory. Tuberculosis patients who were already on treatment for ≤2 months (initiation phase of treatment) and those who still tested positive for *M. tuberculosis* at

current admission were included in the analysis. These patients were included because the tuberculosis infection could still affect the outcome.

Table 2: List and description of tests used for bacteriologic confirmation of tuberculosis during the study period

Test	Type	Description	Source of tuberculosis testing/results and period included
Smear microscopy	LED/ Fluorescent microscopy	• For detecting and monitoring response • Low sensitivity in patients with low bacillary load (HIV–infected and children) • No information on viability and susceptibility of bacilli • Cannot distinguish between *Mycobacterium tuberculosis* complex and non- tuberculosis mycobacteria • Recommended as 1st line diagnosis	• Part of clinical management-2009-2012 • Part of clinical management & systematic testing as part of surveillance-2012-2016
Culture	Liquid (MGIT)	• High sensitivity • Important for monitoring response to treatment for drug resistant *M. tuberculosis* • Results take long- weeks	• Part of clinical management-2009-2012 • Part of clinical management & systematic testing as part of surveillance-2012-2016
PCR-based assays	Xpert® MTB/RIF	• Rapid diagnostic test • Recommended as 1st line of diagnosis • Detects rifampicin resistance • High Sensitivity for rifampicin resistance • Reduced sensitivity in smear negative	• Part of clinical management-2010-2012 • Part of clinical management or systematic testing as part of surveillance-2012-2016

MGIT: Mycobacterium growth indicator

Determination of HIV status

HIV status was obtained from different data sources. Some patients had HIV testing conducted as part of clinical management. This included HIV enzyme-linked immunosorbent assay (ELISA) testing with confirmation by ELISA on a second specimen for patients ≥18 months of age and qualitative HIV PCR testing for confirmation of HIV-infection status in children <18 months of age. In addition, for consenting patients, linked anonymous HIV PCR testing for children less than 18 months of age or ELISA for patients ≥18 months of age was performed using a dried blood spot or whole blood specimen at the NICD-NHLS laboratory[97]. From 2015, linked anonymous testing at NICD was only conducted for patients who gave consent for testing but did not want to know their HIV status. For

patients who had HIV results from both site testing and from NICD, the NICD result was used if the results were discrepant.

Data Management

All clinical and epidemiologic data, laboratory testing and results were managed centrally at NICD. Data were double entered onto a MS Access database.

Case definitions

Here we present the case definitions for the umbrella surveillance programme which provided data for the 3 papers. The case definitions were modified over time; a summary is presented in Table 3. Initially, a case of SARI was defined as a hospitalised individual with onset of illness within 7 days of admission meeting age-specific clinical inclusion criteria (Case definition 1). From July 2010 cases with symptoms duration >7 days were included at enhanced sentinel sites (Edendale and KTHC) (Case definition 2). From 2014, the new World Health Organization (WHO) SARI case definition of fever and history of fever and cough of duration of ≤10 days was adopted in all sentinel sites (Case definition 3) [98] and cases with symptoms duration >10 days were included at enhanced sentinel sites (Case definition 4). From 2015 enrolment of SARI cases with symptoms duration ≥ 10 days was extended to all sites (Case definition 4). The different case definitions in use during the periods when the three studies that used the surveillance data were conducted are summarised in Table 4

Table 3: Case definitions used for enrolment in the main surveillance programme over time and sites where case definitions were applied, 2009-2016

Case definition number	Case Definition	Criteria	Period case definition used for surveillance	Site where case definition applicable
Case definition (CD1)	Severe Acute Respiratory Illness 1 (SARI 1) – Symptoms duration ≤7 days	**2 days - <3 months old**: Child hospitalised with diagnosis of suspected sepsis, or physician-diagnosed lower respiratory tract infection (LRTI) irrespective of signs and symptoms	Feb 2009-April 2014	All sites
		3 months - <5 years old: Hospitalised with physician-diagnosed LRTI including pneumonia, bronchiolitis, bronchitis and pleural effusion		
		≥ 5 years: Any individual hospitalised with acute LRTI with sudden onset of fever (≥38°C) AND cough or sore throat AND shortness of breath or difficulty breathing		
CD2	Severe chronic respiratory illness 1 (SCRI 1) Symptoms duration >7 days	Same criteria as for SARI1 above including cases with suspected tuberculosis and duration >7 days (two sites only)	June 2010-May 2014	Edendale and Klerksdorp Tshepong Hospital Complex (KTHC)
CD3	Severe Acute Respiratory Illness 2 (SARI 2)– Symptoms duration ≤10 days	**2 days - <3 months**: Child hospitalised with diagnosis of suspected sepsis, or physician-diagnosed LRTI irrespective of signs and symptoms	June 2014-to date	All sites
		3 months - <5 years old: Hospitalized with physician-diagnosed LRTI including bronchiolitis, pneumonia, bronchitis and pleural effusion		
		≥ 5 years old: Hospitalized with an acute lower respiratory tract infection with cough AND fever (≥38°C) or history of fever.		
CD4	Severe chronic respiratory illness 2 (SCRI 2) Symptoms duration >10 days	Same criteria as for SARI 2 above, duration of symptoms >10 days	Feb 2015 – to date	All sites, (Edendale & KTHC, chronic cases were included earlier)
		Same criteria as for SARI 2 above with duration of symptoms >10 days including cases with suspected tuberculosis and not meeting any of criteria above	June 2014- to date	(KTHC & Edendale)
CD5 (CD1+CD2+ CD3+CD4)	SRI=SARI1+SCRI + SARI2+SCRI2	Admission with a physician diagnosis of LRTI; e.g., pneumonia, bronchiolitis, bronchitis and pleural effusion, suspected or confirmed tuberculosis) irrespective of symptom duration	2010-2016	Edendale and KTHC

Table 4: Case definitions used for the papers that used surveillance data, period and sites included in the papers

Paper	Case Definition (CD)	Period included	Sites
II	CD1 and CD2 (SARI1 and SCRI1) (Table 2)	2012-2014	2 surveillance sites (Edendale Hospital and Tshepong Hospital)
III	CD1 and CD2 (SARI1 and SCRI1) (Table 2)	June 2010 - December 2011	4 surveillance sites (Chris Hani Baragwanath Hospital, Agincourt (Mapalaneng and Matikwana Hospitals), Edendale Hospital and KTHC)
IV	CD1+CD2+CD3+CD4 (SARI1+SCRI1+SARI2+SCRI2) (Table 2)	June 2010- May 2016	2 Hospitals (Edendale and Tshepong hospital)

SARI Severe Acute Respiratory Illness; SCRI Severe chronic respiratory illness KTHC- Klerksdorp Tshepong Hospital Complex

Using surveillance data to describe factors associated with tuberculosis and influenza co-infection - Objectives II and III

Surveillance data was used in three of the papers included in this PhD. Firstly, it was used to describe the burden of tuberculosis in individuals hospitalised with SRI, secondly to describe factors associated with tuberculosis positivity and factors associated with tuberculosis-influenza coinfection (tuberculosis-influenza coinfection as an outcome). Thirdly surveillance data was used to describe the association between tuberculosis and influenza coinfection and mortality (mortality was an outcome) and factors associated with tuberculosis single infection as compared to influenza single infection or tuberculosis-influenza coinfection. Methods used for these objectives are summarised in Table 1. Different periods were included in the different analyses (Table1 and 4) and for two of the papers we only included individuals aged ≥15 years. Surveillance data includes patients who sought

care and were hospitalised at the participating surveillance sites and only patients who gave consent for participation in the surveillance and among the patients who participated in surveillance only those who had a sample tested for tuberculosis were included for part of the analyses.

Excess mortality associated with influenza among tuberculosis deaths in South Africa, 1999-2009- Objective IV

We conducted an ecologic modelling study using previously described modelling approaches [21, 22]. For data sources, we used mortality data from Statistics South Africa coded according to the International Classification of Diseases, Tenth Revision (ICD-10)[99], obtained population denominators from Stats SA [100], obtained age- and year-specific data on HIV prevalence in the population and antiretroviral treatment (ART) coverage among HIV-infected individuals from the Actuarial Society of South Africa (ASSA) AIDS and Demographic model[101], annual number of tuberculosis cases from the National Strategic Plan for HIV, STI and TB: 2012 to 2016 [102] and influenza virologic data including types and subtypes was obtained from influenza-like-illness surveillance [35].

To estimate the influenza- associated mortality, we fitted age-specific Poisson regression models (with an identity link), to monthly deaths from vital statistics for South Africa [54, 103-105]. This approach is a well-described and accepted method for the indirect estimation of influenza-associated mortality for both respiratory (e.g pneumonia and influenza) and non-respiratory (e.g. myocardial infarction) causes of death [106]. In South Africa >70% of tuberculosis-associated deaths are in individuals who are coinfected with HIV [6], and HIV is a risk factor for influenza-associated mortality [18, 22, 52]. To investigate whether the rate of influenza-associated mortality among individuals dying of PTB is greater than expected simply because of underlying HIV infection, we compared the rate of influenza –associated mortality among PTB deaths to the rate of influenza-associated mortality among non-tuberculosis respiratory deaths in HIV-infected and-uninfected individuals. In addition, we estimated the rates of influenza-associated mortality among PTB and non-tuberculosis respiratory deaths in

individuals aged <65 and ≥65 years. In individuals aged ≥65 years we expected a minimal effect of HIV infection on influenza-associated mortality because of the relatively low population HIV prevalence in this age group (< 3% in individuals aged > 60 years in 2012) [107].

2.2.ETHICAL CONSIDERATIONS

Approval for the thesis was obtained from The Human Research Ethics Committee (Medical) of the University of Witwatersrand, approval number M140827 (Appendix B). In addition, approval for the SARI surveillance protocol, SARI addendum and pneumonia surveillance protocol which contributed data for objective 2 and 3 of the thesis was obtained separately from the ethics committees of the University of Witwatersrand (reference M081042 and M140824) and the University of KwaZulu-Natal's Biomedical Research Committee (reference BF157/08 and BE 494/14).

Objective 4 involved analysis of data available in the public domain and data which formed part of the influenza surveillance at NICD. The ethics for influenza surveillance was approved by the University of Witwatersrand (Wits) Ethics committee Ref M060449.

Written informed consent was obtained from a parent or guardian for individuals <18 years and from each participant >18 years enrolled as part of surveillance. In addition, an assent was obtained from individuals > 7 years and < 18 years (Objectives II & III).

3. RESULTS AND DISCUSSION

Detailed results for each objective are presented in full within the results section of each manuscript (Appendix C). Here I present the important findings and discuss the main themes arising from the results.

3.1.SUMMARY OF PUBLISHED LITERATURE ON INFLUENZA AND TUBERCUOSIS CO-INFECTION- A SYSTEMATIC REVIEW

Summary of results and comparison with other studies

Previous reports have suggested that individuals with tuberculosis were disproportionately affected during the pandemic influenza seasons [65, 90, 91, 108]. We conducted a systematic review of published scientific literature from January 1900 to February 2015 to evaluate the prevalence, presentation and outcome of influenza-tuberculosis coinfection. Our review suggested that there are very few high quality studies exploring the interaction between laboratory-confirmed influenza diseases and clinically diagnosed or laboratory- confirmed tuberculosis disease. We identified 16 published articles reporting on laboratory confirmed influenza disease and laboratory-confirmed or clinically diagnosed tuberculosis disease. Of these, 10 reported data from human studies and six were animal experimental studies. Six of the 10 human studies were more recent, included data from 2009 and later, and were from high tuberculosis burden countries (HBCs) that account for ~80% of the world's tuberculosis cases.

Tuberculosis disease in individuals with influenza illness (exposure is tuberculosis)

Of the six studies that reported on tuberculosis disease in individuals with influenza illness, five were from HBCs[67, 109-112] and three were analytical studies[110-112]. The three analytical studies from HBC, one of high quality[112] reported no association with severe disease among patients with influenza-

tuberculosis coinfection. A case control from Kenya reported that 6% (4/64) of influenza positive cases had tuberculosis compared to <1% (1/190) of neighbourhood-matched controls, unadjusted odds ratio 12 (95% CI 1.34-107.4), this was not significant on multivariate analysis [113]. Less than 1% (23/7180) of patients hospitalised for acute respiratory illness and enrolled in a study from Thailand were coinfected with influenza virus and *M. tuberculosis*. There were no deaths among the 23 cases with influenza-tuberculosis coinfection, whereas 17 (2.8%) deaths occurred among cases in whom only influenza was identified, p=0.1[111]. In a review of 19 cases with laboratory-confirmed influenza A(H1N1)pdm09 virus infection with respiratory failure admitted to an intensive care facility in South Africa, tuberculosis was present in 4/13 (30%) who died vs 0/6 (0%) who survived p=0.5 [110]. Two of the three descriptive studies reported a higher prevalence of tuberculosis disease in cases with severe influenza disease or influenza-associated deaths compared to the prevalence in the general population. However, no inferences could be made on the significance of the association as there were no comparison groups or data was not statistically evaluated[67, 109].

Influenza virus infection in individuals with tuberculosis disease (exposure is influenza)

Of the five papers that reported data on influenza infection in patients with tuberculosis disease [60, 111, 114-116], two were reported as analytical studies by authors[111, 114] and one had suitable data for analysis [115]. Of the three analytical studies, two studies showed no association between influenza infection and tuberculosis disease [111, 114]. A case control study from Indonesia investigating the putative association between tuberculosis disease and influenza virus infection reported no association between the development of clinically active tuberculosis and influenza virus infection as measured by serology using hemaglutinating inhibition (HI) assays in 111 cases with newly diagnosed tuberculosis and 111 community controls with no history of tuberculosis disease or clinical evidence of tuberculosis disease [114]. The proportion of individuals with influenza virus antibody titres ≥10 against influenza A(H3N2) and A(H1N1) viruses in patients with tuberculosis were similar to matched community controls; however, the antibody titre levels for influenza

A(H3N2) virus at time of tuberculosis diagnosis were significantly higher (1.7 times higher, p=0.002) in cases with tuberculosis compared to controls. In addition, the difference in titres between cases with advanced tuberculosis and their controls was significantly higher than in cases with mild to moderate tuberculosis and their controls [114]. Among 23 patients with concurrent *M. tuberculosis* and influenza virus infection from Thailand, none died, compared to 30 (4.7%) deaths among the individuals with only tuberculosis disease; however, this was not statistically significant, p=0.62[111]. In a case series during an influenza B epidemic in a Danish tuberculosis sanatorium, 13% (7/53) of individuals coinfected with influenza virus compared to 2% (3/142) of individuals with tuberculosis only developed tuberculosis complications which included radiological changes or sputum conversion (p=0.005)[115].

Two descriptive studies reported data from influenza outbreaks in sanatoria. Of these, a study from The Netherlands in 1967 among children admitted in a tuberculosis sanatorium with primary tuberculosis of the lungs and hilar lymph nodes reported an increased frequency (20%, 5/20) of developing secondary segmental pulmonary lesions, suggesting progression of tuberculosis disease following serologically diagnosed influenza virus infection (defined as greater than fourfold rise in anti-influenza virus antibody titres) [60]. The other study in a tuberculosis sanatorium in the United States, described the effect of superimposed viral infection on existing tuberculosis disease following an outbreak of the 1957 influenza A pandemic virus, in which two of 31 TB peadiatric cases with influenza virus infection had evidence of worsening of tuberculosis disease on chest radiograph[116].

Quality of human studies

Two of the five analytical studies (one study reporting on both the effect of influenza on tuberculosis and on effect of tuberculosis on influenza) were of high quality (Newcastle Score ≥7) as assessed by the Newcastle Ottawa score[112, 114]. Studies about tuberculosis and influenza virus coinfection were mostly descriptive, mostly included univariable analysis, and a causal relationship could not be demonstrated in any study. Some of the studies used clinical criteria for tuberculosis cases and

among the studies that included laboratory-confirmed tuberculosis, screening for tuberculosis was not done systematically. The review identified a lack of large epidemiological studies using laboratory-confirmed tuberculosis and influenza disease, studies from countries with a high burden of both tuberculosis and HIV, studies including important confounders, like HIV, in the interaction between influenza and tuberculosis disease. Other important areas which were not addressed by the studies reviewed included whether influenza infection is associated with reactivation of latent tuberculosis or not.

Summary from experimental animal models

In murine models, five of the six studies included in our systematic review suggested that influenza and tuberculosis coinfection affected disease progression, presentation or outcome[71, 117-120]. One study showed no effect[121]. All but one of these studies reported on the effect of influenza virus in mice infected with tuberculosis and one study reported on the effect of influenza virus in mice infected with tuberculosis and the effect of tuberculosis in mice infected with influenza virus. The reported effect ranged from tuberculin hypersensitivity in mice being temporarily suppressed following intranasal influenza virus challenge[120] to influenza enhancing susceptibility to tuberculosis disease [119], impairing mycobacterium control and/or decreasing host survival[71, 117-119]. Bernard et al. showed that coinfection compared to tuberculosis only resulted in 50-75% shorter survival time and a higher case-fatality rate in mice [71]. The one study that reported on effect of tuberculosis in mice with influenza showed that 5% of the mice infected with influenza alone died compared to 100% of the mice infected with both influenza and tuberculosis [71].

Summary

In summary, although animal experimental studies suggested an association, specifically that influenza-tuberculosis coinfection in mice resulted in more severe disease than influenza only or tuberculosis only disease, this was not found in four of the five analytical studies in humans. Three of

the five descriptive studies, although not assessed for statistical significance reported either increased frequency of coinfection in severe influenza- or tuberculosis- associated disease compared to prevalence in the community or to the individuals with single infection.

Generalisability and implications of results

Our review only included studies with laboratory-confirmed influenza illness and clinical (4), or laboratory- confirmed tuberculosis (4) or a combination of laboratory-confirmed or clinical (2), as a result this limited the number of studies we could include. The type of tuberculosis included in the papers reviewed differed among the studies, with some studies reporting newly diagnosed tuberculosis, some reporting on cases in tuberculosis sanatorium for a number of months, and some included cases who had completed tuberculosis treatment, thus making it difficult to compare the results of different studies. The numbers of participants in most studies included in the review were small and this could have limited the ability of included studies to detect significant associations. We did not include ecological studies because we wanted to evaluate associations at individual level. In addition, ecological studies did not include data on laboratory-confirmed influenza disease. Studies in Chinese were not included in the review, due to lack of translators, and therefore our review may not reflect the full body of literature on this topic. Lastly, we included case series, outbreak reports and studies that were only descriptive and descriptive studies cannot assess for an association or measure relative severity.

3.2. THE BURDEN AND CLINICAL PRESENTATION OF PULMONARY TUBERCULOSIS IN ADULTS WITH SEVERE RESPIRATORY ILLNESS IN A HIGH HIV PREVALENCE SETTING, 2012-2014

Summary of results and comparison with other papers looking at burden and clinical presentation of tuberculosis in patients admitted with lower respiratory tract illness

The burden of clinical influenza disease in patients admitted with severe respiratory illness in South Africa has been described [15, 18, 49, 51, 52, 122]. However, there is a gap in literature describing tuberculosis in hospitalised patients with SRI. In our study, we wanted to describe the burden and clinical presentation of tuberculosis in this group of patients. We implemented systematic testing for tuberculosis among patients admitted with SRI and showed that the prevalence of tuberculosis was high in patients aged ≥ 15 years hospitalized with SRI irrespective of duration of symptoms, detection rate for laboratory-confirmed tuberculosis, was 28% overall; 18% (133/729) in individuals with acute (≤14 days) and 34% (460/1,368) in individuals with chronic (>14 days) presentation, respectively ($p<0.001$). The finding that tuberculosis was common in patients admitted with pneumonia is similar to other studies that reported a high prevalence of tuberculosis in patients admitted with pneumonia though the prevalence varied between the different studies depending on whether the studies included children, individuals who were immunocompromised or all age groups [78, 123-126]. In a prospective study including 346 non-imunocompromised individuals aged ≥12 years admitted for community acquired pneumonia (CAP) in Indonesia, aetiology was identified in 47% of cases and 4.7% were positive for *M. tuberculosis.* [123]. In a study from Kenya, which investigated 281 adults ≥15 years hospitalised with acute radiologically confirmed pneumonia, aetiology was defined in 65% (182/281), 9% (26) were positive for *M. tuberculosis* [126]. Post-mortem studies have also reported a high prevalence (32%-69%) of tuberculosis in patients dying of respiratory illness or any medical condition, in some cases where tuberculosis was not suspected [127-130]. Unlike in our

study, most of these studies did not differentiate between cases with acute and chronic presentation with one study only including cases with acute presentation, and mostly included HIV-infected cases [126].

Similar to other studies, we showed that tuberculosis among patients admitted with SRI was more commonly identified in individuals with symptoms >14 days[123]. Furthermore, our study showed that clinical presentation differed between individuals with laboratory-confirmed tuberculosis with acute compared to chronic presentation. Compared to patients with a chronic presentation, laboratory-confirmed tuberculosis cases with acute presentation were less likely to present with classical tuberculosis symptoms (cough adjusted odds ratio ((aOR) 0.2, 95%CI 0.1-0.5) or night sweats (aOR 0.4, 95%CI 0.3-0.7)). In addition, they were less likely to be started on tuberculosis treatment by the attending clinician during the admission compared to those with chronic presentation (aOR 0.4, 95%CI 0.3-0.7). The atypical presentation of patients with acute symptoms may have implications for tuberculosis control in that these patients may be missed by tuberculosis screening guidelines and the clinicians may have a low index of suspicion for testing these patients for tuberculosis. It is important to note that although the clinical presentation was different, mortality was not significantly different between laboratory-confirmed tuberculosis patients with acute (8%, 11/131) and chronic (13%, 56/447) presentation.

While the likelihood of tuberculosis disease was higher if symptom duration was >14 days, additional numbers of cases will be identified if laboratory testing for tuberculosis is conducted for adults with SRI irrespective of symptom duration or HIV status. Among the laboratory-confirmed tuberculosis cases in our study, 22% (133) and 78% (460) had an acute and chronic presentation respectively. In addition, among HIV-uninfected laboratory-confirmed cases, 21% (21/98) had an acute presentation. The aetiology study from Kenya described earlier, which reported a tuberculosis prevalence of 9% in patients admitted with pneumonia, only included patients with symptoms ≤ 14 days. [126] .

We also showed that coinfections with respiratory viruses were common, 29% of tuberculosis confirmed cases were infected with a respiratory virus. The common viral coinfection was rhinovirus (16%), followed by adenovirus (7%), RSV (3%), human metapneumovirus (2%), enterovirus, parainfluenza1&3 (1% each) and parainfluenza 2 (<1%). In addition, 4% of tuberculosis cases were coinfected with influenza virus, 7% and 3% among those with acute and chronic presentation respectively. In a Thailand study which only included cases with acute presentation <1% of cases were co-infected with influenza virus and *M. tuberculosis* [111].

Incidence of hospitalization for tuberculosis–associated SRI

We showed that the overall incidence of hospitalized tuberculosis-associated SRI irrespective of symptom duration was high (144/100,000 population; 95% CI 131-158). The annual incidence of tuberculosis-associated SRI in individuals hospitalized with acute presentation was 28 per 100,000 population (95% CI 22-39), increased with increasing age and was highest in individuals ≥65 years (94 cases per 100,000 population, 95% CI 54-153). The incidence of tuberculosis-associated SRI in individuals with chronic presentation was 116 per 100,000 population per annum (95% CI 104-128) and was highest in individuals aged 25-44 years (145 cases per 100,000 population, 95% CI 125-166).

HIV prevalence in our study was high, 81% (424/522) among laboratory- confirmed tuberculosis and it was similar in those with acute (83%, 102/123) and chronic (81%, 322/399, p=0.51) presentation. The high HIV prevalence limited our ability to assess whether HIV affected the clinical characteristics of patient with acute and chronic presentation. In addition, in our setting with such a high prevalence of HIV in patients admitted with severe respiratory illness (76% in our study), it may be advisable to use the same tuberculosis screening guidelines for all patients hospitalised with SRI regardless of HIV status.

Generalisability, limitations and implications of results

In our study, we included known tuberculosis cases during the initiation period of therapy, as we thought that these patients had active tuberculosis and tuberculosis could affect their outcome. However, this may have led to underestimation of mortality in patients classified as having tuberculosis, in that patients already on treatment may be less likely to die during current admission. On the other hand, we may have classified cases as not having tuberculosis if they tested negative for tuberculosis although they may still be experiencing the complications of tuberculosis. We only included patients aged ≥15 years and therefore cannot comment on acute and chronic presentation of tuberculosis in children. Our study was conducted in high HIV prevalence setting and may not be generalisable to settings with low HIV prevalence.

Gap filled by our study and implications following our study

Because we implemented systematic testing for tuberculosis, we had sufficient numbers of individuals with laboratory confirmed tuberculosis (593) and were able to describe the factors associated with acute compared to chronic presentation of tuberculosis. To our knowledge no other study has conducted systematic testing for tuberculosis in patients hospitalised with SRI and addressed this question. Our study allowed us to describe the incidence and clinical presentation of individuals with acute and chronic symptoms and hospitalised with laboratory confirmed tuberculosis. Although HIV prevalence was generally high in patients included in our study, 21% of HIV-uninfected patients with laboratory-confirmed tuberculosis had an acute presentation, this is an important group of patients to highlight as these would have been missed by the South African tuberculosis testing guidelines which recommend only testing HIV-uninfected patients with symptom duration >14 days for tuberculosis. Understanding the burden and spectrum of presentation of tuberculosis and the possibility of identifying early tuberculosis may serve as motivation for clinicians to test patients admitted with SRI for tuberculosis. It may also motivate for extension of policy on intensified case finding to include all hospitalised pneumonia cases. Tuberculosis should be

considered in a differential diagnosis of patients hospitalised with SRI irrespective of the duration of symptoms in high HIV and tuberculosis burden settings such as South Africa.

Remaining gaps

Our study reported the burden and incidence of tuberculosis in individuals ≥15 years, similar studies in children will be useful to assess whether a similar pattern is present among children.

3.3. INCREASED RISK OF MORTALITY AMONG PATIENTS ADMITTED WITH SEVERE RESPIRATORY ILLNESS WHO ARE COINFECTED WITH INFLUENZA VIRUS AND *M.TUBERCULOSIS* COMPARED TO PATIENTS WITH SINGLE INFLUENZA OR TUBERCULOSIS INFECTION

Summary of results and comparison with other studies

To answer this question, we conducted two studies. The first study included patients of all age groups and tuberculosis testing was only conducted as part of clinical care. The second study was conducted two years later and included only individuals aged ≥15 years and we performed systematic testing for tuberculosis for the majority of the period included in the study. The cut-off used for acute presentation in the 1st study was ≤14 days based on guidelines for tuberculosis testing for HIV negative patients. This is a more relevant cut-off for clinicians, which is also used in health promotion messages to encourage patients to seek health care and testing for tuberculosis. For the second study, acute presentation was defined as ≤ 7 days based on the WHO SARI case definition at the time. In the first paper, which relied on tuberculosis testing performed as part of clinical care, overall, among patients with available influenza virus results, only 2959 (38%) were tested for tuberculosis during the current admission for severe respiratory illness. Clinicians were more likely to test patients with a chronic presentation, symptom duration ≥7 days (aOR 1.3, 95%CI 1.1-1.3), patients ≥5 years, especially those aged 25-44 years (aOR 3.5, 95%CI3.0-4.2) as compared to <5 years and HIV infected individuals (aOR 1.5, 95%CI1.3-1.7). The prevalence of tuberculosis in this group of patients was 15% (457), with highest proportion testing positive for tuberculosis in 25-44 year age group. One percent (34/2959) of cases was coinfected with influenza virus and *M. tuberculosis*. The prevalence of coinfection was slightly higher than that reported in a retrospective

study from Thailand (2003-2011) using surveillance data, <1% (23/7180) of cases with acute respiratory illness was co-infected. Similarly in a case series of patients positive for influenza A(H1N1)pdm09 admitted in two hospitals in South Korea from May 2009 through May 2011 <1% (7/12196, 0.06%) were infected with newly diagnosed tuberculosis [68]. Similar to our study, the study from Thailand tested patients who met criteria for severe acute respiratory infection for influenza virus and did not systematically test for *M. tuberculosis* therefore may have missed patients with tuberculosis. In the study from Thailand only 28% of enrolled cases were tested for tuberculosis[111].

In contrast to previous published studies, our study included patients with a more chronic presentation and therefore allowed us to look at the contribution of influenza illness in tuberculosis patients with acute and chronic presentation separately. Among patients with symptoms ≥7 days, patients co-infected with influenza and tuberculosis as compared to patients with tuberculosis only were at increased risk of death (aRRR 5.5, 95% CI 1.2-25.3). This association was only seen in those with chronic presentation. The study from Thailand, which did not show increased risk of mortality in patients co-infected with influenza virus and *M. tuberculosis* compared to influenza only and tuberculosis only cases, only included patients with acute presentation[111]. In a case control study from Kenya examining risk factors for seasonal influenza hospitalization among individuals ≥5 years admitted with respiratory illness, active tuberculosis was associated with influenza hospitalization on bivariate analysis; however, this was not significant on multivariable analysis. Similar to the Thailand study, this study included only patients with acute presentation[112].

Other studies, mostly outbreak reports and case series, which presented individual level data on influenza and tuberculosis disease [67, 109] also suggested increased prevalence of tuberculosis in patients with severe influenza-associated illness (hospitalization or mortality) during influenza

pandemics. These were limited in that they did not have comparison groups and therefore could not compare tuberculosis or influenza single infection to co-infected individuals.

Generalisability, limitations and implications of results

Our study included a mixed group of patients including both children and adults for whom there are substantial differences in diagnosis and presentation. Some sites only recruited cases with acute presentation and other sites included cases with acute and chronic presentation and we did not systematically test patients admitted with SRI for tuberculosis. The differential enrolment criteria and testing procedures for tuberculosis across different participating sites may have introduced selection bias. In addition, tuberculosis cases were defined based on laboratory confirmation and we may have underestimated the prevalence and misclassified cases as not tuberculosis if the result from a sample that was tested was negative. Specific situations where the sensitivity of laboratory confirmation is low include children and HIV-infected individuals where tuberculosis tends to be paucibacillary and smear negative. The study period was18 months which included 2 influenza seasons. The differences in morbidity and mortality between coinfected patients and TB single infection could be due to cases presenting at different times since we did not control for seasonality.

Testing all patients admitted with severe respiratory illness for *M. tuberculosis* may identify more cases of tuberculosis, influenza and cases co-infected with influenza virus and *M. tuberculosis* which in turn may enable us to evaluate specific interactions in the influenza and tuberculosis infected groups separately. Based on the findings from this paper that despite a high HIV prevalence (~70%), very few patients (38%) were tested for *M. tuberculosis* and that influenza virus and *M. tuberculosis* coinfection was associated with increased risk of death among patients presenting with a more chronic presentation we wanted to explore this further. The low prevalence of tuberculosis testing and high HIV prevalence is an important result because based on the national guidelines for tuberculosis testing, active tuberculosis screening for HIV-infected individuals should be

implemented. We instituted systematic tuberculosis testing from June 2012 as part of surveillance; results using these data are presented in paper IV and are summarised below. In this paper (paper IV), because of the increased available sample size we were able to assess factors associated with death among influenza virus positive cases and among *M. tuberculosis* cases separately. From June 2010 to May 2016, 6228 individuals aged ≥15 years were enrolled from the two enhanced sites that included cases with both acute and chronic presentation. Of these, 68% (4253/6228) had results for influenza and tuberculosis of which 6% (239/4253) were positive for influenza virus and 26% (1092/4253) were positive for *M. tuberculosis*. Of these, 25% (1050/4253) were positive for *M. tuberculosis* only, 5% (197/4253) for influenza virus only and 1% (42/4253) of individuals were coinfected with both influenza virus and *M. tuberculosis*. HIV prevalence was high, 76% (4445/5853); 69% (213/308) among influenza virus positive cases and 83% (869/1043) among *M. tuberculosis* positive cases. Similar to the first paper, our study showed that influenza-tuberculosis coinfection was associated with increased risk of death as compared to either influenza only or tuberculosis only cases.

Effect of tuberculosis on influenza

On multivariable analysis, controlling for age group, HIV status and symptom duration, laboratory-confirmed tuberculosis was associated with an increased risk of death in hospitalised patients who tested positive for influenza virus [(8/42 (19%) vs 10/190 (5%)); aOR 4.5, 95% CI 1.5-13.3]. Case series and ecological studies from influenza pandemics suggested that tuberculosis disease predisposed individuals with influenza disease to severe outcomes [62, 67, 109, 110]. As mentioned earlier, these studies were mostly descriptive and tuberculosis prevalence among cases with severe illness or outcomes was compared to the prevalence of *M. tuberculosis* in the general population to infer an association. In addition, in some of the case series tuberculosis diagnosis was not detailed and it was not possible to establish whether tuberculosis was active or not and whether the patients

were on treatment for tuberculosis disease or not [67, 109, 110]. Co-infection with other respiratory viruses was not significantly associated with increased mortality OR1.6 (95% CI 0.4-5.6).

Effect of influenza on tuberculosis

Among hospitalised individuals with laboratory-confirmed tuberculosis disease, factors independently associated with death were age group ≥65 years compared to 15-24 years (aOR 3.6, 95%CI 1.2-11.0) and influenza coinfection (aOR 2.3, 95% CI 1.02-5.2). Patients with longer duration of symptoms compared to those with more acute presentation were at increased risk of tuberculosis-associated death, although this was not statistically significant. As summarised under the systematic review, case series and outbreak reports from tuberculosis sanatoria reported mixed results [67, 109, 114, 115]. Murine studies also suggested that influenza virus infection of mice with tuberculosis reduced long term survival[71, 117, 118]. Bernard et al. showed that the amount of tissue damage among tuberculosis-influenza coinfected mice, increased with increasing time of tuberculosis infection prior to the influenza challenge [71]. Co-infection with other respiratory viruses was not associated with increased mortality, unadjusted odds ratio 0.7 (95% CI 0.4-1.1).

Generalisability, limitations and implications of the results

Patients enrolled in surveillance programmes may not be reflective of patients with respiratory illness who presented at outpatient departments or those who died at home. We were only able to test 69% of patients for *M. tuberculosis*. We may have missed patients with more severe illness who died before *M. tuberculosis* testing or were too ill to expectorate as patients who were tested compared to those not tested were less likely to die during current admission. This could have led to underestimation of case-fatality ratios. HIV-infected individuals made up 73% of participants in our

study. It is known that HIV-infected individuals may be less likely to excrete tuberculosis bacilli and therefore be less likely to have laboratory-confirmed tuberculosis, especially if only tested on microscopy [131-133]. For this reason, we may have misclassified some individuals (particularly those who were HIV infected) as not having tuberculosis following a negative microbiologic result, leading to potential underestimation of association with severe outcome in patients who are coinfected. Although our study used surveillance data, it included sites from different sociodemographic areas, including rural, urban and periurban areas, whereas the other published studies were either restricted to rural or urban areas [67, 109, 111].

Gaps filled by the two studies

We described the prevalence of tuberculosis influenza coinfection and factors associated with coinfection in a high HIV and tuberculosis prevalence setting. Because of the relatively large numbers of patients enrolled in our study and the implementation of systematic tuberculosis testing for part of the study, we were able to assess factors independently associated with death in individuals with laboratory confirmed tuberculosis disease and influenza-associated disease separately and none of the other studies were able to do this. In our studies we tested for other viruses and the associations with increased mortality was only seen with influenza virus and *M. tuberculosis* and not with other viruses.

Remaining gaps and considerations following the two studies on mortality among patients admitted with severe respiratory illness who are co-infected with influenza and tuberculosis

Based on the results from the first paper, South African guidelines on influenza were updated to include tuberculosis disease as a specific risk group for severe influenza disease [134]. Although the WHO advised influenza vaccination for tuberculosis patients during the 2009 influenza pandemic, none of the other influenza guidelines include patients with tuberculosis disease as a group to be

targeted for influenza vaccination. There is limited published data on the effectiveness or efficacy of influenza vaccination in patients with tuberculosis, according to our knowledge none among those co-infected with HIV. Studies of vaccine efficacy in this group of individuals should be implemented. We used the surveillance data, with minimum modifications to existing surveillance, to answer the question on interaction between influenza and tuberculosis. A longitudinal cohort study of newly diagnosed tuberculosis patients would have been better suited to provide good data to infer a causal relationship between coinfection and severe outcomes. However, it would have taken a long time to enrol an adequate sample size and to complete a sufficient follow-up period to look at outcomes of interest. Further work that would strengthen these findings include looking at risk of being hospitalised if a tuberculosis patient is infected with influenza and assessing additional influenza morbidity trough hospitalisations borne by PTB patients.

3.4.EXCESS MORTALITY ASSOCIATED WITH INFLUENZA AMONG TUBERCULOSIS DEATHS IN SOUTH AFRICA, 1999-2009

Summary of results and comparison with other studies

In this study we aimed to estimate the influenza-associated mortality among individuals with PTB in South Africa from 1999–2009. To investigate if there was an increased risk of mortality associated with influenza virus infection in patients with tuberculosis compared to other groups, we estimated the rate of influenza-associated mortality among PTB deaths and compared it to the rate of influenza-associated mortality among non-tuberculosis respiratory deaths.

PTB deaths were highly seasonal, increased each winter, coinciding with influenza virus circulation. This is similar to the data from Switzerland (2016) which showed peaks in PTB mortality coinciding with or slightly after the 1889 and 1918 influenza pandemics[135]. Non-tuberculosis respiratory deaths

were also highly seasonal. In our study, an increased risk of influenza-associated mortality in persons with PTB compared to non-tuberculosis respiratory deaths was observed. Among individuals <65 years of age the mean annual influenza-associated mortality rates per 100,000 person-years were 141 (n = 375) among PTB deaths, 27 (n = 1125) among HIV infected non-tuberculosis respiratory deaths, and 2 (n = 937) among HIV-uninfected non tuberculosis respiratory deaths. In this age group, the risk of influenza-associated mortality was greater among PTB deaths compared to HIV-infected non-tuberculosis respiratory deaths (relative risk (RR): 5.2; 95% CI: 4.6–5.9) and HIV-uninfected non-tuberculosis respiratory deaths (RR: 61.0; CI: 41.4–91.0). The estimated mortality rate associated with influenza virus infection was greater among PTB deaths compared to non-tuberculosis respiratory deaths also among individuals aged ≥65 years (RR: 13.0; 95%CI: 12.0-14.0); an age group where the population HIV prevalence is low. Taken together, these results suggest that the increased risk of influenza-associated death among PTB deaths was not only related to underlying HIV-infection. In our setting, there is a high prevalence of HIV in individuals with tuberculosis[10] and HIV is an important risk factor for both tuberculosis - and influenza –associated mortality[18, 19, 52]. Even with that background, as mentioned earlier, our study found an increased risk of influenza-associated mortality among PTB compared to non-tuberculosis respiratory deaths irrespective of HIV status suggesting that PTB may be an independent risk factor for increased influenza-associated mortality.

The findings of increased risk of influenza-associated mortality in patients with PTB are consistent with ecological data prior to the HIV era, where elevated influenza-related mortality was reported among tuberculosis-infected patients during the 1918 influenza pandemic [65, 108, 135, 136] and the 1889 influenza pandemic [135]. These authors hypothesised that influenza pandemics contributed to the decline of tuberculosis in later years by decreasing the pool of potential transmitters by wiping out cases with tuberculosis (harvesting effect).[65, 90, 108, 136]. A study from Switzerland (2016) using historic data from the 1889 and the1918 influenza pandemics used Poisson regression model to

quantify the excess pulmonary PTB mortality attributable to influenza disease. This study reported that for an increase of 100 influenza deaths (per 100,000 population) monthly PTB mortality rates increased by a factor of 2.0 (95%CI 1.8–2.2, p<0.001) and 1.5 (95%CI 1.1–1.9, p = 0.004) during the 1889 and 1918 influenza pandemic, respectively [135]. Similar to our study, this study did not observe any excess mortality in comparison groups, cancer or extrapulmonary TB mortality. The other studies modelled excess tuberculosis mortality above baseline and did not compare excess mortality to other conditions [63, 108]. Oei et al. (2012) using data from USA, Japan and Netherlands, estimated the age, period and cohort effect of tuberculosis mortality, which showed a spike in tuberculosis mortality during the 1918 pandemic followed by a significant steeper decline in TB mortality than what was observed before the pandemic, suggesting a possible harvesting effect[108]. Housworth and Langmuir (1974) reported excess tuberculosis mortality during intense influenza A epidemics compared to milder influenza B seasons [63]. However Bradshaw et al. (2008), suggested that the excess in tuberculosis mortality among males mortality during the 1918 pandemic was more likely due to war time mobilization rather than influenza illness,[137]. These studies included data from the pandemic influenza whereas our study included seasonal influenza and in addition covered a period when HIV was prevalent. During the period covered by the other studies, influenza illness was clinically diagnosed and there was limited laboratory testing for *M. tuberculosis*. It is possible that tuberculosis cases presenting acutely were misclassified as pneumonia and that influenza cases presenting early in the pandemic were missed.

Gap filled by our study

We described an increased risk of influenza-associated mortality in patients with PTB in a high HIV and tuberculosis setting. The other studies on excess tuberculosis mortality mostly covered pandemic influenza years [65, 90, 108, 135, 136]. Our study complemented these studies in that it included data on seasonal influenza from a high tuberculosis burden country during a period when HIV was prevalent.

In addition, our model stratified the analysis by HIV in order to investigate whether the rate of influenza-associated mortality among PTB deaths was as a result of underlying HIV.

Generalisability and implications for interpreting the results

Because of poor recording of HIV infection on the death register in South Africa [138], indirect methods were utilised to assess the influenza-associated mortality burden among HIV-infected and-uninfected non-tuberculosis respiratory deaths. For PTB and non-tuberculosis respiratory deaths we relied on the diagnosis based on the death certificates with possible misclassification of cause of death. However, this is unlikely to be sufficient to account for the observed increases. Previous studies from South Africa have shown that there is no winter increase in mortality for "control" diagnoses such as cancer for which no association with influenza disease has been demonstrated[15]. Our modelling approach assumed that all excess deaths occurring above the model baseline were attributable to influenza. Influenza-associated mortality could in part be associated with other infections such as *Streptococcus pneumoniae* that have been shown to interact with influenza leading to severe outcomes[139]. However, this effect would be expected to occur among non-tuberculosis respiratory and PTB cases alike. Denominators for the number of individuals with PTB were obtained from national published estimates [102]. Some cases of PTB may not have been recorded on the national register and this could have led to our denominators of numbers of individuals with PTB being too low which would have led to an overestimation of rates of death in individuals with tuberculosis. However, similar estimates of tuberculosis case numbers in the South African population have been found using estimates extrapolated from laboratory-confirmed cases [140]. We were not powered to estimate excess influenza mortality in more refined age groups, owing to the low prevalence (and associated mortality) of tuberculosis in individuals <15years. Among PTB deaths, we could not assess the influenza-associated mortality by HIV status because in our setting the vast majority of tuberculosis patients are positive for HIV and we could not disaggregate the effect of ART and improved TB detection and treatment in this group.

Remaining gaps following our study

We did not have individual level data and were not able to further explore whether the PTB deaths which we observed were in individuals with chronic underlying tuberculosis who experienced an acute exacerbation as a result of superimposed influenza infection.

4. CONCLUSION

Before the data from this PhD, there was limited data on the association between influenza and tuberculosis from analytic studies. Through the work of this PhD I showed that in our setting with high HIV and tuberculosis burden, tuberculosis was common in patients hospitalised for SRI irrespective of symptom duration and that at four surveillance sites clinicians were unlikely to test patients with acute presentation for tuberculosis and therefore tuberculosis maybe missed during influenza season. I also showed that influenza was an independent risk factor for tuberculosis-associated deaths and that tuberculosis was an independent risk factor for influenza-associated deaths. Lastly, I showed increased risk of influenza-associated mortality in persons with PTB compared to non-tuberculosis respiratory deaths in HIV-infected and–uninfected individuals suggesting that the increased risk of death among PTB deaths was independent of HIV. The analyses presented in this submission support the hypothesis from ecological studies that influenza-tuberculosis coinfection results in poor outcomes. These findings may support recommendations for active inclusion of patients with tuberculosis for influenza vaccination (in addition to existing recommendations for vaccination of HIV-infected individuals) and consideration for empiric anti-viral treatment for influenza among patients with tuberculosis hospitalised with acute respiratory symptoms during influenza epidemics. These studies also reinforce the importance of maintaining a high index of suspicion for tuberculosis in SRI patients presenting with acute symptoms. Randomized controlled studies provide the strongest epidemiologic study design for establishing a causal relationship however in the absence of these, evidence from large observational studies does play an important role. Vaccine trials may be needed to assess efficacy of influenza vaccination strategies on outcome and mortality in individuals with tuberculosis in high burden countries.

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7. APPENDICES

Appendix A- Role of the student

Appendix B- Ethical Clearance

Appendix C- Copies of manuscripts making up PhD

Paper I

Paper II

Paper III

Paper IV

Paper V

APPENDIX A: ROLE OF STUDENT

Roles performed by the student in the different papers making up the PHD

Sibongile Walaza, student number 846084/2, had the following roles in the studies and writing of the papers forming part of her thesis entitled: "Interaction between influenza and tuberculosis in South Africa, a country with high prevalence of tuberculosis and HIV"

Paper I and Paper V

Paper I: **Sibongile Walaza**, Cheryl Cohen, Stefano Tempia, Jocelyn Moyes, Athermon Nguweneza, Shabir Madhi; Meredith McMorrow, Adam L. Cohen. **Influenza and tuberculosis co-infection: a systematic review.** Full draft

Paper V: **Sibongile Walaza**, Cheryl Cohen, Ananta Nanoo, Adam L. Cohen, Jo McAnerney, Claire von Mollendorf, Jocelyn Moyes, Stefano Tempia. **Excess mortality associated with influenza among tuberculosis deaths in South Africa, 1999-2009.** PlosOne. DOI 10. 1371/journal.pone 0129173

Role of student: Conceived the studies, obtained data, conducted the analyses, drafted and revised the drafts of the manuscripts.

Paper II-IV

Paper II: **Sibongile Walaza**, Stefano Tempia, Andries Dreyer, Halima Dawood, Ebrahim Variava, Neil A Martinson MD, Jocelyn Moyes, Adam L. Cohen, Nicole Wolter, Claire von Mollendorf, Anne von Gottberg, Sumayya Haffejee, Florette Treurnicht, Orienka Hellferscee, Nazir Ismail, Cheryl Cohen. **The burden and clinical presentation of pulmonary tuberculosis in adults with severe respiratory illness in a high HIV prevalence setting, 2012-2014.** Open Forum Infectious Diseases, Volume 4,Issues 3, 1 July 2017,ofx116, https://doi.org/10.1093/ofid/ofx116

Paper III: **Sibongile Walaza**, Stefano Tempia, Halima Dawood, Ebrahim Variava, Jocelyn Moyes, Adam L. Cohen, Nicole Wolter, Michelle Groome, Claire von Mollendorf, Kathleen Kahn, Marthi Pretorius, Marietjie Venter, Shabir A. Madhi, Cheryl Cohen. **Influenza virus infection is associated with increased risk of death amongst patients hospitalized with confirmed pulmonary tuberculosis in South Africa, 2010-2011.** BMC Infectious Diseases (2015) 15.26; DOI: 10.1186/s12879-015-0746-x

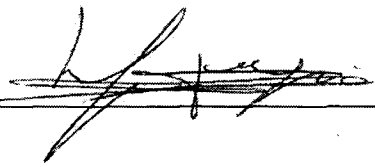
Paper IV: **Sibongile Walaza**, Stefano Tempia, Halima Dawood, Ebrahim Variava, Neil A Martinson, Jocelyn Moyes, Meredith McMorrow, Nicole Wolter, Andries Dreyer, Claire von Mollendorf, Anne von Gottberg, Sumayya Haffejee, Florette Treurnicht, Orienka Hellferscee, Shabir Madhi, Nazir Ismail, Cheryl Cohen. **Interaction of influenza and tuberculosis on mortality among individuals aged ≥ 15 years hospitalised with severe respiratory illness in South Africa, 2010-2016** – Full draft undergoing CDC clearance

Role of student: Conceived the studies, data collection, collation and cleaning, conducted the analyses, drafted and revised the drafts of the manuscripts. In addition to the above, the student was responsible for overall management of all aspects of the surveillance programme from which the data were derived. Compiled the addendum and amended the existing severe acute respiratory illness (SARI) surveillance protocol for the collection of the data relevant to these studies. This included, primarily, the expansion of the study population to include patients with chronic respiratory illness and suspected tuberculosis as well as the introduction of systematic testing for tuberculosis. The student was also responsible for the set-up of a new surveillance site, the recruitment and management of staff, the design of the data collection tools, and the implementation of the protocol.

All co-authors have been informed that the papers are to be used in PhD thesis and agree on their use within the thesis.

Student: Sibongile Walaza: 

Supervisor: Cheryl Cohen: _____

Supervisor: Stefano Tempia: 

APPENDIX B: ETHICAL CLEARANCE

R14/49 Dr Sibongile Walaza et al

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M140827

NAME: (Principal Investigator)	Dr Sibongile Walaza et al
DEPARTMENT:	School of Public Health Edendale Hospital, Klerksdorp-Tshepong Hospital Complex, Chris Hani Baragwanath Academic Hospital, Mapulaneng and Matikwana Hospital
PROJECT TITLE:	Interaction between Influenza and Tuberculosis in South Africa, A Country with High Prevalence of Tuberculosis and HIV
DATE CONSIDERED:	29/08/2014
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Dr Cheryl Cohen and Dr Stefano Tempia

APPROVED BY: Professor P Cleaton-Jones Chairperson, HREC (Medical)

DATE OF APPROVAL: 04/02/2015

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Secretary in Room 10004, 10th floor, Senate House, University.

I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report.**

Principal Investigator Signature Date 06/02/2015

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

PAPER I

Influenza and tuberculosis co-infection: a systematic review

Authors: Sibongile Walaza[1,2], Cheryl Cohen[1,2], Stefano Tempia[3,4], Jocelyn Moyes[1,2] Athermon Nguweneza[1], Shabir A. Madhi[1,6,7]; Meredith McMorrow[3,4,5], Adam L. Cohen[8]

Affiliations

1. Centre for Respiratory Diseases and Meningitis, National Institute for Communicable Diseases of the National Health Laboratory Service, Johannesburg, South Africa
2. School of Public Health, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa
3. Influenza Division, Centers for Disease Control and Prevention, Atlanta, Georgia, United States of America
4. Influenza Program, Centers for Disease Control and Prevention, Pretoria, South Africa
5. U.S. Public Health Service, Rockville, Maryland, United States of America
6. Medical Research Council, Respiratory and Meningeal Pathogens Research Unit, University of the Witwatersrand, Johannesburg, South Africa
7. Department of Science and Technology/National Research Foundation: Vaccine Preventable Diseases, University of the Witwatersrand, Johannesburg, South Africa
8. Strategic Information Group, Expanded Programme on Immunization Department of Immunization, Vaccines and Biologicals World Health Organization

Key words: Tuberculosis, influenza, interaction

Corresponding author

Sibongile Walaza, Centre for Respiratory Diseases and Meningitis, National Institute for Communicable Diseases, Private Bag X4, Sandringham, 2131, Gauteng, South Africa. Email: sibongilew@nicd.ac.za

Alternate corresponding author

Cheryl Cohen, Centre for Respiratory Diseases and Meningitis, National Institute for Communicable Diseases, Private Bag X4, Sandringham, 2131, Gauteng, South Africa. E-mail: cherylc@nicd.ac.za

Key Words: Tuberculosis, HIV, severe respiratory illness, South Africa

Word Count:

Abstract: 367

Main text: 3962

Tables: 3

Figures:1

Abstract

Introduction

Understanding the interaction between tuberculosis and influenza could help inform whether individuals with tuberculosis should be prioritized for seasonal and pandemic influenza vaccination.

Methods

We conducted a systematic review of published scientific literature from January 1900 to February 2015 to evaluate the prevalence, presentation and outcome of influenza-tuberculosis co-infection. Medline, Embase, PsycINFO, CINAHL, Web of Science, Cochrane, CAB Abstracts and Global Health online were searched. Published abstracts and articles that reported on prevalence, disease association, presentation and severity of laboratory-confirmed influenza and clinically diagnosed or laboratory-confirmed tuberculosis co-infection were included.

Results

We assessed 3739 abstracts, reviewed 122 manuscripts and included 16. Of the 16, 10 reported data from human studies and six were animal experimental studies. Six of the 10 human studies were more recent, included data from 2009 or later, and were from high tuberculosis burden countries (HBCs) that account for ~80% of the world's tuberculosis cases, and two were of high quality. Of the six studies that reported on the possible effect of tuberculosis in patients with influenza, three analytical studies reported no association with severe disease among patients with influenza-tuberculosis coinfection; of the three descriptive studies, all of which included data from the 2009 influenza pandemic, two reported a high prevalence of tuberculosis among patients with severe influenza disease or influenza-associated hospitalization. Of the five papers reporting on the possible effect of influenza in individuals with

tuberculosis, an association between influenza and severe tuberculosis disease was found in one of three analytical studies; of the other two studies, both reported a high frequency of tuberculosis disease progression and complications among patients with tuberculosis and seasonal influenza coinfection. In murine models, five studies reported either an increased severity of illness among *Mycobacterium tuberculosis*-infected mice challenged with influenza viruses or temporary suppression of tuberculin hypersensitivity after influenza infection.

Conclusion

Although experimental animal studies suggested that influenza infection increased severity of disease in mice with underlying tuberculosis infection, most analytical studies did not demonstrate a negative impact of co-infection on clinical presentation and outcome. However, descriptive studies suggested increased frequency of influenza-tuberculosis coinfection in patients with severe influenza- or tuberculosis-associated disease. Data are limited from large high quality analytical epidemiological studies with laboratory-confirmed influenza and tuberculosis.

Introduction

Influenza virus infections cause substantial annual morbidity and mortality in humans worldwide [1-3]. Globally, it is estimated that annual influenza epidemics result in three to five million cases of severe illness, and between 250,000-500,000 deaths [4]. In 2015, there were an estimated 10.4 million incident cases of tuberculosis and 1.8 million tuberculosis deaths globally[5]. Despite the availability of effective treatment, in 2015 tuberculosis was the most common cause of infectious disease-related deaths worldwide, with the majority of cases reported in Asia and Africa[5].

Both influenza and tuberculosis impair host immune responses. Specifically influenza can impair T cell immunity and weaken innate immune responses against secondary bacterial infections [6-11]. Lethal synergism associated with viral and bacterial infections can result in increased risk of influenza-associated mortality [12]. Furthermore, patients with tuberculosis, a chronic lung disease, may be at increased risk for severe influenza disease and death. Ecological studies and mathematical modelling of epidemiologic data suggest an increase in the frequency of influenza disease or severe influenza-associated disease in individuals with tuberculosis during influenza pandemics as compared to individuals without tuberculosis. [13-16].

Influenza infection may facilitate the progression of latent *Mycobacterium tuberculosis* infection to tuberculosis disease and alter the clinical presentation of tuberculosis [17].

While chronic lung diseases are a known risk factor for severe outcomes due to influenza infection and influenza vaccination is recommended in this group, tuberculosis is not listed as a separate group and may not be prioritised. In countries with a high burden of tuberculosis and limited resources, individuals

with tuberculosis maybe an important group to target and prioritise for influenza vaccination, and tuberculosis management guidelines could be amended to include influenza vaccination before and during the influenza season. Understanding the interaction between influenza and tuberculosis may assist in determining whether individuals with tuberculosis should be prioritised for influenza vaccination and treatment with antiviral medications. We conducted a systematic review of published literature on the association between laboratory-confirmed influenza and tuberculosis- i.e. influenza in individuals with tuberculosis and tuberculosis in individuals with influenza.

Methods

Search Strategy and study selection criteria

We conducted a systematic review of the scientific literature identified through searches using online databases. For our search we included terms for influenza ("influenza" or "flu") and for tuberculosis ("tuberculosis" or "TB"). The Medline, Embase, PsycINFO, CINAHL, Web of Science, Cochrane, CAB Abstracts and Global Health databases were searched. The search strategy differed slightly by database. In addition, bibliographies of papers that were reviewed were checked for further relevant publications. This review was restricted to published abstracts and articles from January1900 to February 2015 that reported data on the association (burden of disease, transmission and severity) between laboratory-confirmed influenza and clinically diagnosed or laboratory-confirmed tuberculosis. Due to the scarcity of published data, descriptive studies, including studies without comparison groups were included. Articles that included seasonal or pandemic influenza and animal experimental studies were also included. The search was restricted to articles published in English, French, Italian, German, Russian, Finish, Japanese or Portuguese. Articles in languages other than English were screened if the title or the abstract was relevant to the search. We followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines in reporting results [18].

Inclusion and exclusion process

Literature search results (titles and abstracts) were screened independently by two authors (SW and one of the co-authors-CC, AN, JM, ALC or MM) to identify all citations that possibly met the inclusion criteria. Full manuscripts of selected citations were retrieved and assessed by one reviewer (SW) against the inclusion/exclusion criteria and checked independently by a second reviewer, one of the co-authors (ALC, MM, JM, AN, CC, ST). Additional articles were identified from reviewing bibliographies of published articles. Discrepancies in included articles were resolved by consensus between the two reviewers with involvement of a third reviewer (CC) where necessary. Animal experimental studies, observational and analytic studies in humans were included. Studies that reported data on the association between laboratory-confirmed influenza and clinically diagnosed or laboratory-confirmed tuberculosis including the following were included:

- Prevalence and risk for influenza-associated severe disease among patients with tuberculosis disease;
- Prevalence and risk for tuberculosis-associated severe disease among patients with influenza infection;
- Effect of influenza on tuberculosis disease progression
- Clinical presentation of influenza and tuberculosis co-infection; and
- Immune response to co-infection, presentation or outcome of influenza-tuberculosis coinfection in animal studies.

Studies that modelled ecological data on the association between influenza and tuberculosis, individual case reports, vaccine studies and influenza antiviral therapy in patients with tuberculosis were not included. Study selection is summarized in Figure 1.

Data extraction and synthesis

Individual studies were independently assessed for potential bias or confounding. When studies used either cohort or case-control designs, we used the Newcastle-Ottawa Scale to rate the quality of the included papers [19]. Studies were considered high quality if the Newcastle–Ottawa Scale was ≥7 out of 9 and were considered of low quality if the score was ≤ 3 out of 9. Study methods differed; summary measures (odds ratios, relative risks), when reported, were abstracted. Data synthesis consisted of reporting the key findings of the different studies. Where possible in more recent studies the studies were classified according to whether they fall among the 22 high tuberculosis burden countries (HBCs) that account for ~80% of world's tuberculosis cases [3]. No review protocol was registered.

Ethics

Since this study used published data, it was exempt from human subjects ethics review.

Results

The search identified 4321 records; 582 of these were duplicates and were removed. Seven additional records were identified through other sources (Figure 1). The remaining 3739 titles and abstracts were screened. Of these, 122 articles were identified for full review, and 16 articles met the inclusion criteria. Of these, ten were in humans and six were animal experimental studies.

Human studies

Of the 10 human studies, seven used real time reverse transcription polymerase chain reaction (RT-PCR) and three used serology to test for influenza infection. Five of the human studies were analytical[20-24]. Six studies had laboratory-confirmed results for both influenza and tuberculosis (Tables 1 and 2): six studies were from HBC countries including three from Africa. In addition, three studies from Europe reported data from a period with high tuberculosis prevalence. Two of the five analytical studies were of high quality based on the Newcastle–Ottawa Scale ≥7. The human studies included numbers ranging

from 19-7180 individuals. Five studies reported on tuberculosis infection in individuals with influenza [21, 22, 25-27], four on influenza in individuals with tuberculosis [20, 24, 28, 29] and one on both [23] (Tables 1 and 2).

The effect of tuberculosis in patients with influenza

Of the six studies that reported on tuberculosis in individuals with influenza, five were from HBCs (Table 1) [21-23, 25, 27] and three were analytical studies[21-23].

Descriptive studies

Three descriptive studies from HBC using data from the 2009 influenza pandemic reported data on tuberculosis in individuals with influenza. Two of these studies reported a high frequency of tuberculosis in cases with influenza or among influenza deaths. However, no inferences could be made on the significance of the association as there were no comparison groups or data was not statistically evaluated [25, 27]. In a report of individuals that died with influenza A (H1N1)pdm09 virus infection in South Africa, active tuberculosis was identified in seven (10%) of the 72 deaths, a prevalence higher than that in the general population (1%) in 2006 [25]. Similarly, in a hospital-based case series of patients positive for influenza A(H1N1)pdm09 virus in India, 9% of influenza cases had tuberculosis compared to 0.4% tuberculosis prevalence in general population ($p<0.001$) [27]. In a case series of patients infected with influenza A(H1N1)pdm09 virus in South Korea less than 1% (7/12196) had newly diagnosed tuberculosis and there were no deaths among the co-infected (0/7) [26].

Analytical Studies

The three analytical studies from HBC, one of high quality, reported no association with severe disease among patients with influenza-tuberculosis coinfection [21-23]. A case control study from Kenya reported that 6% of hospitalised cases with influenza-associated severe acute respiratory illness had tuberculosis compared to <1% of neighbourhood-matched controls (unadjusted OR 12.0, 95% CI 1.3-107.37); however, active tuberculosis was not associated with influenza hospitalization on multivariable

analysis [22]. Less than 1% (23/7180) of patients hospitalised for acute respiratory illness and enrolled in a study from Thailand were co-infected with influenza virus and tuberculosis. There were no deaths among the 23 cases with co-infection whereas 17 (2.8%), died among the influenza only cases, p=0.1 [23]. In a review of 19 cases with laboratory-confirmed influenza A(H1N1)pdm09 virus infection with respiratory failure admitted to an intensive care facility in South Africa, 21% (4) had a history of tuberculosis (2 had active tuberculosis and 2 had a history of previous pulmonary tuberculosis). Tuberculosis was present in 4/13 who died vs 0/6 who survived p=0.5 [21].

The effect of influenza in patients with tuberculosis

Of the five papers that reported data on influenza in patients with tuberculosis [20, 23, 24, 28, 29](Table 2), two were from tuberculosis HBCs and the other three were from Europe in a period with high tuberculosis prevalence [23, 24]. Two of these papers were reported by the authors as analytical studies[23, 24], and a third[20] had data suitable for analysis.

Descriptive studies

Two descriptive studies reporting on influenza in cases with tuberculosis used data from tuberculosis sanatoria. Of these, one study using data from seasonal influenza, reported on disease progression and complications in patients with coinfection, however, this was not statistically evaluated[28]. This study from The Netherlands in 1967 among children admitted in a tuberculosis sanatorium with primary tuberculosis of the lungs and hilar lymph nodes reported a high frequency of developing secondary segmental pulmonary lesions, suggesting progression of tuberculosis following influenza virus infection defined as greater than fourfold rise in anti-influenza virus antibody titres [28]. One study, using data from the 1957 pandemic, described the effect of superimposed viral infection on existing tuberculosis following an outbreak of influenza A virus in a tuberculosis sanatorium in the United States. It reported

that only two of 31 cases with influenza co-infection had evidence of worsening of tuberculosis on chest radiograph [29]. (Table 2).

Analytical studies

In a case series during an influenza B epidemic in a Danish tuberculosis sanatorium, 13% (7/53) of individuals coinfected with influenza virus compared to 2% (3/142) of individuals with tuberculosis only developed tuberculosis complications which included radiological changes or sputum conversion back to being positive (p=0.005) [20]. A case control study from Indonesia investigating the putative association between tuberculosis and influenza virus infection reported no association between the development of clinically active tuberculosis, through reactivation of latent tuberculosis or directly after exposure to *Mycobacterium tuberculosis*, and influenza virus infection as measured by serology in cases with newly diagnosed tuberculosis and community controls. The proportion of individuals with influenza virus antibody titres $\geq$10 against influenza A(H3N2) and A(H1N1) viruses in patients with tuberculosis were similar to matched community controls; however, the antibody titre levels for influenza A(H3N2) virus at time of tuberculosis diagnosis were significantly higher (1.7 times higher, p=0.002) in cases with tuberculosis compared to controls. In addition, the difference in titres between cases with advanced tuberculosis on chest x-ray and their controls was significantly higher than in cases with mild to moderate tuberculosis and their controls [24]. Among 23 patients with concurrent tuberculosis and influenza infection from Thailand, none died, compared to 30 (4.7%) deaths among the individuals with only tuberculosis; however, this was not statistically significant, p=0.62 [23].

Summary of quality of human studies

Of the five analytical studies, two were high quality studies as assessed by the New Castle Ottawa score and showed no association between coinfection and severe influenza disease [22] or correlation between influenza infection and tuberculosis [24]. Studies about tuberculosis and influenza virus co-infection were

mostly descriptive case series, mostly included univariable analysis, and the causal relationship could not be demonstrated. Some of the studies used clinical criteria for tuberculosis cases; however, the specifics of the criteria used were not always fully described. Among the studies that included laboratory-confirmed tuberculosis, screening for tuberculosis was not done systematically.

Summary of findings from experimental animal models

In murine models, five studies suggested that influenza and tuberculosis co-infection affected tuberculosis and influenza disease presentation or outcome [17, 30-33], and one study showed no effect [34] (Table 3).

Five of the murine studies reported on the effect of influenza on tuberculosis and one study reported on the effect of influenza on tuberculosis and the effect of tuberculosis on influenza.

Effect of influenza on tuberculosis

Volkert et al., (1947) showed that the course of experimental infection with tubercle bacillus in mice was worsened by simultaneous influenza infection (influenza A virus and tubercle bacilli challenge at week 0) and influenza infection superimposed on tuberculosis infection (influenza challenge 3 weeks after TB challenge). Coinfection resulted in more extensive and rapid development of pulmonary tuberculosis lesions in mice than infection with tubercle bacillus only [17]. Florido, et al., (2013) reported that pulmonary Bacille Calmette-Guerine (BCG)-specific CD8 T cell responses were impaired in co-infected mice [32]. Concurrent infection of mice with influenza virus and BCG (challenge on day 0) and sequential infection of mice with TB and influenza virus (TB infection on day 0 and influenza virus 7 weeks later) compared to infection with BCG only resulted in reduction of BCG-specific CD4 and CD8 T cell responses, increased pulmonary disease and a delay in mycobacterium clearance from the lungs of infected mice. For sequential infection with influenza, the reduction in BCG-specific CD8 T cell response was only evident in mice with untreated TB compared to mice that had cleared TB. Concurrent infection

with influenza virus and tuberculosis reduced generation of protective T-cell responses against intracellular mycobacteria but did not affect control of pulmonary influenza viral loads (no difference between coinfected mice compared to the influenza only group) [32]. Redford et al., (2014) demonstrated that influenza A virus infection of mice 28 days before or during (on day1 or 14 days after) *M. tuberculosis* infection enhanced susceptibility to tuberculosis and impaired mycobacterium control and decreased host survival [33]. Bernard et al., (1962) showed that in *Mycobacterium tuberculosis* infected mice, influenza virus challenge 1-5 weeks after *Mycobacterium tuberculosis* infection, compared with *Mycobacterium tuberculosis* only-infected mice, resulted in 50-75% shorter survival time and a higher case-fatality rate [30]. In addition, the effect of influenza virus on tuberculosis severity, measured by amount of tissue damage, increased with increasing time of tuberculosis infection prior to the influenza virus challenge. Five percent of the mice infected with influenza alone died compared to 100% of the mice infected with influenza and tuberculosis.

Massanari (1972) showed that tuberculin hypersensitivity in mice was temporarily suppressed following intranasal influenza virus challenge; however, a normal response resumed after resolution of influenza virus infection. Tuberculin hypersensitivity, tested 4-6 weeks after tuberculosis infection, was temporarily suppressed from day 3 to day 16 post intranasal influenza virus challenge[31]. In contrast, in their mouse model, Co, et al., (2016) showed that influenza virus had little effect on mycobacterial load and did not affect dissemination of tuberculosis [34]. They showed that T cells responding to an acute influenza virus infection can modulate host responses to an on-going BCG infection. Though not statistically significant, acute infection with influenza virus in mice with chronic infection with *M. bovis* BCG moderately increased the acid-fast bacilli load in the liver [34].

Effect of tuberculosis on influenza

In the Bernard et al (1962) study, 5% of the mice infected with influenza alone died compared to 100% of the mice infected with influenza and tuberculosis[30] (Table 3).

Discussion

Our review of the published literature suggests that there are very few analytical studies exploring the interaction between laboratory-confirmed influenza virus infection and clinically diagnosed or laboratory-confirmed tuberculosis. Experimental animal studies suggest an association, specifically that influenza-tuberculosis co-infection in mice results in more severe disease than influenza only or tuberculosis only disease. Observational studies among humans showed mixed results. Most (4/5) of the analytical studies, two of which were of high quality, showed no association between coinfection and progression of disease or severe outcomes. However, three of the descriptive studies, although not assessed for statistical significance reported either a high prevalence of coinfection in cases with severe disease[25, 27] or increased severe disease or progression of disease in co-infected individuals[28] .

Of the five studies reporting on pandemic influenza only, two descriptive studies from HBCs, reported a high prevalence of tuberculosis in cases with severe influenza-associated disease[25, 27]. These studies were limited in that only univariate analyses were presented. Pandemic influenza may behave differently to seasonal influenza because of lack of pre-existing immunity, and the likely interaction between influenza and tuberculosis might be immunologically mediated. High levels of cytokines produced as part of the inflammatory response to infection with a pandemic virus have been reported to result in severe influenza-associated lung damage[35] .Previous studies have demonstrated a higher mortality due to pandemic influenza as compared to seasonal influenza [36-38]. High quality epidemiological studies are required to assess whether the severe disease and outcomes associated with influenza–tuberculosis co-infection is driven by pandemic phenomena as this may have implications for recommendations and prevention strategies. However, even if the association between influenza and tuberculosis is less marked during seasonal influenza epidemics, targeting individuals with active tuberculosis for influenza vaccination and antiviral treatment in HBCs could still potentially prevent significant morbidity and

mortality and might also prevent further spread of tuberculosis if influenza increases coughing. In this review all the analytical studies were conducted from high burden countries. It is important to understand the background prevalence of tuberculosis where studies are conducted for better interpretation of the results. In countries with low tuberculosis burden, it is possible for studies not to identify increased prevalence of coinfection or pick up an association between coinfection and severe outcomes, purely because of low numbers due to low tuberculosis prevalence in the community. In the analytical studies included in our review, lack of association maybe explained by the type of cases included in the studies e.g only including cases with acute presentation and not systematic testing for tuberculosis in patients with severe respiratory illness. Depending on the magnitude of tuberculosis burden, results may have different implications for prioritization in different settings.

Since the 1950s, authors have suggested influenza vaccination among patients with tuberculosis during influenza epidemics [20]. Influenza vaccination is the most effective way to prevent influenza-associated disease. Influenza vaccine has been shown to generate antibody response in patients with tuberculosis that is similar to those without tuberculosis, although these studies were conducted in the 1950s and 1960s and did not include HIV-infected individuals [29]. Antiviral treatment for influenza has been shown to improve outcomes for patients with severe influenza–associated disease [39]. However, both vaccines and antiviral treatment have cost implications and are not easily accessible in low to middle income countries where the burden of tuberculosis and influenza is high [40]. Identifying tuberculosis patients as a risk group for severe influenza-associated disease may assist policy makers in making decisions about prioritising this group of patients for influenza vaccination and treatment with influenza antiviral treatment. More high quality epidemiological data from high tuberculosis burden settings are needed to address this question. In addition, more studies are needed to determine if seasonal or pandemic vaccines or influenza antivirals should be prioritised for tuberculosis patients and whether patients hospitalised with influenza–associated illness should be investigated for tuberculosis.

Although some descriptive studies inferred worsening of tuberculosis in co-infected individuals[28, 29], besides the methodological limitations of the studies, changes reported could have simply reflected a superimposed viral or bacterial infection in cases with underlying tuberculosis rather than worsening of tuberculosis. In addition, there was no comparison of radiological findings in patients with and without co-infection to assess whether changes in the lungs were a factor in the presentation or outcomes of influenza-associated disease.

If cases with more advanced tuberculosis are more at risk of severe influenza disease, this might explain the lack of association in some of the studies which only included patients with an acute presentation [22, 23]. A recent study from South Africa suggested that compared to individuals infected only with tuberculosis, individuals with influenza-tuberculosis co-infection had increased risk of death and this association was not observed in patients with a more acute presentation [41]. If indeed the association with severe disease and poor outcomes is more prevalent in patients with a more chronic presentation, this may further assist in making decisions about which tuberculosis cases to prioritise for interventions, especially in countries where the tuberculosis burden is high and resources are limited.

The mechanism by which influenza-tuberculosis co-infection leads to severe influenza-associated disease may be secondary to underlying lung damage caused by tuberculosis. It is possible that those who had severe outcomes from co-infection already had underlying lung damage from tuberculosis leading to reduced lung capacity to deal with a viral infection like influenza. Seki et al., (2007) suggested that underlying chronic lung diseases like tuberculosis may be an important factor in the increase in frequency of secondary bacterial pneumonia in persons with influenza, which in turn can lead to increased frequency of complications [42].

There were a number of limitations to this systematic review. Many of the observational studies were descriptive, and due to the nature of these studies, an association could not be evaluated. The type of tuberculosis included differed among the studies, with some studies reporting newly diagnosed tuberculosis, some reporting on cases in a tuberculosis sanatorium for a number of months, and some included cases who had completed tuberculosis treatment, thus making it difficult to evaluate the evidence. There were differences in the population tuberculosis incidences where studies were conducted which could affect the power to detect an association. However, the majority of studies were from HBC or were conducted during the period when tuberculosis burden was high.

Many studies did not adequately assess underlying conditions such as HIV and malnutrition. HIV infection is a risk factor for severe influenza disease as well as for tuberculosis and it is an important contributor to the overall burden of severe influenza in high HIV-prevalence settings [43, 44]. However, only a few papers reported data on HIV infection [22, 23]. In one study patients with co-infection of HIV and tuberculosis were at high risk of being hospitalized with influenza; however, the number of co-infected individuals was low and the association was not statistically significant [22]. If the association with severe disease is higher in patients with underlying HIV infection, it may be difficult to differentiate the role played by the individual infection. Other conditions like malnutrition, which like tuberculosis are prevalent in HBC, were not evaluated in included articles, and may be confounders in the association between influenza and tuberculosis. The numbers of participants in most studies were small and this could have limited the ability to detect significant associations. Other important areas which were not addressed by the studies reviewed include whether influenza infection caused reactivation of latent tuberculosis or whether the acute viral infection facilitated a visit to the doctor in patients who already had tuberculosis disease.

Studies in Chinese were not included in the review and therefore our review may not reflect the full body of literature on this topic.

Conclusion

Although experimental animal studies suggest increased severity of disease with coinfection of influenza and tuberculosis, this was not found in most analytical studies on influenza and tuberculosis in humans. However, descriptive studies, although they could not evaluate for an association, reported an increased prevalence of coinfection among cases with severe disease. Data are limited from large epidemiological studies, studies with laboratory-confirmed influenza and tuberculosis, studies from high tuberculosis burden settings and studies that include data on HIV. In order to study the association between influenza and tuberculosis and make inferences about causal associations, more epidemiological studies are needed.

Disclaimer

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the US Centers for Disease Control and Prevention.

Competing interests

No authors have any competing interests.

Financial disclosure

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Supporting information

S1 PRISMA check list

Author Contributions

Conceived and designed the experiments: SW, ALC

Performed the experiments: SW, ST, MM, AN, CC, JM, ALC

Analysed the data: SW, ST, MM, AN, CC, JM, ALC

Wrote and reviewed the paper: SW, ST, MM, AN, CC, SAM, JM, ALC

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Table 1: Summary of studies reporting on effect of tuberculosis in individuals with influenza

Study	Country	Year	Descriptive/ analytical	Objective/hypothesis	Setting	Number studied	Influenza type	Laboratory confirmed influenza	TB diagnosis	Findings	Association*	Newcastle-Ottawa Score **
Archer, (2009)[25]	South Africa	2009	Descriptive	Describe epidemiology of lab confirmed pandemic influenza cases in South Africa	Hospital admissions	72 cases	Pandemic A(H1N1)2009	RT-PCR	Lab methods not specified-As diagnosed/ reported by attending clinician	7/72 (10%) cases who died with influenza A(H1N1)pdm09 had TB vs 1% TB prevalence in 2006 in general community. Not evaluated statistically	NA	NA
Puvanaligam (2011) [27]	India	2009-2010	Descriptive	Describe clinical profile of H1N1 cases	Hospital admissions	442 cases	Pandemic A(H1N1)2009	RT-PCR/culture	Not specified	8.8% influenza A (H1N1)pdm09 cases had TB vs 0.4% TB prevalence in general population ($p<0.001$)	NA	NA
Noh (2013)[26]	South Korea	2009-2011	Descriptive	Describe cases with concurrent TB and influenza	Hospital admissions	12196 subjects	Pandemic A(H1N1) 2009	RT-PCR	Auramine stain, TB PCR and TB culture	No deaths in the 7 cases of concurrent influenza-TB infection. Not evaluated statistically	NA	NA
Ope (2011)[22]	Kenya	2007-2009	Analytical	Describe risk factors for influenza hospitalisation	Hospital admissions	64 cases; 190 controls	Seasonal (AH3N2 &A H1N1 & B)	RT-PCR	Self-report verified by clinician diagnosis and medication	TB associated with influenza hospitalization on bivariate (OR 12.0, 95% CI 1.3-107.37) but not on multivariate (aOR not presented) analysis	No	8

Roth (2013)[23]	Thailand	2003-2011	Analytical	Compare characteristics of TB/influenza to influenza and TB only	Hospital admissions	7180 subjects	Seasonal & pandemic (AH1N1) 2009	RT-PCR	AFB ± culture	Influenza-TB co-infection not associated with increased severity /mortality. Deaths in 0/23 co-infected vs 17/604 (2.8%) with influenza only (p=1.0)	No	6
Koegelenberg, (2009)[21]	South Africa	2009	Analytical	Describe epidemiological characteristics, clinical features and outcome of pandemic H1N1 cases complicated by respiratory failure	Intensive Care Unit admissions	19 cases	Pandemic A(H1N1) 2009	RT-PCR	Lab methods not specified-presence /absence of disease	4/19 ICU cases with influenza A(H1N1)pdm09 had TB. TB in 4/13(31%) who died vs 0/6 who survived, (p=0.5)	No	6

*Association- Evidence of/or association (univariate/multivariable analysis) with increased severity of influenza disease in those with vs. without tuberculosis; or prevalence of coinfection in those with severe influenza disease; ** Score out of a possible score of 9; USA- United States of America; TB-tuberculosis; NA-Not applicable

Table 2: Summary of studies reporting on the effect of influenza in individuals with tuberculosis (TB)

Study	Country	Year	Descriptive/ analytical	Objective/hypothesis	Setting	Number studied	Influenza type	Laboratory confirmed influenza	TB diagnosis	Findings	Association*	Newcastle-Ottawa Score **
Dijkman (1967)[28]	Netherlands	1957-1963	Descriptive	Investigated association between acute respiratory infection (influenza) and unfavourable course of primary pulmonary and hilar tuberculosis among children-	Tuberculosis sanatorium	36 subjects	Seasonal, influenza A & B	Serology, HI antibodies against influenza A. ≥4-fold increase in antibody titres	Clinical& radiological examination AFB towards end of hospitalisation	20% (5/20) of paediatric patients with influenza developed segmental pulmonary lesions. Not evaluated statistically	NA	NA
Sellers (1959)[29]	USA	1957	Descriptive	Assess effect of superimposed viral infection on existing TB	TB sanatorium	31	Pandemic, influenza A, 1957	HI antibodies to PR8 type A & FMI type A	*Tuberculin* conversion, X-ray changes± *M. tuberculosis* culture	2/31 TB cases with influenza had signs of worsening on X-ray (increased perihilar nodes, increased infiltration around cavity and increase in cavity)	NA	NA
Espersen (1954)[20]	Denmark	1952	Analytical	Describe the epidemic of influenza B in a TB sanatorium	Tuberculosis sanatorium	295 subjects	Seasonal, influenza B	HI	Clinical, TB smear± culture	Radiological changes or sputum conversion in 7/53 (13%) co-infected vs 3/142 (2%) TB only (p =0.005).	Yes	6
De Paus (2013) [24]	Indonesia	2001-2004	Analytical	Did newly diagnosed TB patients have a recent influenza virus infection?	Cases from tuberculosis clinic and community controls	111 TB cases; 111 community	Seasonal (AH3N2/AH1N1)	Serology HI-IG and IGM antibodies against influenza A	WHO case definition (clinical +CXR changes	Prevalence of influenza antibodies among TB cases vs controls was 46% vs 41% (p=0.5) for	No	8

				Hypothesis- Influenza virus enhanced the susceptibility to develop active TB/ reactivated latent TB		controls, matched for age, sex and socio economic status		HI titre ≥10	positive microscopy and culture for Mtb)	A/H1N1pdm09 and 82% vs 82% (p=1.0) for A/H3N2.		
Roth (2013)[23]	Thailand	2003-2011	Analytical	Compare characteristics of TB/influenza to influenza and TB only	Hospital admissions	7180 subjects	Seasonal & pandemic	RT-PCR/serology	≥1 sputum AFB or culture positive	Death in 0/23 cases with co-infection vs 30/646 (4.6%) in cases with TB only, p=0.6.	No	6

*Association- Evidence of/or association (univariate/multivariable analysis) with increased severity of tuberculous disease in those with vs without influenza; or increased frequency of coinfection vs single infection in those with severe tuberculosis disease

**Score out of a possible score of 9; USA- United States of America; TB-tuberculosis; NA- Not applicable

Table3: Summary of experimental animal studies exploring the interaction between influenza and tuberculosis

Reference	Objective	Experiment	Influenza strain/TB	Period/ observation period	Findings
Volkert (1947) [17]	Does viral infection of the lungs superimposed on tubercle bacilli infection alter cause and outcome of infection due to bacterium	Model: Mice 1. Experiment group 1 Experimental group • Simultaneous TB bacilli given intraperitoneally and influenza A virus (PR8) intra nasally. Control group 1: Inoculated with TB only Outcomes: • Number of gross tuberculosis pulmonary lesions measured at 3 weeks Experiment group 2: • Inoculation with TB bacilli and influenza 3 weeks later Outcomes: • Number of gross TB pulmonary lesions measured 6 weeks after TB infection (3 weeks after influenza challenge)	Influenza A PR8 / culture of tubercle bacilli	3 weeks and 6 weeks	Experimental group 1 • Increased number and extent of pulmonary lesions compared to control group. Experimental group 2: • Increased number and extent of pulmonary lesions compared to control group.
Florido (2013)[32]	Assessed impact of influenza A virus and mycobacterial respiratory co-infection on development of CD8 T cell responses to each pathogen	Experiment 1 Experimental group: • *Mycobacterium bovis* bacilli Calmette-Guerin (BCG) and influenza A/PR8 at D1 Control groups: • BCG only at D1 Outcomes: • Number of BCG and influenza-specific CD4 and CD8 T cells, number of mycobacteria, viral titres, and number of leukocytes	Influenza A/ PR8; BCG	Experiment 1:7, 14, 21 days post infection; Experiment 2 21 days post influenza challenge (challenge at 7 weeks)	Experiment1 • Experimental (co-infected) group– reduced frequency and magnitude of BCG specific CD8 T cells in the lungs and reduced magnitude of BCG-specific CD4 and CD8-T cell IFN γ – secreting responses; no difference in influenza-specific CD8T cells. • Co-infected group had increased number of viable BCG ova

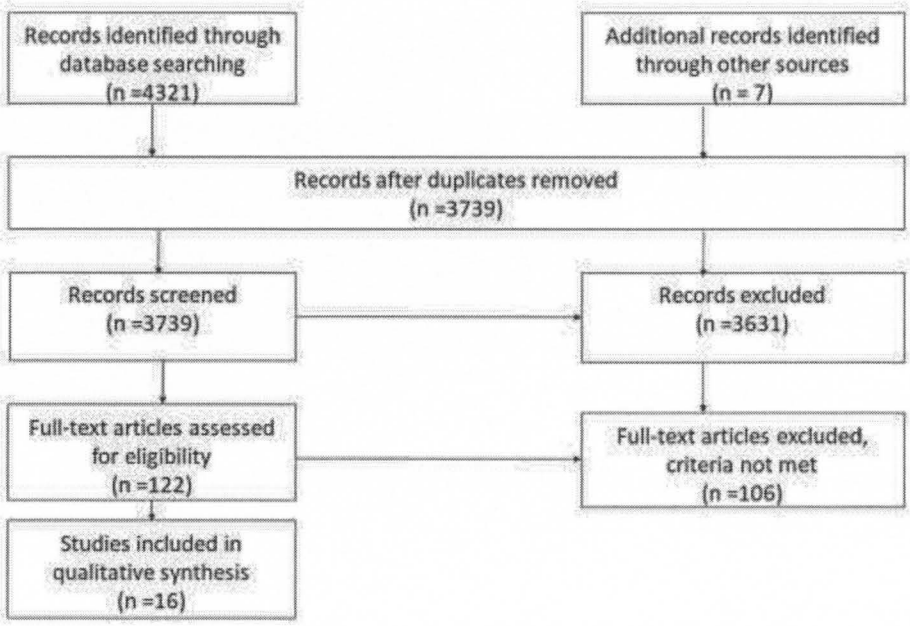
		at D7, D14 and D21 post infection Experiment 2: Experimental group: • BCG at D1, and influenza infection at 7 weeks. Control group: • BCG at D1, TB treatment at week 3 (for 4 weeks) and influenza infection at 7 weeks Outcomes: BCG specific CD8 T cell response D21 post influenza.			• Co-infected group had more extensive/persistent leucocyte accumulation Experiment 2- • Experimental group had reduced BCG- specific CD8 T cell response
Redford (2014)[33]	1.Effect of prior IAV on susceptibility to tuberculosis 2. Effect of IAV/M. *Tuberculosis* coinfection on control of TB	Model: mice 1. Experimental group: • Intranasal IAV on D1, aerosolized MTB on D28. Control group: • Intranasal placebo (phosphate buffered saline (PBS)) on D1, MTB on D28 Outcomes: • lung inflammation, survival, number of viable bacteria in lung tissue 2. Experimental group 2: • Aerosolized MTB D1, intranasal IAV (subtype (Cal/09) on D1 and IAV (subtype X3) on D14 Control group: • Aerosolized MTB D1, placebo (PBS) on D1 and D14 Outcomes: • Number of viable MTB measured on D27	Influenza A Virus/ *M. tuberculosis*	Model 1: 120 days Model 2: 27 days	Model 1 • Experimental group 1 had significant increase in inflammation, decreased survival, higher number viable MTB in lung compared to the control group Model 2 • Experimental group had significantly increased mycobacterial load compared to control group

Bernard, (1962) [30]	Assessed the effect of influenza infection on TB- infected mice. Measured TB bacilla per nodule in the sacrificed mice and time from infection to death for the mice that were not sacrificed	Model: Mice Experimental group Groups 1, 2, 3, 4 and 5 (10 mice in each) • TB challenge at week 1, 2, 3, 4 and 5 respectively. Influenza challenge at week 6. 50% of mice sacrificed 15 days post influenza challenge Group 6 (20 mice)- • influenza only challenge at week 6 for all; Not sacrificed Control groups Groups 1, 2, 3, 4 and 5 (10 mice in each) • TB challenge at week 1, 2, 3, 4 and 5 respectively. No influenza challenge Outcomes: • Number of TB bacilli per nodule in the sacrificed mice • Time from TB infection to death for the mice that were not sacrificed	H 37 RV strain of TB	173 days, 50% of mice in experimental groups 1-5 sacrificed 15 days post influenza challenge.	• Experimental groups had 50-75% lower survival time, had increased number of bacilli per nodules. Effect of influenza infection on TB severity increased with increasing duration of TB infection before influenza challenge • Among non-sacraficed mice, death in 25/25 (100%) coinfected vs 1/20 (5%) infected with influenza only
Massanari (1979)[31]	Examined tuberculin hypersensitivity during superimposed acute influenza infection	Model: Mice Experimental groups1: • Influenza virus, intranasal (i.n) 4-6 weeks after *M. tuberculosis* infection Experimental group 2 • Formalin –inactivated influenza virus (i.n) or intravenous (i.v) live influenza virus 4-6 weeks after *M. tuberculosis* infection Control group Inoculated with phosphate buffered saline (PBS), 4-6 weeks after *M. tuberculosis* infection Outcomes: • Tuberculin hypersensitivity (measured as footpad swelling)	*M. tuberculosis* and influenza A/PR8	4-6 weeks after TB infection	Experimental group 1 • Suppressed tuberculin hypersensitivity from D3 to D16 post intranasal influenza infection. Suppression of immune response preceded presentation of clinical signs of influenza • Reduction of lymphocytes on D2,5,7 post influenza challenge compared to values before influenza infection Experimental group 2 • No immunosuppression in experimental group 2

		6 days after influenza, PBS challenge • Number of circulating lymphpcytes post influenza challenge			
Co (2006) [34]	Tested how BCG-specific and influenza-specific CD4 T cells distribute between the two inflammatory sites (lungs and liver), how these two T cells would interfere with each other and how these interactions affect granuloma formation, dissemination, and control of BCG	Model: Mice Experimental groups: • Mice chronically infected with BCG and intranasal hen egg lysozyme (HEL flu) influenza challenge 5 weeks post BCG • Mice chronically infected with BCG and wild type (wt) influenza challenge 5 weeks post BCG Control group: • Mice infected with BCG alone Outcome: Number of AFB per lesion, number of granuloma lesions and dissemination of mycobacteria from granuloma	HEL-flu or wt. influenza virus virus/ BCG	5 weeks 6 days	Experimental groups • Increase in numbers of granulomas/field (granuloma burden) in experimental groups compared to controls • Slight increase in number of AFB per lesion compared to the control group • No dissemination of mycobacteria from granuloma Interpretation: Co-infection with influenza had little effect on mycobacterial load mycobacteria did not disseminate in either group.

IAV- influenza A virus; IFN- interferon, **HEL-** hen egg lysozyme, BCG- Calmette-Gue´rin, TB-tuberculosis, *M. tuberculosis – Mycobacterium tuberculosis*

Figure1: Flow of information through the different phases of literature search and study



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PAPER II

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MAJOR ARTICLE

The Burden and Clinical Presentation of Pulmonary Tuberculosis in Adults With Severe Respiratory Illness in a High Human Immunodeficiency Virus Prevalence Setting, 2012–2014

Sibongile Walaza,[1,3] Stefano Tempia,[1,7,8] Andries Dreyer,[2] Halima Dawood,[10] Ebrahim Variava,[4,11,12] Neil A. Martinson,[6,12,13] Jocelyn Moyes,[1,3] Adam L. Cohen,[7,8,9] Nicole Wolter,[1,5] Claire von Mollendorf,[1,3] Anne von Gottberg,[1,5] Sumayya Haffejee,[10] Florette Treurnicht,[1] Orienka Hellferscee,[1,5] Nazir Ismail,[2,14] and Cheryl Cohen[1,3]

Centres for [1]Respiratory Diseases and Meningitis and [2]Tuberculosis, National Institute for Communicable Diseases of the National Health Laboratory Service, Johannesburg, South Africa; Schools of [3]Public Health, [4]Clinical Medicine, and [5]Pathology, Faculty of Health Sciences, and [6]DST/NRF Centre of Excellence for Biomedical Tuberculosis Research, University of the Witwatersrand, Johannesburg, South Africa; [7]Influenza Division, Centers for Disease Control and Prevention, Atlanta, Georgia; [8]Influenza Program and [9]Division of Global Health Protection, Centers for Disease Control and Prevention, Pretoria, South Africa; [10]Pietermaritzburg Metropolitan Hospital Complex, KwaZulu-Natal, South Africa; [11]Department of Medicine, Klerksdorp Tshepong Hospital, North West Province; [12]Perinatal HIV Research Unit, MRC Soweto Matlosana Collaborating Centre for HIV/AIDS and TB, Johannesburg, South Africa; [13]Johns Hopkins University Center for TB Research, Baltimore, Maryland; [14]Faculty of Health Sciences, University of Pretoria, Pretoria, South Africa

Background. Understanding the burden and clinical presentation of tuberculosis in patients with severe respiratory illness (SRI) has important implications for anticipating treatment requirements.

Methods. Hospitalized patients aged ≥15 years with SRI at 2 public teaching hospitals in periurban areas in 2 provinces (Edendale Hospital in Pietermaritzburg, KwaZulu-Natal Province and Tshepong Hospital in Klerksdorp, North West Province) were enrolled prospectively from 2012 to 2014. Tuberculosis testing included smear microscopy, culture, or Xpert MTB/Rif.

Results. We enrolled 2486 individuals with SRI. Of these, 2097 (84%) were tested for tuberculosis, 593 (28%) were positive. Tuberculosis detection rate was 18% (133 of 729) in individuals with acute (≤14 days) presentation and 34% (460 of 1368) in those with chronic (>14 days) presentation. Among laboratory-confirmed tuberculosis cases, those with acute presentation were less likely to present with cough (88% [117 of 133] vs 97% [447 of 460]; ajusted odds ratio [aOR] = 0.2, 95% confidence interval [CI] = 0.1–0.5), night sweats (57% [75 of 132] vs 73% [337 of 459]; aOR = 0.4, 95% CI = 0.3–0.7), or be started on tuberculosis treatment on admission (63% [78 of 124] vs 81% [344 of 423]; aOR = 0.4, 95% CI = 0.3–0.7), but they were more likely to be coinfected with pneumococcus (13% [16 of 124] vs 6% [26 of 411]; aOR 2.3, 95% CI 1.3–5.3) than patients with chronic presentation. Annual incidence of acute and chronic tuberculosis-associated SRI per 100 000 population was 28 (95% CI = 22–39) and 116 (95% CI = 104–128), respectively.

Conclusions. In this setting, tuberculosis, including acute presentation, is common in patients hospitalized with SRI.

Keywords. HIV; severe respiratory illness; South Africa; tuberculosis.

Tuberculosis is an important cause of severe respiratory illness (SRI) causing significant morbidity and mortality globally. In 2015, an estimated 10.4 million people developed tuberculosis and 1.8 million died from the disease, 0.4 million (22%) of whom were infected with human immunodeficiency virus (HIV) [1]. In South Africa in the same year, there were an estimated 454 000 incident cases of tuberculosis and 258 000 (57%) were infected with HIV [1].

The 2014, South African national guidelines for tuberculosis management recognized the importance of early diagnosis and treatment of tuberculosis and advocated that sputa be tested in patients with symptoms of tuberculosis, especially those who are infected with HIV irrespective of the duration of cough. However, for HIV-uninfected patients, these guidelines advise testing only patients with chronic duration of symptoms (ie, cough or fever >14 days) [2]. The World Health Organization (WHO) guidelines also call for intensified case finding among HIV-infected individuals and recommend screening for tuberculosis in patients with any current symptoms of tuberculosis [3]. In an earlier publication including data from the same hospitals as the current study, we found that only 38% of individuals admitted for lower respiratory tract infection (LRTI) with any symptom duration were tested for tuberculosis, whereas 80% of admitted patients ≥15 years with LRTI were infected

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Correspondence: S. Walaza, MBBCh, MSc Epidemiology and Biostatistics, Centre for Respiratory Diseases and Meningitis, National Institute for Communicable Diseases, Private Bag X4, 1 Modderfontein Road, Sandringham, 2131, Gauteng, South Africa (sibongilew@nicd.ac.za).

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with HIV [4]. In addition, clinicians were significantly less likely to test patients with acute symptoms for tuberculosis and to start them on tuberculosis treatment [4]. Understanding the spectrum of clinical presentation of tuberculosis may contribute to timely diagnosis and treatment and subsequent better control of the tuberculosis epidemic. Among HIV-infected and -uninfected persons aged ≥15 years hospitalized with SRI in South Africa during 2012–2014, we aimed to describe the following: (1) factors associated with acute compared with chronic presentation of SRI irrespective of laboratory diagnosis; (2) the incidence of laboratory-confirmed acute and chronic presentation of pulmonary tuberculosis; (3) factors associated with laboratory-confirmed pulmonary tuberculosis among patients tested for tuberculosis; and (4) factors associated with acute and chronic presentation among patients with laboratory-confirmed tuberculosis.

METHODS

Study Design

This study used data collected as part of surveillance for SRI [5]. Prospective hospital-based active surveillance was conducted for SRI at 2 sentinel surveillance sites (Edendale Hospital in Pietermaritzburg, Umgungundlovu District, KwaZulu-Natal Province and Tshepong Hospital in Klerksdorp, Matlosana District, North West Province) from July 2012 to August 2014.

Case Definitions

A case of SRI was defined as admission with a physician diagnosis of LRTI (eg, pneumonia, bronchiolitis, bronchitis, and pleural effusion, suspected or confirmed tuberculosis) irrespective of symptom duration and included individuals who met the WHO severe acute respiratory illness (SARI) case definition of cough and fever presenting within 10 days of onset of illness [6].

Study Procedures

Patients admitted from Sunday at 5:00 PM through to 1:00 PM on Fridays were screened for inclusion. Patients who refused or were unable to give consent and patients from outside the catchment area of the respective hospitals were excluded. Data on clinical presentation, previous medical history, antiviral therapy for HIV-infected individuals, inpatient investigations, and management and outcome were collected by interview and medical record review. Patients were followed-up until discharge. Treatment decisions, including initiation of tuberculosis treatment based on laboratory confirmation or empirically, and diagnostic tests were performed according to the attending physician.

In addition, combined nasopharyngeal (NP) and oropharyngeal (OP) swabs, expectorated or induced sputum, and blood specimens were collected from consenting patients. We instituted testing for tuberculosis for all consenting patients, and details of testing for tuberculosis are included in the Supplementary Material.

Laboratory Methods

Nasopharyngeal and OP swabs were transported in universal transport medium at 4–8°C to the National Institute for Communicable Diseases (NICD) within 72 hours of collection. Combined NP and OP swabs were tested for influenza A and B and 8 other respiratory viruses (parainfluenza virus types 1, 2, and 3; respiratory syncytial virus; adenovirus; rhinovirus; human metapneumovirus; and enterovirus) by real-time, reverse-transcription polymerase chain reaction (PCR) [7]. Sputum samples were frozen at −20°C and shipped on dry ice weekly to NICD. Testing for tuberculosis at the hospital laboratory was performed by smear microscopy, culture, and/or XpertMTB/Rif based on the hospital testing algorithm. At NICD, in 2012 testing only included culture and microscopy was added in 2013. Details of tuberculosis testing are included in Supplementary Material. Determination of HIV status is described in detail in Supplementary Material. In brief, HIV testing was not required for participation, and HIV results were obtained from clinical records when available. For consenting patients with unavailable results, either bedside testing was done or a dried blood spot was tested as part of surveillance. Testing included HIV enzyme-linked immunosorbent assay (ELISA) for patients aged ≥18 months and PCR for children aged <18 months if the ELISA was reactive.

Tuberculosis Status

Tuberculosis status was determined using tuberculosis results from specimens tested at the site laboratory and at NICD. A laboratory-confirmed tuberculosis case was defined as an individual with any positive result for acid-fast bacilli on microscopy, *Mycobacterium tuberculosis* on culture, or PCR (Xpert MTB/Rif or MTBDRplus) from the current hospital admission.

Symptom Duration

For duration of symptoms, we used the date of onset of symptoms as reported by the patient and considered cough and fever as the main variables to categorize duration of symptoms as either an acute (≤14 days) or chronic (>14 days) presentation. The cutoff of 14 days was chosen a priori based on South African guidelines for tuberculosis screening for HIV-negative individuals.

Incidence Calculation

Incidence estimates of acute and chronic tuberculosis-associated SRI hospitalization (per 100 000 population) for Pietermaritzburg and Klerksdorp were calculated for 2013. Population denominators for the hospital catchment population were accessed from the 2011 census data as collected by Statistics South Africa [8]. Mid-year population figures by age group were estimated for the districts served by the hospitals. These population numbers were adjusted for proportions of people in the hospital catchment area that attended the surveillance hospital for care for respiratory illness using information from healthcare utilization surveys previously conducted at

each site (Karen Wong and Jo McAnerney, Oral communication, May 2016). Data on all admissions during 2013, including nonenrolled individuals, were collected and used to estimate the number of SRI cases missed during the study period due to nonenrollment over weekends and refusal. The incidence of acute and chronic tuberculosis-associated SRI hospitalizations/100 000 population for 2013 was estimated using the number of patients with acute and chronic presentation testing positive for tuberculosis, adjusting for nonenrollment (due to refusal and over weekend admissions) and patients who did not seek care by age groups divided by the mid-year population estimates for 2013 and multiplied by 100 000. Confidence intervals (CIs) were calculated using the Poisson distribution.

Data Analysis

To identify factors associated with tuberculosis positivity and acute compared with chronic presentation of tuberculosis, we included potential determinants for, as well as outcomes or characteristics of, the primary endpoints of the analysis. We implemented 3 multivariable logistic regression models to identify factors associated with the following: (1) acute compared with chronic presentation among patients hospitalized with SRI irrespective of laboratory diagnosis; (2) laboratory confirmation of tuberculosis among patients admitted with SRI with symptoms of any duration and with available tuberculosis results; and (3) acute compared with chronic presentation among individuals with laboratory-confirmed tuberculosis to assess whether cases with an acute presentation differed in presentation and outcome because they would likely not have been tested for tuberculosis especially if they were HIV negative. We repeated model (3) among HIV-infected and HIV-uninfected individuals separately.

For the multivariate model, all factors significant at $P < .1$ on univariate analysis were evaluated, and nonsignificant factors at $P < .05$ were then dropped from the multivariable model using stepwise forward selection. All 2-way interactions in the final multivariable additive model were evaluated. Two-sided P values <.05 were considered significant throughout. Stata version 13.1 (StataCorp Limited, College Station, TX) was used for the analysis.

RESULTS

From July 2012 through August 2014, 4045 patients fulfilling the SRI case definition were enrolled; 2486 (61%) were aged ≥15 years and were included in the analysis. Of these, 866 (35%) had an acute presentation. A total of 2097 of 2486 (84%) patients were tested for tuberculosis, with equal proportions tested among those with an acute (84%; 729 of 866) and chronic (84%; 1368 of 1620) presentation (Figure 1). Tuberculosis was detected in 28% (593 of 2097) of enrolled individuals, 18% (133 of 729) and 34% (460 of 1368) in those with an acute and chronic presentation, respectively ($P < .001$). Results of different tuberculosis microbiologic tests are included in the Supplementary Material.

Compared with patients tested for tuberculosis, controlling for hospital, patients not tested were less likely to present with cough (ajusted odds ratio [aOR], 0.3; 95% CI, 0.2–0.5) or night

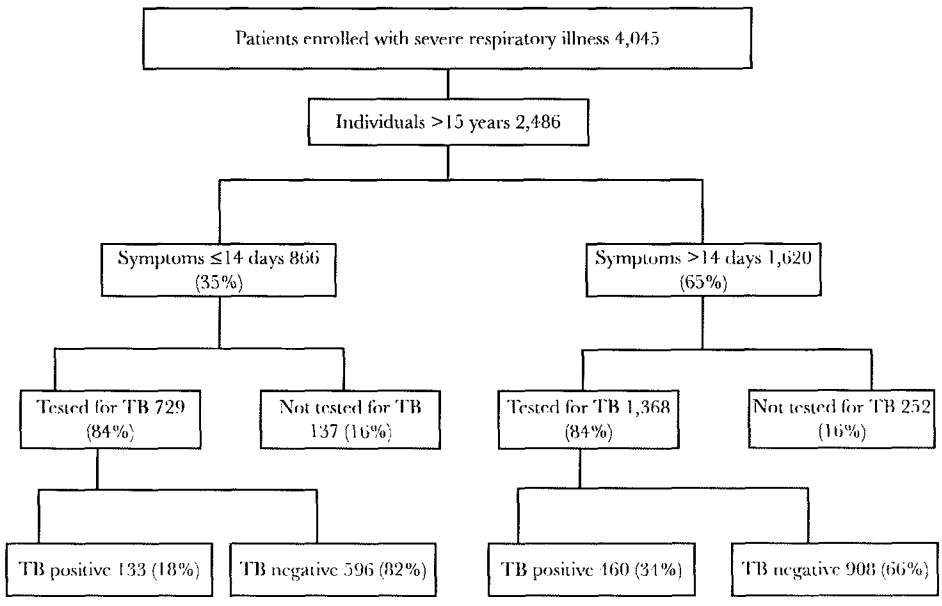

Figure 1. Individuals admitted with severe respiratory illness and consented for enrollment by symptom duration and laboratory-confirmed tuberculosis (TB) status, South Africa, July 2012–August 2014.NOTE: Reasons for not testing for TB ($n = 389$): 54% (210) not able to expectorate and sputum induction contraindicated or too sick or weak to cough up induced sputum, 30% ($n = 117$) induction not successful, 6% ($n = 23$) refused, 10% ($n = 39$) reason not recorded.

sweats (aOR, 0.7; 95% CI, 0.6–0.9) but were more likely to be admitted for >7 days compared with <3 days (aOR, 1.7; 95% CI, 1.1–2.4) and to die (aOR, 3.1; 95% CI, 2.3–4.1) (Supplementary Table 1). There was no significant difference in HIV status among patients tested (77% [1438 of 1877]) and not tested (72% [247 of 344]) for tuberculosis ($P = .06$).

The overall HIV prevalence in patients with SRI was 76% (1685 of 2221) and varied by age group: 72% (134 of 186) in those 15–24 years, 91% (1071 of 1179) in those 25–44 years, 66% (437 of 665) in those 45–64 years, and 23% (43 of 191) in those ≥65 years ($P < .001$). Human immunodeficiency virus status also varied by tuberculosis disease: 75% (1014 of 1355) and 81% (424 of 522) in tuberculosis-uninfected and -infected individuals, respectively ($P = .003$). Of the 1685 HIV-infected SRI patients, 389 (23%) were diagnosed with HIV at the current admission. Of 1482 HIV-infected SRI patients with information on HIV treatment, 45% (674) were receiving HIV treatment at time of admission.

Comparison of Characteristics of Individuals With Acute Presentation to Individuals With Chronic Presentation Hospitalized With Severe Respiratory Illness

Of the patients with acute presentation, 95% (820 of 866) had symptom duration ≤10 days, and 28% (231 of 820) of these met the WHO SARI case definition of LRTI with reported or documented fever, cough, and symptoms ≤10 days [6]. On multivariable analysis, compared with patients with a chronic presentation, patients with an acute presentation were less likely to present with cough or night sweats, to have laboratory-confirmed tuberculosis, to be started on treatment for tuberculosis on current admission, to be hospitalized for a longer duration ≥3 days, and to die (Table 1). The HIV prevalence was similar (73% [568 of 773] vs 77% [1117 of 1448]) in patients with acute and chronic presentation, respectively.

Factors Associated With Laboratory Confirmation of Tuberculosis Among Patients Admitted With Severe Respiratory Illness of Any Duration and Tested for Tuberculosis

Among 2097 patients with available tuberculosis results, 28% (593 of 2097) tested positive for tuberculosis at the current admission. Eighteen percent (33 of 183) of those who met the WHO SARI case definition tested positive for tuberculosis.

On multivariable analysis, compared with tuberculosis-negative patients, patients with laboratory-confirmed tuberculosis of any symptom duration were less likely to be in older age groups >45 year compared with the 15–24 age group, to be coinfected with pneumococcus, and to have received oxygen therapy. They were more likely to present with symptoms >14 days (Table 2). The prevalence of influenza (4% vs 6%, $P = .09$) was similar in patients with and without tuberculosis disease of any duration.

Factors Associated With Acute Presentation Compared to Chronic Presentation in Cases With Laboratory-Confirmed Tuberculosis

Of the 593 laboratory-confirmed tuberculosis cases, 22% (133) and 78% (460) had an acute and chronic presentation, respectively. Human immunodeficiency virus prevalence among laboratory-confirmed cases was 81% (424 of 522), and it was similar in those with acute (83%, 102 of 123) and chronic (81%, 322 of 399) presentation ($P = .51$). Among HIV-uninfected laboratory-confirmed cases, 21% (21 of 98) had an acute presentation. On multivariable analysis, among patients with laboratory-confirmed tuberculosis, cases with acute presentation were less likely to present with cough (aOR, 0.2; 95% CI, 0.1–0.5) or night sweats (aOR, 0.4; 95% CI, 0.3–0.7) or be started on treatment for tuberculosis at current admission (aOR, 0.4; 95% CI, 0.3–0.7) than cases with chronic presentation. In contrast, they were more likely to have pneumococcal coinfection (aOR, 2.6; 95% CI, 1.3–5.3). The case-fatality ratio was not statistically different between laboratory-confirmed tuberculosis patients with acute (8%, 11 of 131) and chronic (13%, 56 of 447) presentation ($P = .194$) (Table 3). Similar results were seen when restricting analysis to HIV-infected patients with laboratory-confirmed tuberculosis (Supplementary Material).

Incidence of Hospitalization for Tuberculosis-Associated Severe Respiratory Illness

The estimated overall incidence (per 100 000 population) of hospitalization for tuberculosis-associated SRI was 144 (95% CI, 131–158), ranged from 43 to 200 per 100 000 among age groups, and was significantly higher in Klerksdorp than in Pietermaritzburg (181 [95% CI, 162–201] vs 93 [95% CI, 77–111]). The annual incidence of tuberculosis-associated SRI in individuals hospitalized with acute presentation was 28 per 100 000 population (95% CI, 22–39), increased with increasing age, and was highest in individuals ≥65 years (94 cases per 100 000 population; 95% CI, 54–153). The incidence of tuberculosis-associated SRI in individuals with chronic presentation was 116 per 100 000 population per annum (95% CI, 104–128) and was highest in individuals aged 25–44 years (145 cases per 100 000 population; 95% CI, 125–166) (Figure 2). The incidence of tuberculosis-associated SRI was lower in individuals with acute compared with chronic presentation, except in individuals aged ≥65 years where there was no significant difference (0.8; 95% CI, 0.4–1.8) (Figure 2).

DISCUSSION

We found that in our setting, a high proportion of adolescents and adults admitted with SRI regardless of symptom duration had active tuberculosis. For patients with acute presentation, 1 in 5 (18%) were found to have active tuberculosis, whereas among those with longer duration of symptoms, 1 in 3 (34%) had active tuberculosis. Although the likelihood of tuberculosis disease was higher if symptom duration was >14 days, additional numbers of cases will be identified if laboratory testing for tuberculosis is conducted for adults with SRI irrespective of symptom duration or HIV status. In particular, 21% of HIV-uninfected patients with laboratory-confirmed tuberculosis had an acute presentation, and these would have been missed by the South African tuberculosis testing guidelines [2]. Although the HIV-uninfected tuberculosis

Table 1. Demographic and Clinical Characteristics of Individuals ≥15 Years Admitted With Severe Respiratory Illness by Symptom Duration (≤ or >14 days), in South Africa, July 2012–August 2014 (*N* = 2486)

Variables	Acute Presentation (≤14 Days) n/N (%)	Chronic Presentation (>14 Days) n/N (%)	Univariate Analysis (Acute Compared With Chronic Presentation)		Multivariable Analysis (Acute Compared With Chronic Presentation)	
			OR (95% CI)	P Value	OR (95% CI)	P Value
Age						
15–24	69/866 (8)	137/1620 (8)	Reference		Reference	
25–44	459/866 (53)	860/1620 (53)	1.1 (0.8–1.4)	.715	1.3 (0.8–2.0)	.221
45–64	244/866 (28)	500/1620 (31)	1.0 (0.7–1.3)	.850	1.2 (0.7–1.9)	.514
65+	94/866 (11)	123/1620 (8)	1.5 (1.1–2.3)	**.038**	1.1 (0.6–2.0)	.721
Female sex	508/866 (57)	816/1619 (50)	1.4 (1.2–1.6)	**<.001**	1.3 (1.0–1.6)	**.027**
Tshepong Hospital	547/866 (63)	1069/1620 (66)	0.9 (0.7–1.1)	.160		
History of cough	748/865 (84)	1564/1620 (96)	0.2 (0.1–0.3)	**<.001**	0.2 (0.1–0.4)	**<.001**
History of fever	374/861 (43)	551/1608 (34)	1.5 (1.2–1.7)	**<.001**	1.4 (1.1–1.8)	**.004**
Night sweats	444/864 (54)	1111/1619 (67)	0.5 (0.4 -0.6)	**<.001**	0.5 (0.4–0.6)	**<.001**
Underlying medical conditions[a]	88/866 (10)	134/1619 (8)	1.3 (0.9–1.7)	.117		
Diabetes	40/865 (5)	37/1619 (2)	2.1 (1.3–3.3)	**.002**	2.1 (1.1–3.8)	**.028**
Mine exposure[c]	83/857 (10)	189/1594 (12)	0.8 (0.6–1.0)	.103		
History of smoking[b]	169/856 (20)	273/1602 (17)	1.2 (1.0–1.5)	.097		
History of alcohol[b]	214/858 (25)	349/1603 (22)	1.2 (1–1.5)	.075		
Tested for TB	729/866 (84)	1368/1620 (84)	1.0 (0.8–1.2)	.863		
Laboratory confirmed TB[g]	133/729 (18)	460/1368 (34)	0.4 (0.3–0.5)	**<.001**	0.7 (0.5–0.9)	**.004**
History of TB treatment[e]	73/863 (8)	189/1612 (12)	0.7 (0.5–0.9)	**.012**		
HIV infected	568/773 (73)	1117/1448 (77)	0.8 (0.7–1.0)	**.055**		
Influenza-positive	59/842 (7)	72/1587 (4)	0.6 (0.4–0.9)	**.011**		
Pneumococcal coinfection[d]	144/790 (18)	144/1457 (10)	2.0 (1.6–2.6)	**<.001**	1.9 (1.5–2.6)	**<.001**
Viral coinfection	231/842 (27)	429/1587 (27)	1.0 (0.8–1.2)	.832		
Required oxygen	300/836 (36)	569/1570(36)	1.0 (0.8–1.2)	.862		
Antibiotics on admission	823/850 (97)	1509/1591 (95)	1.7 (1.1–2.6)	**.026**		
Started on TB treatment[f]	247/803 (31)	809/1457 (56)	0.3 (0.3–0.4)	**<.001**	0.4 (0.3–0.6)	**<.001**
Duration of hospitalization						
<3 days	168/788 (21)	226/1484 (15)	Reference		Reference	
3–7 days	337/788 (43)	605/1484 (41)	0.7 (0.6–0.9)	**.018**	0.7 (0.5–0.9)	**.013**
8+ days	283/788 (34)	653/1484 (44)	0.6 (0.5–0.7)	**<.001**	0.6 (0.4–0.8)	**.001**
Died during admission	91/824 (11)	228/1546 (15)	0.7 (0.5–0.9)	**.012**	0.7 (0.5–0.97)	**.038**

Bold indicates significant variables.

Abbreviations: CI, confidence interval; HIV, human immunodeficiency virus; OR, odds ratio; PCR, polymerase chain reaction; TB, tuberculosis.

[a]Underlying conditions included any of the following: asthma, other chronic lung disease, chronic heart disease (valvular heart disease, coronary artery disease, or heart failure excluding hypertension), liver disease (cirrhosis or liver failure), renal disease (nephrotic syndrome, chronic renal failure), immunocompromising conditions excluding HIV infection (organ transplant, immunosuppressive therapy, immunoglobulin deficiency, malignancy), neurological disease (cerebrovascular accident, spinal cord injury, seizures, neuromuscular conditions), or pregnancy. Comorbidities were considered absent in cases for which the medical records stated that the patient had no underlying medical condition or when there was no direct reference to that condition.

[b]Current history.

[c]Any history of having worked in the mine.

[d]*lytA* PCR positive for *Streptococcus pneumoniae* on blood specimen.

[e]History of TB treatment within the last 12 months of current admission.

[f]Started on TB treatment at current admission; excludes those already on TB treatment at time of admission (*n* = 144). Of the 1056 patients started on TB treatment, 42% (441 of 1056) had a negative TB result, 36% (376 of 1056) tested positive, and 23% (239 of 1056) had missing/pending results at the time of initiation of TB treatment.

[g]6% (38/593) only detected on culture.

cases with acute presentation constituted 3.5% of all tuberculosis cases in this study, in a setting with high tuberculosis prevalence and where tuberculosis is the leading cause of death, any opportunity to diagnose tuberculosis should be used. Studies have reported a high prevalence or incidence of active tuberculosis in South African communities including in cases where tuberculosis was not suspected [9–11]. In a postmortem study conducted in a community served by one of the hospitals included in our study, 31% of patients who died at home with uncategorized cause of death had tuberculosis [10]. Wong et al [11] reported that among HIV-positive individuals, ~30% of microbiologically and histologically proven tuberculosis infections on postmortem were clinically not suspected at the time of death.

In our study, 59% and 27% of laboratory-confirmed tuberculosis cases did not present with fever and chronic cough, respectively, potentially reducing the clinician's suspicion of tuberculosis even further. The atypical clinical presentation of tuberculosis in our study is likely to be related to HIV infection

Table 2. Demographic and Clinical Characteristics Associated With Laboratory-Confirmed Tuberculosis Among Cases Admitted With Severe Respiratory Illness of Any Duration and Tested for Tuberculosis at Two Sites in South Africa, 2012–2014 (*N* = 2097)

Variables	Tuberculosis Negative n/N (%)	Tuberculosis Positive n/N (%)	Univariate Analysis		Multivariable Analysis	
			OR (95% CI)	P Value	OR (95% CI)	P Value
Age						
15–24	107/1504 (7)	63/593 (11)	Reference		Reference	
25–44	780/1504 (52)	365/593 (62)	0.8 (0.6–1.1)	.179	0.7 (0.5–1.1)	.114
45–64	475/1504 (32)	144/593 (24)	0.5 (0.4–0.7)	**<.001**	0.6 (0.4–0.9)	**.007**
65+	142/1504 (9)	21/593 (3)	0.3 (0.1–0.4)	**<.001**	0.3 (0.1–0.5)	**<.001**
Female gender	801/1503 (53)	397/593 (51)	0.9 (0.7–1.1)	.295		
Klerksdorp Tshepong	991/1504 (66)	397/593 (67)	1.04 (0.9–1.3)	.645		
Duration of symptoms >14 days	908/1504 (60)	460/593 (76)	2.3 (1.8–2.8)	**<.001**	1.6 (1.2–2.0)	**.001**
History of cough (any duration)	1416/1503 (94)	564/593 (95)	1.2 (0.8–1.8)	.419		
Chronic cough >14 days	833/1503 (55)	833/592 (73)	2.2 (1.8–2.7)	**<.001**		
History of fever	544/1493 (37)	239/590 (41)	1.2 (0.9–1.4)	.084		
Night sweats	927/1503 (62)	412/591 (70)	1.4 (1.2–1.8)	**<.001**		
Underlying medical condition[a]	161/1503 (11)	27/593 (5)	0.4 (0.3–0.6)	**<.001**		
Diabetes	48/1502 (3)	12/593 (2)	0.6 (0.3–1.2)	.151		
History of TB treatment[e]	142/1499 (9)	68/589 (28)	1.2 (0.9–1.7)	.157		
HIV infected	1014/1355 (75)	424/522 (81)	1.5 (1.1–1.9)	**.003**		
Worked in mine[c]	173/1484 (12)	68/583 (12)	1.0 (0.7–1.3)	.997		
History of smoking[b]	281/1488 (19)	113/586 (19)	1.0 (0.8–1.3)	.835		
History of alcohol[b]	374/1489 (25)	128/586 (22)	0.8 (0.6–1.0)	.117		
Influenza-positive	89/1471 (6)	24/582 (4)	0.7 (0.4–1.06)	.086		
Pneumococcal coinfection[d]	212/1366 (16)	42/535 (8)	0.5 (0.3–0.7)	**<.001**	0.6 (0.4–0.9)	**.024**
Viral coinfection	419/1471 (28)	169/582 (29)	1.03 (0.8–1.3)	.802		
Invasive bacterial infection on culture[g]	7/251 (3)	2/122 (2)	0.6 (0.1–2.8)	.502		
Required oxygen	547/1445 (38)	171/584(29)	0.7 (0.5–0.8)	**<.001**	0.7 (0.6–0.9)	**.005**
Antibiotics on admission	1421/1468 (97)	554/588 (94)	0.5 (0.3–0.8)	**.007**		
TB treatment started[f]	470/1371 (34)	422/547 (77)	6.5 (5.1–8.1)	**<.001**	5.6 (4.-7.2)	**<.001**
Duration of hospitalization						
<3 days	247/1364 (18)	102/554 (18)	Reference			
3–7 days	557/1364 (41)	248/554 (45)	1.1 (0.8–1.4)	.592		
8+ days	560/1364 (41)	204/554 (37)	0.9 (0.7–1.2)	.382		
Died during admission	152/1423 (11)	67/578 (12)	1.1 (0.8–1.5)	.555		

Bold indicates significant variables.

Abbreviations: CI, confidence interval; HIV, human immunodeficiency virus; OR, odds ratio; PCR, polymerase chain reaction; TB, tuberculosis.

[a]Underlying conditions included any of the following: asthma, other chronic lung disease, chronic heart disease (valvular heart disease, coronary artery disease, or heart failure excluding hypertension), liver disease (cirrhosis or liver failure), renal disease (nephrotic syndrome, chronic renal failure), immunocompromising conditions excluding HIV infection (organ transplant, immunosuppressive therapy, immunoglobulin deficiency, malignancy), neurological disease (cerebrovascular accident, spinal cord injury, seizures, neuromuscular conditions), or pregnancy. Comorbidities were considered absent in cases for which the medical records stated that the patient had no underlying medical condition or when there was no direct reference to that condition.

[b]Current history.

[c]Any history of having worked in the mine.

[d]*lytA* PCR positive for *Streptococcus pneumoniae* on blood specimen.

[e]History of TB treatment within the last 12 months of current admission.

[f]Started on TB treatment at current admission; excludes those already on TB treatment at time of admission (n = 108).

[g]Invasive isolates were defined as a bacterial pathogen isolated from blood or pleural fluid from a specimen taken within 48 hours of hospitalization; organisms viewed as likely contaminants were excluded. Two percent (9 of 373) of patients had a positive blood culture (5 *S pneumoniae*, 2 *Staphylococcus aureus*, and 1 *Escherischia coli*). Two percent (2 of 122) of laboratory-confirmed TB patients had a positive blood culture (1 *E coli* and 1 *S aureus*).

because 81% of patients in our study were infected with HIV. Symptom screening has been reported to perform poorly in HIV-infected patients, particularly in those on antiretroviral therapy [12, 13]. Early detection and prompt treatment are key to the success of tuberculosis infection control [14]. To facilitate diagnosis, minimize delays to treatment initiation, interrupt transmission, and improve patient outcomes, studies have advocated for rapid microbiological diagnosis of tuberculosis, which could be achieved through using rapid diagnostic tests including Xpert and urine assay for lipoarabinomannan (LAM) [15–20]. Urine LAM tests, although not currently part of standard of care in our setting, have been shown to be beneficial especially among HIV-infected individuals with advanced disease where systematic screening of admitted individuals is advised [16–19, 21].

Tuberculosis patients with acute presentation compared with chronic presentation were less likely to present with typical

Table 3. Demographic and Clinical Factors Associated With Acute (≤14 Days) vs Chronic (>14 Days) Presentation Among Individuals ≥15 Years With Laboratory-Confirmed Tuberculosis, July 2012–August 2014 (*N* = 593)

Variables	Acute Presentation n/N (%)	Chronic Presentation n/N (%)	Univariate Analysis		Multivariable Analysis	
			OR (95% CI)	P Value	OR (95% CI)	P Value
Age						
15–24	11/133 (8)	52/460 (11)	Reference		Reference	
25–44	82/133 (62)	283/460 (62)	1.4 (0.7–2.7)	.375		
45–64	33/133 (24)	111/460 (24)	1.4 (0.7–2.9)	.379		
65+	7/133 (5)	14/460 (3)	2.4 (0.8–7.2)	.131		
Female gender	73/133 (55)	228/460 (50)	1.2 (0.8–1.8)	.280		
Tshepong Hospital	89/133 (67)	308/460 (67)	1.0 (0.7–1.5)	.993		
History of any cough	117/133 (88)	447/460 (97)	0.2 (0.1–0.5)	**<.001**	0.2 (0.1–0.5)	**<.001**
History of fever	60/133 (45)	179/457 (41)	1.3 (0.9–1.9)	.220		
Night sweats	75/132 (57)	337/459 (73)	0.5 (0.3–0.7)	**<.001**	0.4 (0.3–0.7)	**<.001**
Underlying medical condition[a]	6/133 (5)	21/460 (5)	0.98 (0.4–2.5)	.979		
History of TB treatment[c]	51/4457(11)	17/132 (13)	1.2 (0.7–2.1)	.586		
Diabetes	5/433 (5)	7/460 (2)	2.5 (0.8–8.1)	.118		
HIV infected	102/123 (83)	322/399 (81)	1.2 (0.7–1.9)	.581		
Influenza positive	9/130 (7)	15/452 (3)	2.2 (0.9–5.1)	.075		
Pneumococcal coinfection[b]	16/124 (13)	26/411 (6)	2.2 (1.1–4.2)	**.019**	2.6 (1.3–5.3)	**.008**
Viral coinfection	40/130 (31)	129/452 (29)	1.1 (0.7–1.7)	.622		
Required oxygen	48/131 (37)	123/453 (27)	1.6 (1.1–2.3)	**.036**		
Started on antibiotics	125/131 (95)	429/457 (94)	1.4 (0.6–3.4)	.505		
Started on TB treatment[d]	78/124 (63)	344/423 (81)	0.4 (0.3–0.6)	**<.001**	0.4 (0.3–0.7)	**.001**
Duration of hospitalization						
<3	21/124 (21)	89/430 (19)	Reference			
3–7	54/124 (42)	194/430 (45)	1.1 (0.6–1.9)	.806		
8+	49/124 (37)	155/430 (36)	1.2 (0.7–2.2)	.501		
Died during admission	11/131 (8)	56/447 (13)	0.6 (0.3–1.3)	.197		

Bold indicates significant variables.

Abbreviations: CI, confidence interval; HIV, human immunodeficiency virus; OR, odds ratio; PCR, polymerase chain reaction; TB, tuberculosis.

[a]Underlying conditions included any of the following: Asthma, other chronic lung disease, chronic heart disease (valvular heart disease, coronary artery disease, or heart failure excluding hypertension), liver disease (cirrhosis or liver failure), renal disease (nephrotic syndrome, chronic renal failure), immunocompromising conditions excluding HIV infection (organ transplant, immunosuppressive therapy, immunoglobulin deficiency, malignancy), neurological disease (cerebrovascular accident, spinal cord injury, seizures, neuromuscular conditions) or pregnancy. Comorbidities were considered absent in cases for which the medical records stated that the patient had no underlying medical condition or when there was no direct reference to that condition.

[b]*lytA* PCR positive for *Streptococcus pneumoniae* on blood specimen.

[c]History of TB treatment within the last 12 months of current admission.

[d]Started on TB treatment at current admission; excludes those already on TB treatment at time of admission (*n* = 39).

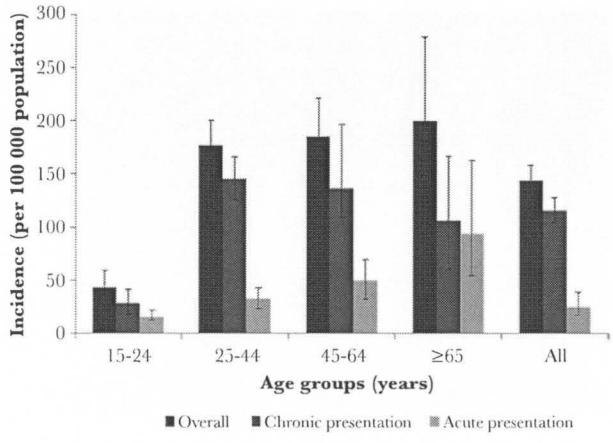

Figure 2. Incidence of tuberculosis-associated severe respiratory illness in acute (≤14 days) vs chronic (>14 days) presentation per 100 000 population, South Africa 2013.

symptoms of tuberculosis such as night sweats and cough and were less likely to be started on tuberculosis treatment by the attending clinician. The delay in initiating tuberculosis treatment, especially among patients with acute presentation, may reflect the clinicians' level of suspicion for tuberculosis in this group of patients. Patients with undiagnosed tuberculosis may continue to spread tuberculosis to other patients and healthcare workers [22–24]. Healthcare service delays have been cited as major contributors to total delay to initiation of tuberculosis treatment [25–27]. A postmortem study in patients dying in hospital found that among patients not suspected of having tuberculosis at the time of death, 42% (40 of 96) were culture positive for tuberculosis and that pneumonia was the leading admission diagnosis (25%) in those not suspected of tuberculosis [28]. This study supports the importance of maintaining a high clinical index of suspicion for tuberculosis in patients presenting with acute SRI symptoms in high HIV and tuberculosis burden settings.

Coinfection with respiratory viruses was common (29%) among tuberculosis-confirmed cases. Four percent were coinfected with influenza: 7% and 3% in patients with an acute and chronic presentation, respectively. This may be an important group to target for influenza vaccination because increased risk of influenza-associated mortality in patients with tuberculosis has been reported [4, 29–31]. Pneumococcal coinfection was identified in 8% of tuberculosis cases and more commonly identified in tuberculosis cases with an acute than chronic presentation. Among cases with an acute presentation, pneumococcus was the most commonly detected copathogen. Studies have postulated an interaction between tuberculosis and pneumococcus [32–36]. This may suggest severe comorbidity as the reason for admission to hospital in patients with tuberculosis. Blood cultures were performed in <20% of enrolled patients with tuberculosis results, which limited our ability to comment on the proportion with bacteremic pneumonia.

The overall incidence of hospitalized tuberculosis-associated SRI was high (144; 95% CI, 131–158), and it was twice as high in the Matlosana district compared with areas around Pietermaritzburg (181 [95% CI, 162–201] vs 93 [95% CI, 77–111]). Because gold mining is a major industry in Klerksdorp, tuberculosis incidence may be higher compared with Pietermaritzburg, which is a nonmining town. Silicosis is a frequent occurrence among miners, and gold miners are at higher risk for acquiring tuberculosis [37]. In addition, mining areas could fuel transmission among general population. Both Matlosana and UMgungundlovu Districts are high HIV-prevalence areas, and SRI cases at Tshepong and Edendale hospital had a similar high HIV prevalence. Additional results on incidence are discussed in the Supplementary Material.

Our study has a number of limitations. This study included hospitalized individuals only and may not be reflective of patients with respiratory symptoms treated in outpatient facilities. Although our study aimed to test all patients admitted with SRI for tuberculosis, only 84% of patients were tested. Tested patients were less likely to die, and were more likely to die, and this could have biased our study. More than half (54%) of patients that were not tested were not able to expectorate and induced sputum was contraindicated or they were either too sick or weak to cough up sputum. In addition, 18% of patients in our study were tested on tuberculosis PCR only and 6% were tested on smear only. It is possible that patients diagnosed as not having tuberculosis did actually have tuberculosis because the sensitivity of diagnostic tests is <100%. Guidelines for testing with the Xpert MTB/Rif advise that a second sample be tested with microscopy and culture especially in HIV-infected patients who test negative for tuberculosis on PCR. For tuberculosis testing at site, we included testing conducted as part of clinical care, and additional testing was conducted as part of surveillance. Surveillance testing initiated in July 2012 used the same systems as clinician-initiated testing and coincided with a campaign to encourage clinician testing because we wanted to make sure that all results would be available to clinicians. For this reason we were not able to tell whether the proportion of clinician-initiated testing for individual cases differed between patients with acute and chronic presentation. In a previous study that included the same sites from 2010 to 2011 when no systematic testing was implemented, we found that testing for tuberculosis was less common in individuals with a duration of symptoms <7 days [4].

Data on cryptococcosis was not collected as part of the main study; therefore, we were not able to assess its contribution. For mortality, we used in-hospital outcome, and the 12% case-fatality ratio reported in our study would likely be higher if patients were followed up after discharge from hospital. According to death notification forms in South Africa, of the 458 933 tuberculosis deaths that occurred in 2013, 106 554 (23%) occurred at home [38]. For incidence calculation, uncertainty may have been introduced through adjustment for nonenrollment, and this was not included in the estimation of CIs. Finally, we only included patients aged ≥15 years in our study and therefore cannot comment on acute and chronic presentation of tuberculosis in children.

CONCLUSIONS

Tuberculosis should be considered in a differential diagnosis of patients presenting with SRI irrespective of the duration of symptoms in high HIV and tuberculosis burden settings such as South Africa. The absence of classic tuberculosis symptoms and an acute presentation should not preclude the possibility of a diagnosis of tuberculosis.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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Potential conflicts of interest. All authors: No reported conflicts of interest.

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The burden and clinical presentation of pulmonary tuberculosis in adults with severe respiratory illness in a high HIV prevalence setting, 2012-2014- (Supplementary Material)

Methods

Information on testing for tuberculosis

For enrolled patients who were not tested for tuberculosis as part of clinical management, expectorated sputum (or induced sputum if the patient not able to expectorate) was collected by study staff for tuberculosis testing at the hospital laboratory in addition to the sample tested at the National Institute for Communicable Diseases (NICD), Johannesburg. Initially testing for tuberculosis at NICD was only conducted if sample was not available for site testing and from 2013 all samples received at NICD were tested. Sputum was not collected if the patient was not able to expectorate and sputum induction was contraindicated (e.g. acute respiratory distress, oxygen saturation <90% on room air, haemoptysis, severe respiratory distress, unstable cardiovascular status (arrhythmia, angina), deemed inappropriate by attending clinician) or the patient was too weak to cough up induced sputum. Results of tuberculosis testing from the site laboratory were available to clinicians through the hospitals laboratory information system and positive tuberculosis results from NICD testing were communicated to the clinicians as results became available.

Laboratory methods

Smear microscopy of sputum samples was performed using fluorescent auramine-O staining for acid fast bacilli (AFB). Culture was performed using liquid media with the Bactec MGIT 960 (Becton Dickinson, USA) system. Positive cultures were identified as *M. tuberculosis* complex using Ziehl-Neelsen staining and MPT64 antigen testing (Becton Dickinson, USA). Genotypic resistance to isoniazid and rifampicin in tuberculosis-positive patients was tested

using the Genotype MTBDR*plus* v2 assay (Hain Lifesciences, Germany). Whole blood was tested for the presence of pneumococcal DNA (*lyt*A gene) using quantitative real-time PCR.

Tuberculosis Status

The study focused on pulmonary tuberculosis, and we included patients with positive results from sputum and pleural effusion. We did not include cases of extra-pulmonary tuberculosis without pulmonary disease. Tuberculosis patients who were already on treatment for ≤ 2 months (initiation phase of treatment) and those who still tested positive for tuberculosis at current admission were included in the analysis. The reason for including these patients was because we felt that the tuberculosis was still active and could still affect the outcome.

Symptom Duration

Data on symptoms of tuberculosis was collected by interview using a structured questionnaire. History of any cough, any fever, night sweats, weight loss, chronic cough (>14 days) and chronic fever (>14 days) was collected.

Determination of HIV status

HIV status was obtained from two data sources. For some patients, HIV testing was requested by admitting physicians as part of clinical management. This included HIV enzyme-linked immunosorbent assay (ELISA) testing with confirmation by ELISA on a second specimen. For consenting patients who were not tested by the attending clinician, pre-test counselling and bedside testing for HIV was offered by study staff. In addition, for consenting patients who refused HIV testing by admitting physicians and did not want to receive their results, linked anonymous ELISA(1) was performed using a dried blood spot or whole blood specimen. CD4+ cell count per cubic millimeter was collected for HIV-infected patients as part of clinical management or part of the study if not requested by the clinician.

Age categories used in analysis

For the different analysis, age was categorized into four age groups (15-24, 25-44, 45-64 and ≥65 years) based on HIV prevalence in the different age groups.

Results

Testing for tuberculosis

Of the 2,097 tested, tuberculosis microscopy results were available for 1,709 (82%), of which 241 (14 %) tested tuberculosis positive; PCR results for 1,646 (78%), of which 448 (27%) tested tuberculosis positive; and culture results for 1,300 (62%), of which 214 (16%) tested tuberculosis positive. Of the 2,097 tested, 127 (6%) were tested by microscopy only and 30 (24%) were positive, three (<1%) were tested on culture only and none were positive, 380 (18%) were tested on PCR only and 114 (30%) were positive and 1,587 (76%) were tested on ≥2 tests and 449 (28%) were positive for tuberculosis.

On multivariable analysis among HIV-infected patients with laboratory-confirmed tuberculosis, individuals with acute presentation were less likely to present with cough (aOR 0.2, 95% CI 0.1-0.7), night sweats (aOR 0.4, 95% CI 0.2-0.7), and low CD4 count (aOR 0.3, 95% CI 0.2-0.6) and be started on treatment for tuberculosis at current admission (aOR 0.4, 95% CI 0.2-0.7) compared to patients with chronic presentation (Supplementary table 2). Overall 10% (43/412) of HIV-infected tuberculosis patients died. There was no difference in outcome, among HIV-infected patients with chronic and acute presentation [case fatality ration (CFR) 11% (35/312) and 8% (8/100), respectively, p=0.36]. Among the 98 HIV-

uninfected laboratory-confirmed tuberculosis patients, there was no significant difference in presentation and outcome in patients with acute and chronic presentation (data not presented).

Discussion

Pneumococcus and influenza are considered to be common causes of community-acquired pneumonia [38]. In our study, in SRI patients with symptoms for ≤14 days, tuberculosis was as commonly detected as pneumococcus and more commonly identified than influenza. The finding of tuberculosis in cases admitted with acute presentation of LRTI is similar to that reported by others in African settings.[34, 39, 40].

The overall incidence was high among persons aged ≥65 years and they had the highest incidence of tuberculosis-associated acute SRI, though this was not statistically different from persons aged 45-64 years. This may reflect tuberculosis testing practices in outpatient departments, in that clinicians may not suspect tuberculosis in elderly and in particular those with acute presentation or that rapidly progressing tuberculosis frequently will require hospitalization. In a previous study, the elderly were less likely to be tested for tuberculosis, 39% tested compared to 56% in the 25-44 year age group [4]. If the elderly, who are also at increased risk for severe influenza illness, are more likely to present acutely and are less likely to be tested for tuberculosis, this may be an important target group to prioritize for testing for tuberculosis in efforts to curb transmission.

SARI surveillance is a recommended approach for influenza surveillance. Such surveillance platforms, with modified case definitions and expanded laboratory testing, may provide an opportunity to monitor severe respiratory illness due to other etiologies. Given the high frequency of tuberculosis detection in acute presentation in our study (18%; 133/279), even in

patients meeting the WHO SARI case definition with symptoms for ≤10 days (18%; 33/183), tuberculosis testing may be useful in routine SARI surveillance in sub-Saharan Africa [6].

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Supplementary table1: Comparison of characteristics and presentation of individuals≥ 15 years of age tested and not tested for tuberculosis (N=2486)

		Tested for tuberculosis	Not tested for tuberculosis	Univariate analysis		Multivariable analysis	
				OR (95% CI)	P-value	OR (95% CI)	P-value
Age	15-24	170/2097 (8)	236/389 (9)	Reference		Reference	
	25-44	1145/2097 (55)	174/389 (45)	0.7 (0.5-1.1)	0.098	0.7 (0.5-1.2)	0.184
	45-64	619 /2097 (30)	125/389 (32)	0.9 (0.6-1.4)	0.819	1.0 (0.6-1.6)	0.945
	65+	163 /2097 (8)	54/389 (14)	1.6 (1.0-2.5)	0.064	1.4 (0.8-2.3)	0.256
Female gender		1102/2096 (53)	222/389 (57)	1.2 (1.0-1.5)	0.103		
Tshepong		1388/2097 (66)	228/389 (59)	0.7 (0.6-0.9)	0.004	0.7 (0.5-0.9)	0.002
Duration of symptoms >14 days		1368 /2097 (65)	252/389 (65)	1.0 (0.8-1.3)	0.863		
History of any cough		1980/2096 (94)	332/389 (85)	0.3 (0.2-0.5)	<0.001	0.3 (0.2-0.5)	<0.001

History of fever	798/2083 (38)	145/386 (38)	1.0 (0.8-1.2)	0.782		
Night sweats	1339/2094 (64)	216/389 (74)	0.7 (0.6-0.9)	0.002	0.7 (0.6-0.9)	0.023
Underlying medical condition*	228/2096 (11)	47/389 (4)	1.1 (0.8-1.6)	0.487		
Diabetes	60/2095 (3)	17/389 (4)	1.5 (0.9-2.7)	0.118		
HIV-infected	1437/1877 (77)	247/344(72)	0.8 (0.6-1.0)	0.056		
Mine Exposure	241/2067 (12)	31/384 (8)	0.7 (0.5-1.9)	0.041		
Influenza- positive	113/2053 (6)	18/376 (5)	0.9 (0.5-1.4)	0.572		
Pneumococal coinfection***	254/1901 (13)	34/346 (10)	0.7 (0.5-1.0)	0.072		
Viral co-infection	588/2053 (29)	72/376 (19)	0.6 (0.4-0.8)	<0.001		
Required oxygen	718/2029 (35)	151/377 (40)	1.2 (0.9-1.5)	0.084		
Started on antibiotics	1975/2056 (96)	357/385 (93)	0.5 (0.3-0.8)	0.004		
Started on TB treatment***	892/1918 (46)	164/342 (48)	1.1 (0.8-1.3)	0.621		
Duration of hospitalization						

<3 days	349/1918 (18)	45/354 (13)	Reference			
3-7 days	805/1918 (42)	137/354 (39)	1.3 (0.9-1.9)	0.130	1.3 (0.9-1.9)	0.167
8+ days	764/1918 (40)	172/354 (49)	1.8 (1.2-2.9)	0.002	1.7 (1.1-2.4)	0.007
Died during admission	219/2001 (11)	100/369 (27)	3.0 (2.3-3.9)	<0.001	3.0 (2.3-4.1)	<0.001

Underlying conditions included any of the following: Asthma, other chronic lung disease, chronic heart disease (valvular heart disease, coronary artery disease, or heart failure excluding hypertension), liver disease (cirrhosis or liver failure), renal disease (nephrotic syndrome, chronic renal failure), immunocompromising conditions excluding HIV infection (organ transplant, immunosuppressive therapy, immunoglobulin deficiency, malignancy), neurological disease (cerebrovascular accident, spinal cord injury, seizures, neuromuscular conditions) or pregnancy. Comorbidities were considered absent in cases for which the medical records stated that the patient had no underlying medical condition or when there was no direct reference to that condition.

***lytA* PCR positive for *Streptococcus pneumoniae* on blood specimen

*** started on TB treatment at current admission (excludes those already on TB treatment at time of admission (n=144))

Supplementary table 2: Demographic and clinical factors associated with acute (≤14 days) vs. chronic (>14 days) presentation among HIV-infected individuals ≥ 15 years with laboratory- confirmed tuberculosis, July 2012-August 2014 (N=424)

		Acute presentation n/N (%)	Chronic presentation n/N (%)	Univariate analysis OR (95% CI)	P-value	Multivariable analysis OR (95% CI)	P-value
Age	15-24	7/102 (7)	26/322 (8.1)	Reference		Reference	
	25-44	78/102 (76)	222/ 322 (69)	1.3 (0.5-3.1)	0.550	1.3 (0.4-3.9)	
	45-64	16/102 (16)	69/322 (21)	0.9 (0.3-2.3)	0.769	0.6 (0.2-2.2)	
	65+	1/102 (1)	5/322 (2)	0.7 (0.1-7.4)	0.800	2.4 (0.2-35.5)	
Female gender		60/102 (59)	167/322 (52)	1.3 (0.8-2.1)	0.220		
Tshepong Hospital		69/102 (68)	220/322 (68)	1.0 (0.6-1.6)	0.898		
History of any cough		89/102 (87)	312/322 (97)	0.2 (0.1-0.5)	0.001	0.2 (0.1-0.7)	0.009
History of fever		82/178 (46)	205/322 (42)	1.3 (0.8-2.0)	0.249		

Night sweats	61/101 (60)	238/321 (74)	0.5 (0.3-0.9)	0.009	0.4 (0.2-0.7)	0.005
Underlying medical condition*	2/102 (2)	12/322 (4)	0.5 (0.1-2.3)	0.393		
Diabetes	1/102 (1)	2/322 (1)	1.2 (0.1-17.6)	0.708		
Low CD4 count**	44/72 (61)	184/238 (77)	0.5 (0.3-0.8)	0.007	0.4 (0.2-0.7)	0.003
Mine Exposure[€]	7/101 (7)	40/316 (13)	0.5 (0.2-1.2)	0.119		
Influenza- positive	8/100 (8)	11/317 (3)	2.4 (0.9-6.2)	0.066		
Pneumococcal coinfection***	13/97 (13)	18/292 (6)	2.4 (1.1-5.0)	0.026		
Viral co-infection	35/100 (35)	92/317 (29)	1.3 (0.8-2.1)	0.258		
Required oxygen	38/100 (38)	81/317 (25)	1.2 (1.1-2.9)	0.017		
Started on antibiotics	96/100 (96)	302/320 (94)	1.4 (0.5-4.3)	0.526		
Started on TB treatment****	61/95 (64)	244/296 (82)	0.4 (0.2-0.6)	<0.001	0.4 (0.2-0.7)	0.003
Receiving HAART	33/72 (46)	146/244 (46)	0.9 (0.6-1.7)	0.943		
Duration of hospitalization						

<3 days	14/94 (15)	49/302 (16)	Reference	
3-7 days	42/94 (45)	37/302 (45)	1.1 (0.5-2.1)	0.841
8+ days	38/94 (40)	116/302 (38)	1.1 (0.6-2.3)	0.701
Died during admission	8/100 (8)	35/312 (11)	0.7 (0.3-1.5)	0.362

Underlying conditions included any of the following: Asthma, other chronic lung disease, chronic heart disease (valvular heart disease, coronary artery disease, or heart failure excluding hypertension), liver disease (cirrhosis or liver failure), renal disease (nephrotic syndrome, chronic renal failure), immunocompromising conditions excluding HIV infection (organ transplant, immunosuppressive therapy, immunoglobulin deficiency, malignancy), neurological disease (cerebrovascular accident, spinal cord injury, seizures, neuromuscular conditions) or pregnancy. Comorbidities were considered absent in cases for which the medical records stated that the patient had no underlying medical condition or when there was no direct reference to that condition.

*****lytA*** PCR positive for *Streptococcus pneumoniae* on blood specimen

*** CD4 categorized as Normal (CD4+ T-lymphocytes ≥200) or low (CD4+ T-lymphocytes <200/mm3)

**** Started on TB treatment at current admission; excludes those already on TB treatment at time of admission (n=28)

$^{\epsilon}$ Any history of having worked in the mine

PAPER III

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RESEARCH ARTICLE Open Access

Influenza virus infection is associated with increased risk of death amongst patients hospitalized with confirmed pulmonary tuberculosis in South Africa, 2010–2011

Sibongile Walaza[1,2*], Stefano Tempia[3,4], Halima Dawood[6], Ebrahim Variava[8,9], Jocelyn Moyes[1,2], Adam L Cohen[3,4], Nicole Wolter[1], Michelle Groome[5], Claire von Mollendorf[1,2], Kathleen Kahn[7], Marthi Pretorius[1], Marietjie Venter[1,10], Shabir A Madhi[1,5] and Cheryl Cohen[1,2*]

Abstract

Background: Data on the association between influenza and tuberculosis are limited. We describe the characteristics of patients with laboratory-confirmed tuberculosis, laboratory-confirmed influenza and tuberculosis-influenza co-infection.

Methods: Patients hospitalized with severe respiratory illness (acute and chronic) were enrolled prospectively in four provinces in South Africa. Naso/oropharyngeal specimens were tested for influenza virus by real time reverse transcriptase polymerase chain reaction. Tuberculosis testing was conducted as part of clinical management.

Results: From June 2010 through December 2011, 8032 patients were enrolled and influenza testing was conducted on 7863 (98%). Influenza virus was detected in 765 (10%) patients. Among 2959 patients with tuberculosis and influenza results, 2227 (75%) were negative for both pathogens, 423 (14%) were positive for tuberculosis alone, 275 (9%) were positive for influenza alone and 34 (1%) had influenza and tuberculosis co-infection. On multivariable analysis amongst individuals with symptoms for ≥7 days, tuberculosis influenza co-infection was associated with increased risk of death, (adjusted relative risk ratio (aRRR) (6.1, 95% confidence interval (CI) 1.6-23.4), as compared to tuberculosis only infection. This association was not observed in individuals with symptoms for <7 days (aRRR.0.8, 95% CI 0.1-7.0).

Conclusion: Tuberculosis and influenza co-infection compared to tuberculosis single infection was associated with increased risk of death in individuals with symptoms ≥7 days. The potential public health impact of influenza vaccination among persons with laboratory-confirmed tuberculosis should be explored.

Keywords: Influenza, Tuberculosis, Co-infection, South Africa

Background

Despite the availability of efficacious treatment, tuberculosis remains the second most common cause of infectious disease–related deaths worldwide, after human immunodeficiency virus (HIV) and acquired immune deficiency syndrome (AIDS) [1]. In 2010, there were an estimated 8.8 million incident cases of tuberculosis globally [2]. HIV infection is an important risk factor for tuberculosis, and HIV-infected individuals have a 20 times greater risk of developing tuberculosis as compared to HIV-uninfected individuals [3]. In South Africa, there were an estimated 390,000 incident cases of tuberculosis in 2011 and 65% were co-infected with HIV [1].

Similarly, despite the availability of effective vaccines, influenza virus infection causes substantial annual morbidity and mortality worldwide and in South Africa [4-6]. Globally, it is estimated that influenza epidemics result in three to five million cases of severe illness, and about

* Correspondence: sibongilew@nicd.ac.za; cherylc@nicd.ac.za
[1]Centre for Respiratory Disease and Meningitis, National Institute for Communicable Diseases (NICD) of the National Health Laboratory Service (NHLS), Private Bag X4, Sandringham, 2131 Johannesburg, Gauteng, South Africa
[2]School of Public Health, Faculty of Health Science, University of the Witwatersrand, Johannesburg, South Africa
Full list of author information is available at the end of the article

250,000-500,000 deaths annually [4]. In 2010 an estimated 12 million episodes of severe and 3 million episodes of very severe acute lower respiratory tract infection resulted in hospital admissions in children <5 years worldwide [5]. A number of risk factors are described for severe outcomes among patients with influenza; these include extremes of age, chronic underlying diseases, pregnancy and immune-suppression [6]. HIV-infected individuals have an increased risk of influenza-associated hospitalisations and mortality [7].

While chronic lung diseases are a known risk factor for severe outcome due to influenza infection, there are few published data on the association between influenza and tuberculosis. Review of data from the 1918 influenza pandemic suggests that individuals with tuberculosis had a disproportionately higher mortality than the general population [8,9] and that underlying tuberculosis infection may have contributed to the elevated mortality observed in young adults [8,10]. There is little information about the interaction between seasonal or pandemic influenza and tuberculosis in recent years. In South Africa, 10% of cases that died during the 2009 A(H1N1)pdm09 pandemic were co-infected with tuberculosis [11]. In South Korea, concurrent tuberculosis and influenza A(H1N1) pdm09 infection was reported in 7 immunocompetent patients, none resulting in fatal outcome [12].

We describe the epidemiological and clinical characteristics of patients admitted with laboratory-confirmed tuberculosis, influenza and tuberculosis-influenza co-infection among patients hospitalized with severe respiratory illness (SRI) in South Africa, a country with a high prevalence of HIV.

Methods

Study design

We conducted prospective hospital-based active surveillance for Severe Acute Respiratory Illness (SARI) surveillance at 6 public hospitals in 4 surveillance sites situated across the country: Chris Hani Baragwanath Academic Hospital (CHBAH), Gauteng Province; Edendale Hospital, KwaZulu-Natal Province; the two hospitals of the Klerksdorp–Tshepong Hospital Complex (KTHC), Northwest Province; and Mapulaneng and Matikwana Hospitals, Mpumalanga Province [13]. At each sentinel hospital trained surveillance officers screened all admitted patients for surveillance inclusion criteria from Monday to Friday, except at CHBAH where adult patients were screened on 2 of every 5 days per week because of large patient numbers.

Case definitions

A case of SARI was defined as a hospitalised individual with onset of illness within seven days of admission meeting age-specific clinical inclusion criteria. We included children aged two days through <3 months with physician-diagnosed sepsis or acute lower respiratory tract infection (ALRI), children aged three months through <5 years with physician-diagnosed ALRI (including, for example bronchitis, bronchiolitis, pneumonia and pleural effusion) and patients aged ≥5 years meeting a modified World Health Organization (WHO) case definition for SARI including sudden onset of fever (>38°C) or reported fever, cough or sore throat, and shortness of breath or difficulty breathing [14]. In addition expanded case definitions were applied at three of the hospitals in two of the surveillance sites, (Edendale Hospital and KTHC) to include individuals meeting the above case definition but with symptoms for >7 days and individuals with suspected or confirmed tuberculosis; these patients were defined as having severe chronic respiratory illness (SCRI). For this analysis severe respiratory illness (SRI) was used to refer to all enrolled patients i.e. patients admitted with an acute or chronic respiratory illness.

The South African National Department of Health tuberculosis case definitions were modified to define suspected tuberculosis [15]. A suspected tuberculosis case in children <12 years was defined as: i) any child presenting with a history of exposure to an infectious tuberculosis case or with a positive tuberculin skin test and symptoms of tuberculosis (chronic cough, weight loss and fever) with or without an abnormal chest x-ray suggestive of tuberculosis, or ii) admitted with a physician diagnosis of tuberculosis or suspected tuberculosis, or iii) initiated on tuberculosis treatment on admission; or iv) was on tuberculosis treatment for <2 months at the time of admission. A suspected tuberculosis case in individuals ≥12 years of age was defined as: i) any patient presenting with night sweats, chronic cough, weight loss or haemoptysis lasting for >2 weeks, or ii) had a physician diagnosis of tuberculosis or suspected tuberculosis, or iii) was initiated on tuberculosis treatment on admission, or iv) was on tuberculosis treatment for <2 months at the time of admission.

Patients who were on tuberculosis treatment for ≥2 months at the time of admission were not enrolled into the surveillance programme as cases of suspected or confirmed tuberculosis (because we assumed that after two months of treatment tuberculosis was no longer the reason for their clinical presentation). These patients were, however, enrolled if they met the criteria for SARI. The tuberculosis status of the enrolled patients was based on results of tuberculosis testing at current admission.

For this analysis, a laboratory-confirmed tuberculosis case was defined as an individual with a positive result for *Mycobacterium tuberculosis* on microscopy, culture or polymerase chain reaction (PCR) by GeneXpert MTB/RIF test (Cepheid, Sunnyvale, California) from the current hospital admission or from a specimen taken within two weeks preceding or following the admission. An influenza

case was defined as an individual with a positive PCR test for influenza. An influenza-tuberculosis co-infection case met criteria for both laboratory-confirmed tuberculosis and influenza during the same admission.

Data collection

A standardized questionnaire was used to collect demographic and clinical data, medical history of the patient and in-hospital outcome. Hospital and intensive care unit (ICU) admission and collection of specimens for bacterial culture, tuberculosis testing and CD4+ T-cell counts were performed according to attending-physician discretion.

Sample collection and processing

Respiratory specimens (oropharyngeal and nasopharyngeal swabs for patients ≥5 years of age or nasopharyngeal aspirates for children <5 years of age), were collected and placed in 4 ml virus transport medium. Whole blood samples were collected in EDTA-containing vacutainer tubes within 24 hours of hospital admission for the detection of *Streptococcus pneumoniae* and HIV infection.

After collection, respiratory and blood samples were kept at 4°C at the sentinel site, and transported on ice at least twice per week to the National Institute for Communicable Diseases of the National Health Laboratory Services (NICD-NHLS) for testing.

Detection of respiratory viruses and *S. pneumoniae*

Respiratory samples were tested by multiplex real-time reverse-transcription polymerase chain reaction (PCR) (rRT-PCR) for influenza type A and B, adenovirus, enterovirus, rhinovirus, human metapneumovirus, respiratory syncytial virus and parainfluenza virus types 1–3 [16]. The influenza A positive specimens were subtyped using the Centers for Disease Control and Prevention (CDC) rRT-PCR protocol for detection and characterization of influenza. DNA was extracted from whole blood specimens using the MagNA Pure LC 2.0 instrument and DNA Isolation kit III for bacteria (Roche, Mannheim, Germany) and were tested for the *S. pneumoniae lytA* gene by a quantitative real-time PCR [17].

Determination of HIV infection

HIV status data were obtained from two data sources. Some patients had HIV testing requested by admitting physicians as part of clinical care. This included HIV enzyme-linked immunosorbent assay (ELISA) testing with confirmation by ELISA on a second specimen for patients ≥18 months of age and qualitative HIV PCR testing for confirmation of HIV-infection status in children <18 months of age. In addition, for consenting patients, linked anonymous HIV PCR testing for children <18 months of age or ELISA for patients ≥18 months of age was performed using a dried blood spot or whole blood specimen at the NICD-NHLS laboratory.

Determination of tuberculosis infection

Testing for *M. tuberculosis*, including microscopy, culture, GeneXpert MTB/RIF test, or a combination of these, was undertaken at the discretion of the attending-physician and performed at the laboratory serving the hospital where the patient presented. Specimens for tuberculosis testing were examined by light microscopy for the presence of acid fast bacilli. Tuberculosis culture was performed using the BACTEC MGIT automated culture system (Becton Dickinson, Franklin Lakes, New Jersey). Tuberculosis PCR was performed using the Xpert MTB/RIF system (Cepheid, Sunnyvale, California). The GeneXpert MTB/RIF is an automated sample processing and nucleic acid amplification test for detection of *M. tuberculosis* and is able to detect resistance to rifampicin. The National Department of Health tuberculosis treatment guidelines recommended treatment for patients with positive smears and that patients suspected of tuberculosis should have two specimens tested on microscopy and if these were negative then a 3rd sample should be tested for smear and culture [15].

Data analysis

To identify factors associated with tuberculosis testing, tuberculosis positivity and tuberculosis-influenza co-infection we included both potential determinants for, as well as outcomes or characteristics of the primary endpoints of the analysis. Univariate comparisons were performed using logistic or multinomial regression. In addition, we implemented three multivariable models to identify factors associated with: (i) tuberculosis testing among enrolled patients; (ii) tuberculosis positivity among enrolled patients tested for tuberculosis; and (iii) tuberculosis single infection compared to influenza single infection or tuberculosis-influenza co-infection. The tuberculosis testing and positivity models were implemented using stepwise forward selection logistic regression. Multinomial regression was used for the comparison of tuberculosis only, influenza only and tuberculosis-influenza co-infected groups and this analysis was performed separately in individuals with symptoms for <7 days and ≥7 days. Multinomial regression allows modelling of outcome variables with >2 categories and relates the probability of being in category j to the probability of being in a baseline category. A complete set of coefficients are estimated for each of the j levels being compared with the baseline and the effect of each predictor in the model is measured as relative risk ratio (RRR). For this analysis, we used the tuberculosis single infection group as the baseline category and compared it with the influenza single infection and tuberculosis-influenza co-infection

groups and we restricted the analysis to laboratory confirmed cases for both influenza and tuberculosis. The general form of the multivariable multinomial model with dichotomous predictors was as follows:

$$\ln\frac{p(Y_j)}{p(Y_1)} = \beta_0^{(j)} + \beta_1^{(j)}X_1 + \ldots + \beta_n^{(j)}X_n + \varepsilon \quad (1)$$

Where $p(Y_1)$ is the probability of the outcome variable in the base category (tuberculosis single infection) and $p(Y_j)$ is the probability of the outcome variable in the other categories; j represents the two other categories ($j = 2$: influenza single infection; and $j = 3$: tuberculosis-influenza coinfection) that are compared to the base category; $\beta_0^{(j)}$ is the model constant for category j; $\beta_1^{(j)}$ to $\beta_n^{(j)}$ are coefficients associated with predictors X_1 to X_n in category j; and ε is the error term.

The model in equation 1 was fitted to any enrolled case with available tuberculosis and influenza results irrespective of duration of symptoms as well as separately among individuals with acute (duration of symptoms <7 days) and chronic (duration of symptoms ≥7 days) clinical presentation to specifically assess the clinical characteristics of individuals within these two groups. In order to allow for direct comparison of the final multivariable models for acute and chronic patients we elected to retain any variable which was significant at 0.05 in either one of these models in the final multivariable model. Covariates with a p-value <0.2 at the univariate analysis were assessed for significance at the multivariable analysis and statistical significance was assessed at $p < 0.05$ for all models. Statistically significant variables were retained in the multivariable models. HIV status was retained in the multinomial models a priori as this is a potential confounder of the interaction between influenza and tuberculosis. Two-way interactions were assessed by inclusion of product terms for all variables remaining in the final additive models. The statistical analysis was implemented using STATA® version 12 (StataCorp, Texas, USA).

Ethics

Ethical approval for the study was obtained from the University of the Witwatersrand, Human Research Ethics committee (reference M081042) and the University of KwaZulu-Natal Biomedical Research Ethics committee (reference BF157/08). The United States Centers for Disease Control and Prevention deemed this surveillance not to be research and therefore this project did not need human subjects review.

Results

Patient enrolment and testing

From June 2010 through December 2011, we enrolled 8032 patients, and influenza results were obtained from 7863 (98%) cases (Figure 1). Of these 4077 (52%) were females and 3291 (42%) were children ≤5 years. Influenza

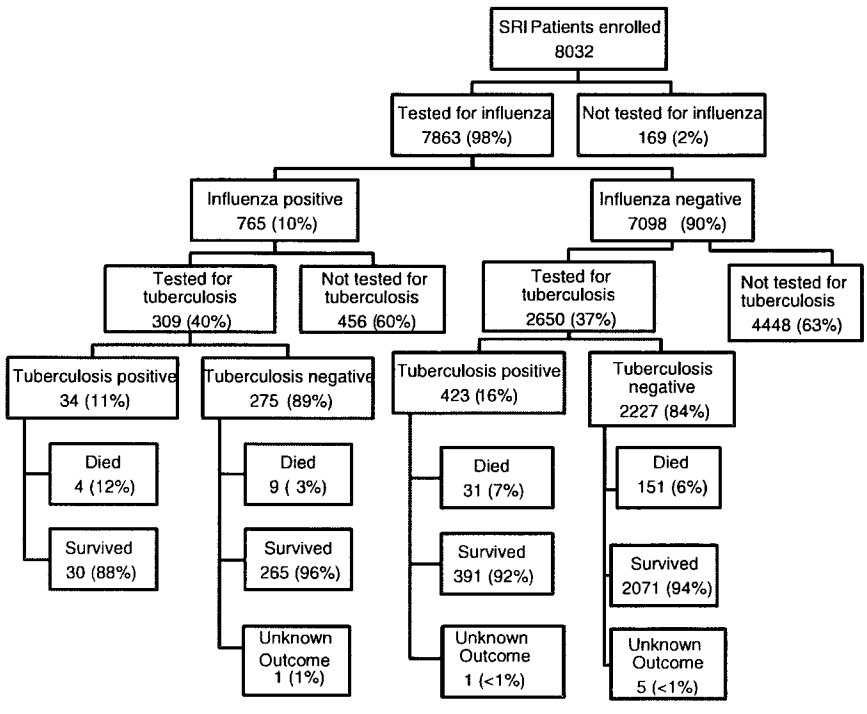

Figure 1 Flow chart of patients enrolled into the severe respiratory illness (acute and non acute) surveillance, South Africa 2009-2011.

virus was detected in 765 (10%) patients. An influenza subtype was characterized from 764 (99.8%) influenza positive samples, including 219 (29%) influenza A(H1N1)pdm09, 195 (25%) A(H3N2) and 350 (46%) influenza B. Among patients with available influenza results, 2959 (38%) were tested for tuberculosis during admission. Of these, 69% (2029) and 31% (930) met the SARI and SCRI case definitions, respectively. HIV results were available for 2739 (93%) of 2959 patients with available tuberculosis results and 1877 (69%) were HIV-infected. Among influenza tested patients with available data <1% (6/7863) received oseltamivir and <1% (4/7857) were vaccinated against influenza. Two percent (179/7863) of enrolled patients with results for influenza and 2% (49/2959) of those tested for influenza and tuberculosis were already receiving tuberculosis treatment at time of current admission.

Factors associated with tuberculosis testing on admission

Among patients with available influenza results, on multivariable analysis factors associated with tuberculosis testing were age ≥5 years, with the highest proportion tested (56%, 1378/2473) in the 25–44 age group (adjusted odds ratio (aOR) 3.5, 95% CI 3.0-4.2) as compared to age <5 years, HIV infection (aOR 1.5, 95% CI 1.3- 1.7), symptom duration ≥7 days (aOR 1.3, 95% CI 1.1-1.5), hospitalisation duration >7 days (aOR 1.3, 95% CI 1.1- 1.4), requiring oxygen therapy (aOR 1.2, 95% CI 1.0-1.3) and antibiotic (aOR 1.9, 95% CI 1.4-2.5) or tuberculosis (aOR 1.2, 95% CI 1.0-1.4) treatment started during current admission (Table 1). The proportion of patients tested for tuberculosis was similar for patients testing influenza-positive and influenza-negative and for those who died in-hospital and those who survived.

Factors associated with laboratory-diagnosed tuberculosis

Among 2959 patients with available tuberculosis and influenza results, 15% (457/2959) tested positive for tuberculosis on the current admission and 2% (10/457) of these were receiving tuberculosis treatment at time of current admission. The highest percentage testing tuberculosis positive was in the 25–44 years age group (18%, 249/1378) (Table 2). Of the patients testing positive for tuberculosis, 332 (73%) were positive on microscopy only. The tuberculosis detection rate was 10% among those with symptoms for <7 days and 25% among those with symptoms for ≥7 days. Of the 457 laboratory-confirmed tuberculosis cases, 198 (43%) and 259 (57%) met the SARI and SCRI case definitions respectively. On multivariable analysis, patients with laboratory-diagnosed tuberculosis were less likely to be co-infected with *S. pneumoniae* (aOR 0.5, 95% CI 0.3-0.9) and to have received oxygen therapy (aOR 0.7, 95% CI 0.5- 0.9). They were more likely to be admitted at KTHC as compared to CHBAH (aOR 1.9, 95% CI 1.4-2.6) and to be started on tuberculosis treatment at current admission (aOR 4.6, 95% CI 3.5-6.0) (Table 2).

HIV results were available for 93% (424/457) of tuberculosis-positive and 92% (2315/2502) of tuberculosis-negative individuals. Of these, 335 (79%) of tuberculosis-positive and 1542 (67%) of tuberculosis-negative individuals were HIV infected. Amongst tuberculosis-positive patients tested for HIV, age-specific HIV prevalence were 36% (15/42), 79% (35/44), 95% (222/234), 65% (58/89), 33% (5/15) in the <5 years, 5–24 years, 25–44 years, 45–64 years and ≥65 years age groups, respectively. Amongst tuberculosis-negative patients tested for HIV, age-specific HIV prevalence were 19% (78/417), 71% (147/206), 89% (966/1081), 65% (325/502) and 24% (26/109) in the <5 years, 5–24 years, 25–44 years, 45–64 years and ≥65 years age groups, respectively.

Factors associated with tuberculosis and influenza infection

Among the 2959 patients with available tuberculosis and influenza results, 2227 (75%) were negative for both pathogens, 423 (14%) were positive for tuberculosis alone, 275 (9%) were positive for influenza alone and 34 (1%) had influenza and tuberculosis co-infection. Tuberculosis treatment was being received at the time of admission for 2% (n = 10) of patients positive for tuberculosis only and <1% (n = 2) of patients positive for influenza only. None of the patients with influenza and tuberculosis co-infection were on tuberculosis treatment at the time of admission.

On multivariable analysis adjusting for age group, patients infected with influenza only compared to patients with tuberculosis only were less likely to be HIV infected (adjusted relative risk ratio (aRRR) 0.6, 95% CI 0.4-0.9), to present with duration of symptoms ≥7 days (aRRR 0.3, 95% CI 0.2-0.4), to be admitted for longer than 7 days (aRRR 0.6, 95% CI 0.4-0.9) while they were more likely to be started on oxygen therapy (aRRR 1.9, 95% CI 1.3-2.8) (Table 3). Patients co-infected with tuberculosis and influenza compared to patients with tuberculosis only were less likely to present with symptoms ≥7 days (aRRR 0.3, 95% CI 0.1-0.7), but were at increased risk of death (aRRR 3.1, 95% CI 1.1-10.1).

Of the 720 patients testing positive for influenza and/or tuberculosis, 384 (53%) presented with symptom duration <7 days. Of these, 166 (43%) were positive for tuberculosis alone, 194 (50%) were positive for influenza alone and 24 (6%) had tuberculosis-influenza co-infection. Of the 336/720 (47%) patients testing positive for influenza and/or tuberculosis with symptom duration ≥7 days, 246 (73%) were positive for tuberculosis alone, 81 (24%) were positive for influenza alone and 9 (3%) had tuberculosis-influenza co-infection.

Table 1 Demographic and clinical characteristics of patients admitted with severe respiratory illness (acute and chronic), by tuberculosis testing status at four sites in South Africa, June 2010- December 2011

Characteristic		Tested for tuberculosis n/N (%)	Unadjusted odds ratio (95% CI)	P value	Adjusted odds ratios (95% CI)	P value
Age group (years)	0-4	571/3291 (17)	Reference		Reference	
	5-24	270 /587 (46)	4.05 (3.4-4.9)	<0.001	3.3 (2.6-4.1)	<0.001
	25-44	1378/2473 (56)	5.99 (5.3-6.8)	<0.001	3.5 (3.0-4.2)	<0.001
	45-64	611/1197 (51)	4.96 (4.3-5.7)	<0.001	3.2 (2.7-3.8)	<0.001
	≥65	129/315 (39)	3.30 (2.6-4.2)	<0.001	2.6 (2.0-3.4)	<0.001
Sex	Female	1664/4077 (41)	Reference			
	Male	1295/3786 (34)	0.8 (0.7-0.8)	<0.001		
Site	Chris Hani Baragwanath	1618/4437 (36)	Reference		Reference	
	Mapulaneng & Matikwana	144/749 (19)	0.4 (0.3-0.5)	<0.001	0.4 (0.3-0.5)	<0.001
	Edendale	385/1156 (33)	0.9 (0.8-0.99)	0.046	0.7 (0.6-0.8)	<0.001
	Klerksdorp/Tshepong	812/1521 (53)	1.99 (1.8-2.2)	<0.001	1.1 (0.95-1.3)	0.152
Influenza	Negative	2650/7098 (37)	Reference			
	Positive	309/765 (40)	1.1 (0.9-1.3)	0.097		
HIV status	Negative	862/3410 (25)	Reference		Reference	
	Positive	1877/3523 (53)	3.4 (3.0-3.7)	<0.001	1.5 (1.3-1.7)	<0.001
Underlying medical condition*	No	2652/7041 (38)	Reference			
	Yes	307/821 (37)	0.98 (0.85-1.14)	0.879		
Duration of symptoms prior to admission (days)	<7	1884/5957(32)	Reference		Reference	
	≥7	1012/1765 (57)	2.9 (2.6-3.2)	<0.001	1.3 (1.1-1.5)	0.003
Concurrent invasive bacterial infection**	No	653/1832 (36)	Reference			
	Yes	24/46(52)	1.96 (1.1- 3.5)	0.023		
Pneumococcal	No	2435/6284 (39)	Reference			
Infection***	Yes	214/434 (49)	1.5 (1.3-1.9)	<0.001		
Receiving tuberculosis treatment at time of admission	No	158/292 (54)	Reference	<0.001		
	Yes	49/179 (27)	0.3 (0.2-0.5)			
Started on treatment for tuberculosis	No	2301/6716 (34)	Reference		Reference	
	Yes	647/1108 (58)	2.7 (2.4-3.1)	<0.001	1.2 (1.0-1.4)	0.036

Table 1 Demographic and clinical characteristics of patients admitted with severe respiratory illness (acute and chronic), by tuberculosis testing status at four sites in South Africa, June 2010- December 2011 *(Continued)*

Antibiotics prescribed	No	77/332 (23)	Reference		Reference	
	Yes	2877/7515 (38)	2.1 (1.6-2.7)	<0.001	1.9 (1.4-2.5)	<0.001
Oxygen therapy	No	1869/5145 (36)	Reference			
	Yes	1086/2698 (40)	1.2 (1.1-1.3)	0.001	1.2 (1.0-1.3)	0.018
ICU admission	No	2936/7772 (38)	Reference			
	Yes	15/65 (23)	0.5 (0.3-0.9)	0.017		
Duration of hospitalisation (days)	≤7	1913/5666 (34)	Reference		Reference	
	>7	1029/2143 (48)	2.9 (2.6-3.2)	<0.001	1.2 (1.1-1.4)	0.002
Died	No	2757/7321 (38)	Reference	Reference		
	Yes	195/509 (38)	1.02 (0.9-1.2)	0.769		

HIV – human immunodeficiency virus; ICU– intensive care unit, TB-Tuberculosis.

*Underlying conditions included any of the following: Asthma, other chronic lung disease, chronic heart disease (valvular heart disease, coronary artery disease, or heart failure excluding hypertension), liver disease (cirrhosis or liver failure), renal disease (nephrotic syndrome, chronic renal failure), diabetes mellitus, immunocompromising conditions excluding HIV infection (organ transplant, immunosuppressive therapy, immunoglobulin deficiency, malignancy), neurological disease (cerebrovascular accident, spinal cord injury, seizures, neuromuscular conditions) or pregnancy. Comorbidities were considered absent in cases for which the medical records stated that the patient had no underlying medical condition or when there was no direct reference to that condition.

**Concurrent invasive bacterial infections were defined as a bacterial pathogen isolated from blood, cerebrospinal fluid or another sterile site from a specimen taken within 48 hours of hospitalisation; organisms viewed as likely contaminants were excluded.

***Pneumococcal co-infection (lyt A PCR positive for *Streptococcus pneumoniae* on blood specimen).

Other variables evaluated but not presented in the table because they were not significant on univariate analysis were, Oseltamivir treatment prescribed and smoking and alcohol intake for patients ≥12 years.

Table 2 Demographic and clinical characteristics associated with laboratory-confirmed tuberculosis among patients admitted with severe respiratory illness (acute and chronic) that were tested for tuberculosis and influenza at four sites in South Africa, June 2010- December 2011

Characteristic		Laboratory confirmed tuberculosis n/N (%)	Unadjusted odds ratio (95% CI)	P value	Adjusted odds ratios (95% CI)	P value
Age group (years)	0-4	55/571 (10)	Reference		Reference	
	5-24	47/270 (17)	1.97 (1.3-3.0)	0.001	1.36 (0.8-2.3)	0.233
	25-44	249/1378 (18)	2.1 (1.5-2.8)	<0.001	1.37 (0.9-2.06)	0.120
	45-64	90/611 (15)	1.6 (1.1-2.3)	0.008	1.11 (0.7-1.7)	0.628
	65+	16/129 (12)	1.3 (0.7-2.4)	0.348	0.94 (0.5-1.9)	0.872
Site	Chris Hani Baragwanath	141/1618 (9)	Reference		Reference	
	Mapulaneng & Matikwana	23/144 (16)	1.99 (1.2-3.2)	0.005	1.4 (0.8-2.3)	0.228
	Edendale	49/385 (13)	1.5 (1.1-2.2)	0.016	0.9 (0.6-1.4)	0.668
	Klerksdorp/Tshepong	244/812 (30)	4.5 (3.6-5.6)	<0.001	1.9 (1.4-2.6)	<0.001
Influenza status	Negative	423/2650 (16)	Reference			
	Positive	34/309 (11)	0.7 (0.5-0.9)	0.023		
HIV status	Negative	89/862 (10)	Reference			
	Positive	335/1877 (18)	1.9 (1.5-2.4)	<0.001		
Underlying medical condition*	No	426/2652 (16)	Reference			
	Yes	31/307 (10)	0.6 (0.4-0.9)	0.007		
Duration of symptoms prior to admission	<7	190/1884 (10)	Reference			
	≥7	255/1012 (25)	3.0 (2.4-3.7)	<0.001		
Pneumococcal co-infection on PCR**	No	400/2435 (16)	Reference		Reference	
	Yes	18/214 (8)	0.5 (0.3-0.8)	0.003	0.5 (0.3-0.9)	0.016
Oxygen therapy	No	337/1869 (18)	Reference		Reference	
	Yes	120/1086 (11)	0.6 (0.4-0.7)	<0.001	0.7 (0.5- 0.9)	0.007
Receiving TB treatment at time of admission	No	25/158 (16)	Reference			
	Yes	10/49 (20)	1.4 (0.6-3.1)	0.456		
Started on TB treatment	No	205/2301 (9)	Reference		Reference	
	Yes	251/647 (39)	6.5 (5.2-8.0)	<0.001	4.6 (3.5-6.0)	<0.001
Duration of hospitalisation (days)	≤7	279/1913 (14)	Reference			
	>7	174/1029 (17)	1.2 (0.96-1.6)	0.096		
Season	Summer	57/366 (15)	Reference			
	Autumn	78/449 (17)	1.1 (0.8-1.6)	0.492		
	Winter	178/1027 (17)	1.1 (0.8-1.6)	0.441		
	Spring	144/1117 (13)	0.8 (0.6-1.1)	0.194		
Died	No	421/2757 (15)	Reference			
	Yes	35/195 (18)	1.2 (0.8-1.8)	0.318		

HIV – human immunodeficiency virus; ICU– Intensive Care Unit, TB-Tuberculosis, CI – confidence interval.
Analysis includes 2029 individuals with severe acute respiratory illness and 930 with severe chronic respiratory illness.
*Underlying conditions included any of the following: Asthma, other chronic lung disease, chronic heart disease (valvular heart disease, coronary artery disease, or heart failure excluding hypertension), liver disease (cirrhosis or liver failure), renal disease (nephrotic syndrome, chronic renal failure), diabetes mellitus, immunocompromising conditions excluding HIV infection (organ transplant, immunosuppressive therapy, immunoglobulin deficiency, malignancy), neurological disease (cerebrovascular accident, spinal cord injury, seizures, neuromuscular conditions) or pregnancy. Comorbidities were considered absent in cases for which the medical records stated that the patient had no underlying medical condition or when there was no direct reference to that condition.
**_lyt A_ PCR positive for *Streptococcus pneumoniae* on blood specimen.
Other variables that were evaluated but not presented in the table because they were not significant on univariate analysis were sex, influenza type, concurrent bacterial infection, antibiotic prescribed on admission, admission to intensive care unit, and smoking and alcohol intake for patients ≥12 years.

Table 3 Factors associated with influenza infection and tuberculosis-influenza co-infection compared with tuberculosis infection only in patients admitted with severe respiratory illness (acute and chronic) and tested for tuberculosis and influenza at four sites in South Africa, June 2010- December 2011

Variables		Laboratory confirmed tuberculosis Reference[a]	Laboratory confirmed influenza			Laboratory confirmed tuberculosis-influenza co-infection		
			n/N (%)	RRR[b]	ARRR[c] (95% CI)	n/N (%)	RRR[b]	ARRR[c] (95% CI)
Age group (years)	0-4	47/423 (11)	46/275 (10)	Reference	Reference	8/34 (22)	Reference	Reference
	5-24	44/423 (10)	28/275 (11)	0.6 (0.3-1.2)	1.3 (0.6-2.9)	3/34 (9)	0.4 (0.1-1.6)	0.5 (0.1-2.5)
	25-44	231/423 (55)	121/275 (44)	0.5 (0.3-0.8)	1.5 (0.8-2.8)	18/34 (53)	0.5 (0.2-1.2)	0.5 (0.2-1.7)
	45-64	86/423 (20)	57/275 (21)	0.7 (0.4-1.1)	1.3 (0.7-2.5)	4/34 (12)	0.3 (0.1-0.9)	0.3 (0.1-2.5)
	65+	15/423 (3)	23/275 (8)	1.6 (0.7-3.4)	3.9 (1.6-9.8)	1/34 (3)	0.3 (0.04-3.4)	9.53e-07
Sex	Female	247/423 (58)	176/275 (64)	Reference		20/34 (59)	Reference	
	Male	176/176 (42)	99/275 (36)	0.8 (0.6-1.1)		14/34 (41)	1.0 (0.5-1.9)	
Site	Chris Hani Baragwanath	124/423 (29)	169/275 (61)	Reference		17/34 (50)	Reference	
	Mapulaneng & Matikwana	21/423 (5)	11/275 (4)	0.4 (0.2-0.8)		2/34 (6)	0.7 (0.1-3.2)	
	Edendale	48/423 (11)	22/275 (8)	0.3 (0.2-0.6)		1/34 (3)	0.2 (0.01-1.2)	
	Klerksdorp/ Tshepong	230/423 (54)	73/275 (27)	0.2 (0.2-0.3)		14/34 (41)	0.4 (0.2-0.9)	
HIV status	Negative	82/392 (21)	99/257 (39)	Reference	Reference	7/32 (22)	Reference	Reference
	Positive	310/392 (79)	158/257 (61	0.4 (0.3-0.6)	0.6 (0.4-0.9)	25/32 (78)	0.9 (0.4-2.3)	1.6 (0.5-4.9)
Underlying medical conditions[d]	No	394/423 (93)	240/275 (87)	Reference		32/34 (94)	Reference	
	Yes	29/423 (7)	35/275 (13)	1.98 (1.2-3.3)		2/34 (6)	0.8 (0.2-3.7)	
Duration of symptoms prior to admission	<7	166/412 (40)	194/275 (71)	Reference	Reference	24/33 (73)	Reference	Reference
	≥7	246/412 (60)	81/275 (29)	0.3 (0.2-0.4)	0.4 (0.2-0.4)	9/33 (27)	0.3 (0.1-0.6)	0.3 (0.1-0.7)
Pneumoccocal co-infection on PCR[e]	No	371/388 (96)	233/246 (95)	Reference	Reference	29/30 (97)		
	Yes	17/388 (4)	13/246 (5)	1.2 (0.6-2.6)		1/30 (3)	0.8 (0.1-5.8)	
Oxygen therapy	No	313/423 (74)	158/275 (57)	Reference	Reference	24/34 (71)	Reference	Reference
	Yes	110/423 (26)	117/275 (43)	2.1 (1.5-2.9)	1.9 (1.3-2.8)	10/34 (29)	1.2 (0.5-2.6)	0.97 (0.4-2.3)
Tuberculosis treatment	No	185/422 (44)	243/274 (89)	Reference		20/34 (59)	Reference	
	Yes	237/422 (56)	31/274 (11)	0.1 (0.1-0.2)		14/34 (41)	0.5 (0.3-1.1)	
Hospital duration (days)	≤	257/420 (61)	198/274 (72)	Reference		22/33 (67)	Reference	Reference
	>7	163/420 (39)	76/274 (28)	0.6 (0.4-0.8)	0.6 (0.4-0.9)	11/33 (33)	0.8 (0.4-1.7)	0.7 (0.3-1.5)
Died	No	391/422 (73)	265/274	Reference	Reference	30/34 (88)	Reference	Reference
	Yes	31/422 (7)	9/274	0.4 (0.2-0.9)	0.5 (0.2-1.1)	4/34 (12)	1.7 (0.6-5.1)	3.2 (1.1-10.0)

[a]Tuberculosis only group used as reference for multinomial model analysis (i.e. comparing influenza only to tuberculosis only and tuberculosis–influenza co-infection to tuberculosis only group).
[b]Unadjusted relative risk ratio (RRR) at multivariable analysis.
[c]Adjusted relative risk ratio (ARRR) at multivariable analysis.
[d]Underlying conditions included any of the following: Asthma, other chronic lung disease, chronic heart disease (valvular heart disease, coronary artery disease, or heart failure excluding hypertension), liver disease (cirrhosis or liver failure), renal disease (nephrotic syndrome, chronic renal failure), diabetes mellitus, immunocompromising conditions excluding HIV infection (organ transplant, immunosuppressive therapy, immunoglobulindeficiency, malignancy), neurological disease (cerebrovascular accident, spinal cord injury, seizures, neuromuscular conditions) or pregnancy. Comorbidities were considered absent in cases for which the medical records stated that the patient had no underlying medical condition or when there was no direct reference to that condition.
[e]*lyt* A PCR positive for *Streptococcus pneumoniae* on blood specimen.

The median duration of symptoms was 2 (range 0–6) and 14 (7–195) days in patients presenting with symptoms <7 days and ≥7 days respectively.

On multivariable analysis, among patients presenting with symptoms <7 days, patients with influenza only compared to patients with tuberculosis only were less likely to be admitted for >7 days (aRRR 0.4, 95% CI 0.3-0.7). No differences were found in patients co-infected with tuberculosis and influenza compared to patients with tuberculosis only and there was no increased risk of death

Table 4 Factors associated with influenza infection and tuberculosis-influenza co-infection compared with tuberculosis infection only among patients presenting with symptoms duration <7 days admitted with severe respiratory illness and tested for tuberculosis and influenza at four sites in South Africa, June 2010-2011

Variables		Laboratory confirmed tuberculosis Reference[a]	Laboratory confirmed influenza			Laboratory confirmed tuberculosis-influenza co-infection		
			n/N (%)	ARRR[b]	P-value	n/N (%)	ARRR[b]	P-value
Duration of symptoms	<7	100/165 (61)	148/194 (76)	Reference		6/9 (67)	Reference	
	≥7 days	65/165 (39)	46/194 (24)	0.4 (0.3-0.7)	0.001	3/9 (33)	0.5 (0.2-1.5)	0.240
HIV status	Negative	46/148 (31)	74/179 (41)	Reference		5/22 (23)	Reference	
	Positive	102/148 (69)	105/ 179 (59)	0.8 (0.5-1.2)	0.280	17/22 (77)	1.6 (0.5-4.7)	0.404
Oxygen therapy	No	101/166 (61)	108/194 (56)	Reference		17/24 (71)	Reference	
	Yes	65/166 (39)	86/194 (44)	1.4 (0.9-2.3)	0.141	7/24 (29)	0.7 (0.3-2.0)	0.560
Died	No	157/166 (95)	191/194 (98)	0.5(0.2-1.5)		24/24 (96)	Reference	
	Yes	9/166 (5)	3/194 (2)	0.2 (0.04-1.2)	0.081	1/24 (4)	0.98 (0.1-8.6)	0.986

HIV – human immunodeficiency virus.
[a]Tuberculosis only group used as reference for multinomial model analysis (i.e. comparing influenza only to tuberculosis only and tuberculosis–influenza co-infection to tuberculosis only group).
[b]Adjusted relative risk ratio (ARRR) at multivariable analysis.

in influenza tuberculosis co-infected individuals (aRRR 0.8, 95% CI 0.1-8.6) (Table 4).

On multivariable analysis among patients presenting with symptoms ≥7 days, patients with influenza only compared to tuberculosis only were less likely to be HIV-infected (aRRR 0.4, 95% CI 0.2-0.7) and more likely to receive oxygen (aRRR 1.7, 95% CI 1.7-5.4). Patients co-infected with tuberculosis and influenza as compared to patients with tuberculosis only were at increased risk of death (aRRR 5.5, 95% CI 1.2-25.3) (Table 5).

Discussion

We describe an increased risk of death associated with influenza co-infection among patients with symptoms ≥7 days hospitalized with laboratory-confirmed tuberculosis in South Africa. Mortality was not increased in patients co-infected with influenza and tuberculosis presenting with a more recent symptom onset. This suggests that influenza infection may contribute to a proportion of the mortality burden amongst patients with tuberculosis, in particular amongst those with a longer duration of symptoms. Because influenza illness is considered to be an acute infection it is possible that it is not considered as a possible diagnosis in patients with longer duration of symptoms. Both influenza and tuberculosis should be considered and tested for among patients hospitalized with pneumonia in settings with high tuberculosis prevalence such as South Africa. Based on the findings of this study our surveillance programme has been amended at two sites to include systematic testing for tuberculosis

Table 5 Factors associated with influenza infection and tuberculosis-influenza co-infection compared with tuberculosis infection only among patients admitted with severe respiratory illness presenting with symptoms duration ≥7 days and tested for tuberculosis and influenza at four sites in South Africa, June 2010- December 2011

Variables		Laboratory confirmed tuberculosis Reference[a]	Laboratory confirmed influenza			Laboratory confirmed tuberculosis-influenza co-infection		
			n/N (%)	ARRR[b]	P-value	n/N (%)	ARRR[b]	P-value
Hospital duration	<7	150/244 (61)	50/80 (62.5)	Reference		6/9 (67)	Reference	
	≥7 days	94/244 (39)	30/80 (37.7)	0.99 (0.6-1.7)	0.991	3/9 (33)	0.6 (0.1-2.7)	0.520
HIV status	Negative	34/233 (83)	25/78 (32)	Reference		1/9 (11)	Reference	
	Positive	199/233 (17)	53/78 (68)	0.4 (0.2-0.7)	0.002	8/9 (89)	2.2 (0.2-20.5)	0.486
Oxygen therapy	No	203/246 (83)	50/81 (62)	Reference		6/9 (67)	Reference	
	Yes	43/246 (17)	31/81 (38)	3.0 (1.7-5.5)	<0.001	3/9 (33)	2.1 (0.5-9.3)	0.315
Died	No	224/245 (91)	74/80 (92.5)	Reference		6/9 (67)	Reference	
	Yes	21/245 (9)	6/80 (7.5)	0.54 (0.2-1.5)	0.875	3/9 (33)	5.48 (1.2-25.4)	0.029

HIV – human immunodeficiency virus.
[a]Tuberculosis only group used as reference for multinomial model analysis (i.e. comparing influenza only to tuberculosis only and tuberculosis–influenza co-infection to tuberculosis only group).
[b]Adjusted relative risk ratio (ARRR) at multivariable analysis.

in patients admitted with severe respiratory illness. In addition, we suggest that where possible tuberculosis testing should be included in SARI programmes in Sub-Saharan Africa where HIV prevalence is high.

A recent study from Thailand did not identify an increased risk of severe outcomes or mortality in patients co-infected with tuberculosis and seasonal influenza when compared to tuberculosis and influenza single infection [18]. The number of patients co-infected with influenza and tuberculosis in this study were, however, small, and patients with a more chronic presentation were not enrolled in the surveillance system. Similar to the findings of our study, ecological and record review studies have reported elevated mortality among tuberculosis-infected individuals during previous influenza pandemics [9,10,19] and 10% of A(H1N1)pdm09 laboratory-confirmed deaths in South Africa in 2009, were co-infected with tuberculosis [11].

In patients presenting with longer duration of symptoms, tuberculosis may have caused lung damage leading to reduced lung capacity and thus impairing the ability to cope with viral infections such as influenza and potentially increasing the severity of illness associated with influenza virus infection. In addition, influenza infection impacts on antibacterial host defenses by either increased production of pro-inflammatory cytokines, causing cell damage [20], or due to inadequate pro-inflammatory chemokine response [21,22], which could increase susceptibility to tuberculosis. Lastly, immunosuppression induced by influenza virus through production of glucocorticoids [23], production of immunosuppressive cytokines [20] or, suppression of T-cell immunity [24] could trigger reactivation of latent tuberculosis and aggravate pulmonary tuberculosis.

Persons with AIDS experience a significant excess mortality due to influenza infection as compared to HIV-uninfected individuals [7,25]. The relative risk of *M. tuberculosis* infection among HIV-infected compared to -uninfected persons varies between 21–37 depending on the country's HIV prevalence [2]. In our study, a high proportion of patients with tuberculosis were also HIV-infected; however the association with increased mortality in patients co-infected with tuberculosis and influenza virus remained statistically significant even when controlling for concurrent HIV infection in the model.

Despite the high prevalence of tuberculosis in South Africa, in our study, only 37% (3018/8032) of patients admitted with SRI were tested for tuberculosis. Even amongst patients who were initiated on tuberculosis treatment during the current admission, only 58% (656/1123) were tested before initiation of treatment. A study conducted in South Africa reported tuberculosis as the most common cause (40%) of community acquired pneumonia in both HIV-infected and-uninfected patients [26]. A post mortem study in patients dying in hospital found that among patients not suspected of having tuberculosis at time of death, 42% (40/96) were culture positive for tuberculosis and that pneumonia, was the leading admission diagnosis (25%) in those not suspected of tuberculosis [27]. Only 32% of patients with symptom duration of <7 days were tested for tuberculosis, as compared to 57% in those with a chronic presentation, but amongst those tested, 10% had laboratory confirmed-tuberculosis. National guidelines for tuberculosis management advise screening for tuberculosis in patients presenting with persistent chronic symptoms (cough or fever >14 days) [28], however results from a pooled analysis of sensitivity of symptoms for active pulmonary tuberculosis, showed that in studies from sub-Saharan countries, with high burden of HIV, sensitivity of any cough was higher 62% than that of chronic cough >2 weeks, 49% [29]. Failure to test for tuberculosis could lead to delayed or missed diagnosis of tuberculosis, an increase in nosocomial tuberculosis transmission and missed opportunities to identify drug resistance when testing with the GeneXpert assay. Because of the long duration of tuberculosis treatment and possible associated adverse events, a microbiologic diagnosis of tuberculosis should be sought wherever possible [30]. The majority of tuberculosis-positive patients in our study were aged >5 years. Microbiological confirmation of tuberculosis in children is difficult [14,31-34], because childhood tuberculosis tends to be paucibacillary and smear negative. This difficulty can be further compounded by HIV co-infection.

Less than 1% of patients in our study received influenza antiviral treatment or influenza vaccination despite being recommended in national guidelines [35,36]. The association between influenza and tuberculosis seen in our study may be different in settings where influenza vaccination and antivirals are widely used. In South Africa, while there are recommendations for annual influenza vaccination, tuberculosis is not specifically listed as an underlying co-morbid illness to be targeted for vaccination [37]. Tuberculosis treatment guidelines do not routinely include influenza vaccination as part of management [15]. A randomised controlled study of trivalent inactivated influenza vaccine conducted in HIV-infected adults with CD4+ T cell counts >200 μg/ml in South Africa reported 75% (95% CI 9.2-95.6) efficacy in adults [38]. However, patients with tuberculosis co-infection or low CD4+ T cell counts were not included. Although vaccination remains the most effective method to prevent influenza infection, tuberculosis infection suppresses immune responses and thus vaccine effectiveness may be lower in this patient group. There are no published data on the effectiveness of influenza vaccination in patients with tuberculosis, particularly those co-infected with HIV (79% of individuals in our study).

Our study has limitations that warrant discussion. Firstly, we restricted our analysis to patients with tuberculosis laboratory results at current admission, and our case definition of laboratory-confirmed tuberculosis included smear-positive results. As testing for tuberculosis is not 100% sensitive, especially for extra pulmonary tuberculosis which is common in patients with HIV, we may have misclassified a proportion of tuberculosis-positive individuals as not having tuberculosis; however there is no reason to suspect that sensitivity would differ between influenza-positive and -negative patients. The majority of patients with laboratory-confirmed tuberculosis only had microscopy results and it is possible that some may have been infected with mycobacteria other than tuberculosis in a high HIV prevalence setting such as South Africa. However, current South African guidelines recommend initiation of tuberculosis treatment based on smear microscopy results [11]. Secondly we enrolled acute and chronic cases only at two enhanced sites and we didn't systematically test all enrolled cases for tuberculosis. The patients tested for tuberculosis differed in several characteristics from those not tested. These differences in enrolment and testing procedures as well as the low proportion of patients tested for tuberculosis may have introduced potential selection bias in the population included in the study. Testing all patients admitted with SRI will improve identification of tuberculosis cases and the ability to detect significant differences between groups. We did not collect data on chest X- ray findings. Availability of data on radiological changes in the lungs could have strengthened the evidence in support of our hypothesis regarding changes in the lungs resulting in severe outcome in patients with underlying tuberculosis co-infected with influenza.

Conclusion

In conclusion, we observed an increased risk of death among tuberculosis-infected patients co-infected with influenza with a symptom history of ≥7 days. The potential public health impact of influenza vaccination among persons with laboratory-confirmed tuberculosis should be explored.

Abbreviations

AIDS: Acquired immune deficiency syndrome; ALRI: Acute lower respiratory tract infection; aOR: adjusted odds ratio; aRRR: Adjusted relative risk ratio; CDC: Centers for Disease Control; CHBAH: Chris Hani Baragwanath Academic Hospital; ELISA: Enzyme-linked immunosorbent assay; HIV: Human immunodeficiency virus; KTHC: Klerksdorp Tshepong Hospital Complex; ICU: Intensive care unit; *M. tuberculosis*: *Mycobacterium tuberculosis*; NICD-NHLS: National Institute for Communicable Diseases of the National Health Laboratory Services; PCR: Polymerase chain reaction; RR: Relative risk; RRR: Relative risk ratio; SARI: Severe acute respiratory illness; SCRI: Severe chronic respiratory illness; *S. pneumoniae*: *Streptococcus. pneumoniae*; SRI: Severe respiratory illness; WHO: World Health Organization.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

SW, CC, ST, JM– contributed to conception and the study design, acquisition of data, analysis and interpretation of data, and drafting of manuscript. CMV, ALC contributed to interpretation of data, drafting of the manuscript and review of manuscript. SAM, MG, HD, EV, KK contributed to acquisition of data and review of manuscript. MV, MP and NW contributed to laboratory testing, acquisition of laboratory data and review of the manuscript. All authors read and approved the final manuscript.

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Author details

[1]Centre for Respiratory Disease and Meningitis, National Institute for Communicable Diseases (NICD) of the National Health Laboratory Service (NHLS), Private Bag X4, Sandringham, 2131 Johannesburg, Gauteng, South Africa. [2]School of Public Health, Faculty of Health Science, University of the Witwatersrand, Johannesburg, South Africa. [3]US Centres for Disease Control and Prevention, Atlanta, GA, USA. [4]US Centres for Disease Control and Prevention, Pretoria, South Africa. [5]Medical Research Council: Respiratory and Meningeal Pathogens Research Unit and Department of Science and Technology/National Research Foundation: Vaccine-Preventable Diseases, University of the Witwatersrand, Johannesburg, South Africa. [6]Pietermaritzburg Metropolitan Hospital Complex, KwaZulu- Natal, South Africa. [7]MRC/Wits Rural Public Health and Health Transition Research Unit (Agincourt), Bushbuckridge, South Africa. [8]Tshepong Hospital, North West Province, South Africa. [9]Faculty of Medicine, University of Witwatersrand, Johannesburg, South Africa. [10]Zoonosis Research Unit, Department of Medical Virology, University of Pretoria, Pretoria, South Africa.

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PAPER IV

The impact of influenza and tuberculosis interaction on mortality among individuals aged ≥15 years hospitalised with severe respiratory illness in South Africa, 2010-2016

Sibongile Walaza[1,2], Stefano Tempia[1,3,4], Halima Dawood[5], Ebrahim Variava[6,7,8], Nicole Wolter[1,9], Andries Dreyer[10], Jocelyn Moyes[1,2], Claire von Mollendorf[1,2], Meredith McMorrow[3,4,11], Anne von Gottberg1,9, Sumayya Haffejee[5], Marietje Venter[15], Florette K. Treurnicht[1], Orienka Hellferscee[1,9], Neil A Martinson[8,12,13], Nazir Ismail[10,14], Cheryl Cohen[1,2]

Affiliations

1. Centre for Respiratory Diseases and Meningitis, National Institute for Communicable Diseases of the National Health Laboratory Service, Johannesburg, South Africa
2. School of Public Health, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa
3. Influenza Division, Centers for Disease Control and Prevention, Atlanta, Georgia, United States of America
4. Influenza Program, Centers for Disease Control and Prevention, Pretoria, South Africa
5. Pietermaritzburg Metropolitan Hospital Complex, KwaZulu- Natal, South Africa
6. Department of Medicine, Klerksdorp Tshepong Hospital, North West Province,
7. School of Clinical Medicine, Faculty of Health Sciences, University of Witwatersrand, Johannesburg, South Africa
8. Perinatal HIV Research Unit, MRC Soweto Matlosana Collaborating Centre for HIV/AIDS and TB
9. School of Pathology, Faculty of Health Sciences, University of the Witwatersrand
10. Centre for tuberculosis, National Institute for Communicable Diseases of the National Health Laboratory Service, Johannesburg, South Africa
11. Division of Global Health Protection, Centers for Disease Control and Prevention, Pretoria, South Africa
12. DST/NRF Centre of Excellence for Biomedical Tuberculosis Research, University of the Witwatersrand, Johannesburg, South Africa

13. Johns Hopkins University Center for TB Research, Baltimore, MD
14. Faculty of Health Sciences, University of Pretoria, Pretoria
15. Zoonosis Research Program, Department of Medical Virology, University of Pretoria, Pretoria, South Africa

Corresponding author

Sibongile Walaza, Centre for Respiratory Diseases and Meningitis, National Institute for Communicable Diseases, Private Bag X4, Sandringham, 2131, Gauteng, South Africa.

Telephone: 27 11 386 6410, Fax: 27 11 3866580, E-mail: sibongilew@nicd.ac.za or sbowalaza@gmail.com

Alternate corresponding author

Cheryl Cohen, Centre for Respiratory Diseases and Meningitis, National Institute for Communicable Diseases, Private Bag X4, Sandringham, 2131, Gauteng, South Africa.

Telephone: 27 11 386 6593, Fax: 27 11 882 9979, E-mail: cherylc@nicd.ac.za

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Abstract

Background

Data on the prevalence and impact of influenza-tuberculosis co-infection on clinical outcomes from high HIV and tuberculosis burden settings are limited. We explored the impact of influenza and tuberculosis co-infection on mortality among hospitalised adults with lower respiratory tract infection (LRTI).

Methods

We prospectively enrolled patients aged ≥15 years admitted with physician-diagnosed LRTI or suspected tuberculosis at two hospitals in South Africa from June 2010- May 2016. Combined nasopharyngeal and oropharyngeal swabs were tested for influenza and 8 other respiratory viruses. Tuberculosis testing of sputum included smear microscopy, culture and/or Xpert MTB/Rif. Blood was tested for pneumococcus by *lyt* A polymerase chain reaction (PCR).

Results

Among 6228 enrolled individuals aged ≥15 years, 4253 (68%) were tested for both influenza and tuberculosis. Of these, the detection rate was 6% (239/4253) for influenza, 26% (1092/4253) for tuberculosis and 77% (3113/4053) for HIV. One percent (42/4253) was positive for both influenza and tuberculosis. On multivariable analysis, among tuberculosis-positive patients, factors independently associated with death were age group ≥65 years compared to 15-24 years (adjusted odds ratio [aOR] 3.6, 95% confidence interval [CI] 1.2-11.0) and influenza co-infection (aOR 2.3, 95% CI 1.02-5.2). Among influenza-positive patients, laboratory-confirmed tuberculosis was associated with an increased risk of death (aOR 4.5, 95% CI 1.5-13.3).

Conclusion

Tuberculosis co-infection is associated with increased mortality in individuals with influenza and influenza co-infection is also associated with increased mortality in individuals with tuberculosis. These data may help in making decisions on whether influenza vaccines or influenza antivirals should be prioritised for tuberculosis patients.

Introduction

Both influenza and tuberculosis cause significant morbidity and mortality globally [1-4]. Data on the impact of influenza and tuberculosis co-infection, particularly from high HIV and tuberculosis burden settings are limited. Most available data are from ecologic or individual level studies usually conducted during pandemic influenza years, frequently descriptive, with limited sample size and lacking data on important confounders such as HIV infection [5-11]. A better understanding of influenza and tuberculosis interaction is important to assist policy makers to make decisions regarding priority target groups for influenza immunization.

HIV is a risk factor for both tuberculosis and influenza-associated morbidity and mortality [12-15]. In South Africa 11.6%-12.7% of the general population and approximately one fifth (22.2%) of women in reproductive age were living with HIV between 2010 and 2016 [16]. In 2015, there were an estimated 450 000 incident cases of tuberculosis of which 57% were HIV-infected. An estimated 25000 HIV-negative tuberculosis cases died in 2015 and an estimated 73000 deaths occurred among tuberculosis cases co-infected with HIV [17].

Similarly, an increased risk of influenza-associated hospitalisation and mortality has been reported among individuals with AIDS. [13, 14, 18, 19]. We previously reported an increased risk of mortality in patients with influenza-tuberculosis co-infection from six hospitals in South Africa. [20] However, this earlier study was limited by differential enrolment criteria across different participating sites and sample size which did not allow us to evaluate specific interactions in the influenza and tuberculosis infected groups separately.

In this study we assess the effect of influenza-tuberculosis co-infection on mortality among patients hospitalized with severe respiratory illness, first among those that were tuberculosis--positive and second among those that were influenza-positive.

Methods

Study Design

We conducted prospective hospital-based sentinel surveillance for severe respiratory illness (SRI) at two hospitals which form part of enhanced surveillance for pneumonia in two provinces; Edendale Hospital in the KwaZulu-Natal Province and Tshepong Hospital in the North West Province from June 2010 through May 2016.

Case definitions

A case of SRI was defined as admission with physician-diagnosed lower respiratory tract infection (LRTI) including suspected tuberculosis, of any duration. Patients aged ≥15 years from the hospital catchment area were included in our analysis.

Study procedures

SRI cases admitted from 17H00 on Sunday through 13H00 on Friday were eligible for enrolment. Data on clinical presentation, previous medical history, antiretroviral therapy for HIV-infected individuals, inpatient investigations, clinical management and outcome were collected through structured interviews and record review. Each patient was followed-up until discharge. Treatment decisions and diagnostic tests for patient management were determined by the attending physician.

Sample collection and laboratory procedures

Combined nasopharyngeal (NP) and oropharyngeal (OP) swabs and blood specimens were systematically collected from consenting patients by study staff. Combined NP and OP swabs were transported in universal transport medium (Copan, Brescia, Italy) on cooled ice packs to the National Institute for Communicable Diseases (NICD) within 72 hours of collection and were tested for influenza A and B viruses, respiratory syncytial virus, parainfluenza virus types 1, 2 and 3, adenoviruses, human metapneumovirus, enterovirus and rhinoviruses, using

either a multiplex real-time reverse transcription polymerase chain reaction assay[21] from 2010 through to 2014 or the commercial Allplex Respiratory Assay (panel 2 and 3) (Seegene, Seoul, Korea) from 2015. Influenza A and B and respiratory syncytial viruses were tested using the commercial FTD Flu/RSV assay (Fast Track Diagnostics, Luxembourg) from 2015-2016. Parainfluenza virus types 1, 2 and 3, adenoviruses, human metapneumovirus, enteroviruses and rhinoviruses were only tested for period 2010-2015.

From June 2010-May 2012, tuberculosis testing of expectorated sputum was conducted at the discretion of the attending clinician. Systematic testing for tuberculosis was instituted in June 2012. For this period, enrolled patients who were not tested for tuberculosis as part of clinical management, expectorated sputum (or induced sputum if the patient was not able to expectorate) was collected by study staff for tuberculosis testing at the hospital laboratory and a second sample was collected and sent to NICD in Johannesburg for tuberculosis and other bacterial pathogens testing. Sputum samples were frozen at -20°C and stored at the site laboratory until shipped on dry ice on a weekly basis to NICD. Microbiological investigation for tuberculosis at the site laboratory was performed by smear microscopy, culture and/or XpertMTB/Rif (Cepheid, California, USA). At NICD, tuberculosis was identified by microscopy and culture only. Smear microscopy of sputum samples was performed using fluorescent auramine-O staining for acid fast bacilli (AFB). Culture was performed at the site laboratory and at NICD using liquid media with the Bactec MGIT 960 (Becton Dickinson, New Jersey, USA) system. Positive cultures were identified as *Mycobacterium tuberculosis* complex using Ziehl-Neelsen staining and MPT64 antigen testing (Becton Dickinson, New Jersey, USA) [22]. Due to logistic limitations including contraindications to induced sputum collection, we were not able to offer all patients sputum induction and not all patients provided an expectorated sputum specimen. A further proportion of patients were not able to

produce a sputum specimen even after sputum induction procedures. All patients without an available sputum for testing were considered not tested for tuberculosis. Amongst patients tested for tuberculosis, a patient was considered tuberculosis positive if at least one tuberculosis test (smear, culture or PCR) was positive. Whole blood specimens were tested for the presence of pneumococcal DNA (*lyt*A gene) using quantitative real-time PCR [23].

Determination of HIV status and CD4 count testing for HIV-infected patients

HIV status was obtained from several data sources. HIV testing was requested by admitting physicians as part of clinical management for most patients. This included HIV enzyme-linked immunosorbent assay (ELISA) testing with confirmation by ELISA on a second specimen if the first sample was positive. For consenting patients who were not tested by the attending clinician, pre-test counselling and bedside testing for HIV was offered by NICD study staff. Homemed's HIV1/2 point of care test (POCT) was used as the first screening test and ABON™ HIV1/2 Tri-Line test kit was used as second confirmatory test for all participants testing positive on the first screening test. Discrepant rapid test results were confirmed by an ELISA test. In addition, for consenting patients who refused HIV testing by admitting physicians and did not want to receive their results, linked anonymous[24] ELISA was performed at NICD using a dried blood spot or whole blood specimen. When results were available from multiple sources or discrepant, the NICD result was used. CD4+ cell count per cubic millimeter was collected for HIV-infected patients as part of clinical management or part of the study if not requested by the clinician.

Ethical Consideration

The protocol was approved by the University of the Witwatersrand Human Research Ethics Committee (reference M081042 and M1400824) and the University of KwaZulu-Natal Human Biomedical Research Ethics Committee (BF157/08 and BE 496/14).

Analysis

We implemented multivariable logistic models to assess:

i. Factors associated with testing for tuberculosis to assess for potential bias in the population who received tuberculosis testing
ii. The association between tuberculosis and influenza co-infection on mortality in two different populations:
    a. All individuals testing tuberculosis positive (who also were tested for influenza)
    b. All individuals testing influenza positive (who also were tested for tuberculosis)

Symptom duration, HIV serostatus and age were included a-priori in models (ii. a) and (ii. b) as important potential confounders. Symptom duration was included based on our previous findings demonstrating that influenza-tuberculosis co-infection was associated with increased risk of death compared to tuberculosis or influenza single infection in patients with symptoms >7 days, but not in patients with acute presentation [20].

Factors significant at $p<0.1$ on univariate analysis were evaluated in the multivariable analysis and non-significant factors at $p\geq0.05$ were dropped from the multivariable model using stepwise forward selection. All two-way interactions in the final multivariable additive model were evaluated. Two-sided p-values <0.05 were considered significant throughout. Stata version 14 (StataCorp Limited, College Station, Texas, United States) was used for the analysis.

Results

From June 2010 through May 2016, we enrolled 6228 individuals aged ≥15 years with SRI. Of these, 6149 (99%) and 4300 (69%) were tested for influenza and tuberculosis, respectively. The overall HIV prevalence among 5853 patients aged ≥15 years with SRI and known HIV results was 76% (4445/5853), 69% (213/308) among influenza positive cases and 83% (869/1043) among tuberculosis positive cases. Overall mortality amongst enrolled patients was 13% (777/6064); 11% (119/1088) among tuberculosis positive, 12% (37/318) among influenza positive and 19% (8/42) among influenza tuberculosis coinfected.

Compared to patients not tested for tuberculosis, on multivariable analysis, patients tested for tuberculosis were more likely to present with cough [adjusted odds ratio (aOR), 2.1, 95% confidence interval (CI) 1.5-2.8] and night sweats (aOR 1.3, 95% CI 1.1-1.6), to have a history of working in a mine (aOR, 1.6, 95% CI 1.2-2.2), to have pneumococcal coinfection identified by *lyt A* PCR (aOR, 1.3, 95% CI 1.0-1.8) and to be admitted at Tshepong Hospital (aOR1.5, 95% CI 1.2-1.8); however, they were less likely to be admitted for >7 days compared to <3 days (aOR 0.8, 95% CI 0.6-0.9) and to die during admission (aOR 0.5, 95% CI 0.4-0.6) (Table1).

In total, 68% (4253/6228) of participants were tested for both pathogens (Figure 1). Among these individuals, the overall detection rate was 6% (239/4253) for influenza, 26% (1092/4253) for tuberculosis and 77% (3113/4053) for HIV. Of these, 25% (1050/4253) were positive for tuberculosis only, 5% (197/4253) for influenza only and 1% (42/4253) were co-infected with influenza and tuberculosis. Of the 1092 individuals positive for tuberculosis, 444 (41%), 202 (19%), 114 (10%) and 332 (30%) were positive on PCR only, microscopy only, culture only and on ≥2 tests respectively. Of the 1087 individuals positive for

tuberculosis with data on previous tuberculosis treatment, 118 (11%) had a history of being on tuberculosis treatment within 12 months of current admission.

Factors associated with mortality in patients testing tuberculosis positive

Of the 1092 patients aged ≥15 years tested for influenza with laboratory-confirmed tuberculosis, 116/1075 (11%) died. Mortality increased with increasing age, 11% (69/656; aOR 1.5, 95% CI 0.7-3.3), 12% (32/258; aOR 1.8, 95% CI 0.8-4.0) and 19% (7/36; aOR 3.6, 95% CI 1.2-11.0) among individuals aged 25-44, 45-64 and ≥65 years compared to 15-24 year age group (6%, 8/129), although this was only significant in the ≥65 years age group (Table 2). Patients with symptom duration longer than 7 days were more likely to die (12%, 94/786 vs 8%, 20/258; aOR 1.5, 95% CI 0.9-2.5), although this was not statistically significant on multivariable analysis. On multivariable analysis controlling for HIV status and duration of symptoms, factors independently associated with death among tuberculosis positive patients were age group ≥65 years compared to 15-24 years (aOR 3.6, 95% CI 1.2-11.0) and influenza co-infection (aOR 2.3 (95% CI 1.1-5.2).

Factors associated with mortality in patients testing influenza positive

Among 239 hospitalised patients aged ≥15 years tested for tuberculosis with laboratory-confirmed influenza, 18/232 (8%) died. Mortality increased with increasing age, 6% (8/131; aOR 1.0, 95% CI 0.1-8.9), 10% (6/57; aOR 2.5, 95% CI 0.3-24.2) and 12% (3/25; aOR 5.1, 95% CI 0.4-65.9) among individuals aged 25-44, 45-64 and ≥65 years compared to 15-24-year age group (5%,1/19) and was higher in individuals with symptom duration >7 days

(10%, 12/120 vs 5%, 6/109; aOR 1.6, 95% CI 0.6-4.7), although these were not statistically significant on multivariable analysis (Table 3).

On multivariable analysis controlling for age, HIV status and symptom duration, laboratory-confirmed tuberculosis was associated with an increased risk of death in patients who tested positive for influenza [aOR 4.5, 95% CI 1.5-13.3] (Table 3).

Discussion

Influenza and tuberculosis are both common aetiologies identified among adults hospitalised with LRTI in South Africa and both are associated with high proportion of in-hospital mortality (12% and 11%, respectively). Although co-infection with influenza and tuberculosis was relatively rare (1%) of which 19% died (8/42), among patients with influenza, tuberculosis co-infection was associated with elevated mortality risk (aOR 4.5) and influenza co-infection, was associated with increased mortality (aOR 2.3) among patients with tuberculosis. These results suggest that when influenza and tuberculosis disease coexist in an individual patient, their co-presence is associated with a worse prognosis than either infection alone.

In our study we found an increased risk of mortality among tuberculosis-positive patients that were co-infected with influenza viruses. Ecological studies have suggested excess tuberculosis mortality during influenza pandemics and seasonal epidemics; however, a causal relationship could not be demonstrated [6, 25-28]. Our results are similar to reports from descriptive studies from tuberculosis sanatoria in the 1950s which also reported increased tuberculosis mortality or increased complications following influenza infection [29, 30]. However, these studies were not statistically evaluated. Animal experimental studies have suggested a causal relationship between influenza infection and tuberculosis mortality,

suggesting that influenza infection of mice with tuberculosis reduced long term survival [31-33]. Bernard et al. showed that in mice, an influenza challenge 1-5 weeks after tuberculosis infection, compared with tuberculosis only-infected mice resulted in 50-75% shorter survival time and a higher case-fatality ratio.[32] In the same study, sequential infection of mice with tuberculosis and influenza, with tuberculosis infection on day 0 and influenza infection 7 weeks later, resulted in increased pulmonary disease and a delay in mycobacterium clearance from the lungs of infected mice [33].

In our study we also found an increased risk of mortality among influenza-positive patients that were co-infected with tuberculosis. Our results are similar to those from studies conducted during the 2009 influenza pandemic, which reported either a high prevalence of tuberculosis in individuals hospitalised or who died of influenza compared to the general population or described tuberculosis as a risk factor for influenza-associated severe disease [8-10]. However, these studies were mostly descriptive. In animal studies, Redford *et al.* demonstrated that influenza A virus infection of mice 28 days before or during *M. tuberculosis* infection impaired mycobacterium control and decreased host survival [34]. Co-infection or superinfection with other bacterial pathogens, *Streptococcus pneumoniae, Staphylococcus aureus* and *Haemophilus influenzae* have also been reported as a risk factor for severe influenza-associated disease and death[35, 36].

We had previously reported an increased risk of death among patients with tuberculosis that were co-infected with influenza , which was only significant among patients with symptoms >7 days [20]. In the current study, we used a different analytic approach to explore the effects of tuberculosis and influenza separately in the groups of individuals with each pathogen infection. Using this analytic approach, although not statistically significant, patients with longer duration of symptoms compared to those with more acute presentation were at increased risk of death. It is possible that the association with severe outcomes in

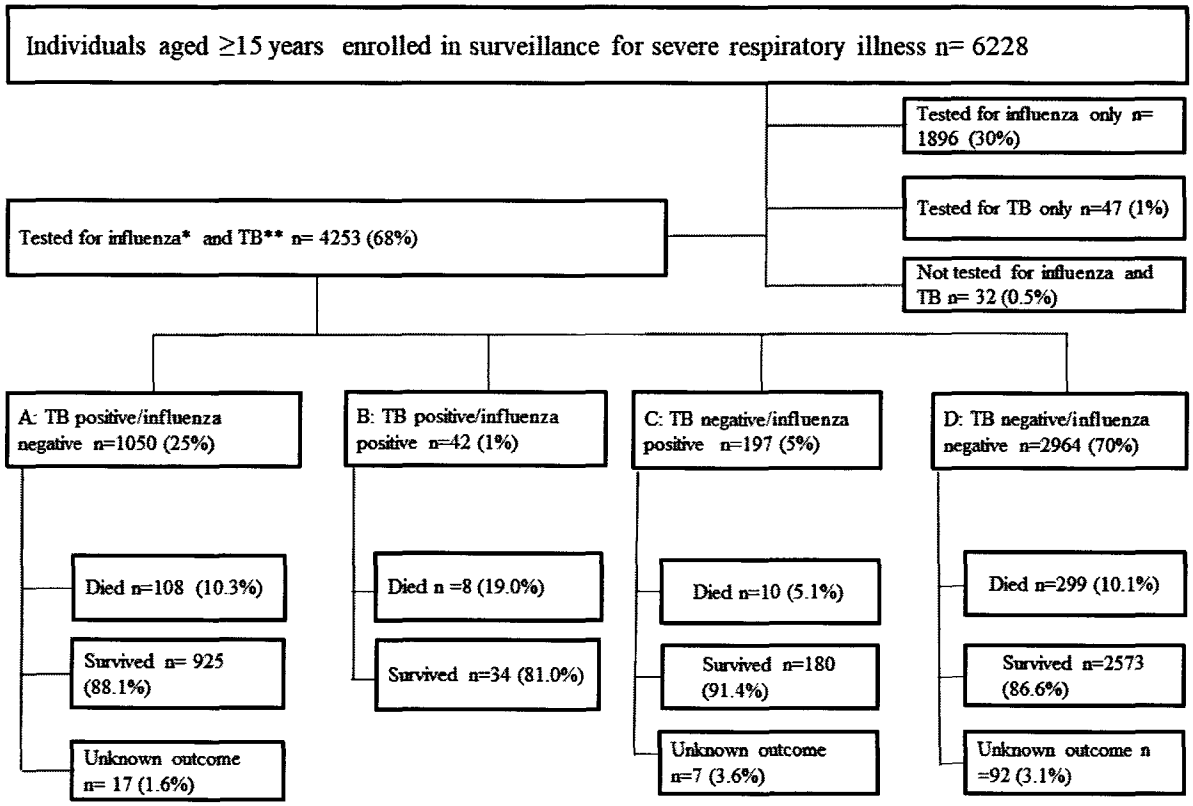
tuberculosis-positive patients who are co-infected with influenza is in part due to the chronic lung damage caused by tuberculosis. In an experimental study, Bernard *et al.* demonstrated that the amount of tissue damage among tuberculosis-influenza co-infected mice, increased with increasing time of tuberculosis infection prior to the influenza challenge [32]. Studies restricted to influenza patients with an acute presentation did not find an association between co-infection and severe outcomes, although in one such study, tuberculosis was associated with severe disease on univariate analysis [37, 38]. A study from Thailand did not find an increased risk of clinical worsening or mortality among hospitalised tuberculosis patients co-infected with influenza presenting with acute respiratory illness [38]. This may suggest that chronic lung damage secondary to tuberculosis plays an important role in disease presentation and outcome. However, in our analysis we did not have data on radiological changes in the lungs to assess whether chronic lung damage was associated with severe outcomes.

Our study has limitations that warrant discussion. First, whereas we obtained a tuberculosis result in 69% of patients admitted with SRI, we may have missed patients with more severe illness who died before tuberculosis testing or were too ill to expectorate as patients who were tested compared to those not tested were less likely to die during current admission. This could have led to underestimation of case-fatality ratios. Second, microbiologic confirmation of a diagnosis of tuberculosis is challenging especially in patients who are infected with HIV [39, 40]. HIV-infected individuals made up 77% of participants in our study, therefore we may have misclassified some cases as not having tuberculosis following a negative microbiologic result, leading to potential underestimation of association with severe outcome in patients who are co-infected.

In conclusion, we observed an increased risk of mortality in patients co-infected with influenza and tuberculosis, compared to individuals with either tuberculosis or influenza alone. This study, using laboratory-confirmed, individual-level data supports the hypothesis

from ecological studies suggesting that influenza-tuberculosis co-infection is associated with increased mortality. These data may be useful to guide clinicians and policy makers, especially in countries with high burden of tuberculosis and limited resources, in making decisions on whether influenza vaccines or influenza antivirals should be prioritised for tuberculosis patients.

Figure 1: Individuals aged ≥15 years enrolled in surveillance for severe respiratory illness (SRI) and tested for influenza and tuberculosis, Tshepong and Edendale Hospitals, South Africa, June 2010-May2016



TB=tuberculosis;*239 (6%) positive for influenza, **1092 (26%) positive for TB; A & B= included in TB–associated mortality analysis; B&C included in influenza-associated mortality

Table 1: Demographic and clinical characteristics of individuals aged ≥15 years admitted with severe respiratory illness, by tuberculosis testing status, at Tshepong and Edendale Hospitals, South Africa, June 2010-May2016

Characteristic		Tested for tuberculosis n/N (%)	Unadjusted odds ratio (95% CI)	P value	Adjusted odds ratios (95% CI)	P value
Age group (years)	15-24	373/537 (69)	Reference	Reference		
	25-44	2346/3334 (70)	1.0 (0.9-1.3)	0.670		
	45-64	1253/1805 (69)	1.0 (0.8-1.2)	0.985		
	≥65	322/537(60)	0.7 (0.5-0.8)	0.001		
Sex	Male	1986/2837 (70)	Reference			
	Female	2306/3374 (68)	0.9 (0.8-1.0)	0.159		
History of smoking	No	3438/5029 (68)	Reference			
	Yes	802/1107 (72)	1.2 (1.1-1.4)	0.008		
History of alcohol	No	3284/4848 (68)	Reference			
	Yes	958/1293 (74)	1.4 (1.2-1.6)	<0.001		
Site	Edendale	1442/2247 (64)	Reference			
	Tshepong	2857/3980 (72)	1.4 (1.3-1.6)	<0.001	1.4 (1.2-1.7)	<0.001
Cough	No	156/253 (62)	Reference			
	Yes	3696/5051 (73)	1.7 (1.3-2.2)	<0.001	2.1 (1.5-2.8)	<0.001
Night sweats	No	1481/2145 (69)	Reference			
	Yes	2644/3659 (72)	1.2 (1.0-1.3)	0.009	1.3 (1.1-1.6)	0.001
Influenza	Negative	4013/5823 (69)	Reference			
	Positive	239/325 (74)	1.3 (1.0-1.6)	0.080		
Viral coinfection	No	2994/4342 (69)	Reference			
	Yes	1258/1806 (70)	1.0 (0.9-1.2)	0.591		
HIV status	Negative	945/1407 (67)	Reference			
	Positive	3134/4445 (71)	1.2 (1.0-1.3)	0.017		
Underlying medical condition other than HIV*	No	3931/5643 (70)	Reference			
	Yes	363/570 (64)	0.7 (0.6-0.8)	<0.001		
Diabetes	No	3812/5432 (70)	Reference			
	Yes	120/212 (57)	0.5 (0.4-0.7)	<0.001	0.5 (0.4-0.8)	0.001

Worked in mine	No	2751/3609 (76)	Reference			
	Yes	351/416 (84)	1.7 (1.3-2.2)	<0.001	1.6 (1.1-2.2)	0.007
Pneumococcal	No	3532/5174 (68)	Reference			
coinfection**	Yes	456/605 (75)	1.4 (1.2-1.7)	<0.001	1.3 (1.0-1.8)	0.039
Duration of	≤7	1586/2324 (68)	Reference			
symptoms prior to admission (days)	>7	2579/3673 (70)	1.1 (1.0-1.2)	0.107		
Oxygen	No	2474/3625 (68)	Reference			
	Yes	1404/1944 (72)	1.2 (1.1-1.3)	0.003		
On antibiotics	No	166/282 (59)	Reference			
	Yes	4077/5784 (70)	1.7 (1.3-2.1)	<0.001		
ICU admission	No	4212/6094 (69)	Reference			
	Yes	18/32 (56)	0.6 (0.3-1.2)	0.121		
Duration of	<3	715/1087 (66)	Reference			
hospitalisation	3-7	1802/2503 (72)	1.3 (1.1-1.6)	<0.001	1.1 (0.9-1.5)	0.275
(days)	>7	1551/2326 (67)	1.0 (0.9-1.2)	0.603	0.8 (0.6-0.9)	0.036
Died	No	3749/5286 (71)	Reference			
	Yes	433/777 (56)	0.5 (0.4-0.6)	<0.001	0.5 (0.4-0.6)	<0.001

HIV – human immunodeficiency virus; ICU– intensive care unit, TB-Tuberculosis

*Underlying conditions included any of the following: Asthma, other chronic lung disease, chronic heart disease (valvular heart disease, coronary artery disease, or heart failure excluding hypertension), liver disease (cirrhosis or liver failure), renal disease (nephrotic syndrome, chronic renal failure), immunocompromising conditions excluding HIV infection (organ transplant, immunosuppressive therapy, immunoglobulin deficiency, malignancy), neurological disease (cerebrovascular accident, spinal cord injury, seizures, neuromuscular conditions) or pregnancy. Comorbidities were considered absent in cases for which the medical records stated that the patient had no underlying medical condition or when there was no direct reference to that condition

** Positive on *lyt*A PCR

Table 2: Factors associated with death among hospitalised individuals aged ≥15 years with severe respiratory illness testing tuberculosis positive at two sentinel surveillance sites, Edendale Hospital and Tshepong Hospital, South Africa, 2010-2016 (n=1075)

Characteristics		Case-fatality ratio (%)	Univariate analysis		Multivariable analysis	
			OR (95% CI)	P value	OR (95% CI)	P value
Demographic characteristics			Reference			
Age group (years)	15-24	8/125 (6)	Reference			
	25-44	69/656 (11)	1.7 (0.8-3.7)	0.161	1.5 (0.7-3.3)	0.286
	45-64	32/258 (12)	2.1 (1.0-4.6)	0.077	1.8 (0.8-4.0)	0.181
	≥65	7/36 (19)	3.5 (1.2-10.5)	0.024	3.6 (1.2-11.0)	0.026
Sex	Male	71/512 (14)	Reference			
	Female	45/563 (8)	0.5 (0.4-0.8)	0.002		
Site	Edendale	39/301 (13)	Reference			
	Tshepong	77/774 (10)	0.7 (0.5-1.1)	0.155		
Cough	No	3/32 (9)	Reference			
	Yes	104/960 (11)	1.2 (0.4-3.9)	0.794		
Night sweats	No	39/306 (13)	Reference			
	No	74/746 (10)	0.8 (0.5-1.1)	0.180		
HIV status	Negative	20/168 (12)	Reference			
	Positive	87/848 (10)	0.8 (0.5-1.4)	0.526	0.9 (0.5-1.6)	0.756
Underlying medical condition*	No	111/1032 (11)	Reference			
	Yes	5/43(12)	1.1 (0.4-2.8)	0.857		
Diabetes	No	113/1056 (11)	Reference			
	Yes	3/19 (16)	1.6 (0.5-5.5)	0.482		
Smoking	No	92/858 (11)	Reference			
	Yes	24/202 (12)	1.1 (0.7-1.8)	0.635		

Alcohol	No	95/831 (11)	Reference			
	Yes	21/229 (9)	0.8 (0.5-1.3)	0.333		
Influenza infection	No	108/1033 (10)	Reference			
	Yes	8/42 (19)	2.0 (0.9-4.5)	0.084	2.3 (1.0-5.2)	0.045
Pneumococcal	No	100/922 (11)	Reference			
coinfection**	Yes	9/73 (12)	1.2 (0.6-2.4)	0.696		
Viral co-infection***	No	92/773 (12)	Reference			
	Yes	22/269 (8)	0.7 (0.4-1.1)	0.094		
Duration of symptoms	≤7 days	20/258 (8)	Reference			
prior to admission	>7 days	94/786 (12)	1.6 (1.0-2.7)	0.062	1.5 (0.9-2.5)	0.114
Duration of	<3	20/189 (11)	Reference			
hospitalisation (days)	3-7	52/480 (11)	1.0 (0.6-1.8)	0.925		
	>7	42/371 (11)	1.1(0.6-1.9)	0.792		

OR – Odds ratio, CI – confidence interval, HIV – human immunodeficiency virus

*Asthma, other chronic lung disease, chronic heart disease (valvular heart disease, coronary artery disease, or heart failure excluding hypertension), liver disease (cirrhosis or liver failure), renal disease (nephrotic syndrome, chronic renal failure), immunocompromising conditions excluding HIV infection (organ transplant, immunosuppressive therapy, immunoglobulin deficiency, malignancy), neurological disease (cerebrovascular accident, spinal cord injury, seizures, neuromuscular conditions) or pregnancy. Comorbidities were considered absent in cases for which the medical records stated that the patient had no underlying medical condition or when there was no direct reference to that condition.

**Positive on *lytA* PCR

***Co-infection with at least one of parainfluenza virus 1, 2 and 3; respiratory syncytial virus; enterovirus; human metapneumovirus; adenovirus; rhinovirus.

Table 3: Factors associated with death among hospitalised individuals aged ≥15 years with severe respiratory illness and testing influenza positive at two sentinel sites, Tshepong and Edendale Hospitals, South Africa, 2010-2016 (n=232)

Characteristics		Case-fatality ratio (%)	Univariate analysis		Multivariable analysis	
			OR (95% CI)	P value	OR (95% CI)	P value
Demographic characteristics						
Age group (years)	15-24	1/19 (5)	Reference			
	25-44	8/131 (6)	1.2 (0.1-9.9)	0.885	1.0 (0.1-8.9)	0.989
	45-64	6/57 (11)	2.1 (0.2-18.8)	0.501	2.5 (0.3-24.3)	0.417
	≥65	3/25 (12)	2.5 (0.2-25.7)	0.453	5.1 (0.4-65.9)	0.210
Sex	Male	9/91 (10)	Reference			
	Female	9/141 (6)	0.6 (0.2-1.6)	0.333		
Site	Edendale	3/70 (4)	Reference			
	Tshepong	15/162 (9)	2.3 (0.6-8.1)	0.205		
Cough	No	0/4 (0)	Reference			
	Yes	15/188(8)	1.0	<0.001		
Night sweats	No	8/93 (9)	Reference			
	Yes	10/127 (8)	0.9 (0.3-2.4)	0.846		
HIV status	Negative	4/66 (6)	Reference			
	Positive	14/156 (9)	1.5 (0.5-4.8)	0.470	2.0 (0.5-8.8)	0.337
Underlying medical condition other than HIV*	No	18/205 (9)	Reference			
	Yes	0/26 (0)	1.0			
Diabetes	No	17/223 (8)	Reference			
	Yes	1/7 (14)	2.0 (0.2-17.8)	0.526		
Smoking	No	11/189 (6)	Reference			

	Yes	7/43 (16)	3.1 (1.1-8.7)	0.027		
Alcohol	No	15/192 (9)	Reference			
	Yes	3/40 (8)	0.9 (0.3-3.5)	0.946		
Worked in mine	No	8/129 (6)	Reference			
	Yes	2/9 (22)	4.3 (0.8-24.3)	0.097		
Tuberculosis	No	10/190 (5)	Reference			
	Yes	8/42 (19)	4.2 (1.6-11.5)	0.005	4.5 (1.5-13.3)	0.007
Pneumococcal	No	15/179 (8)	Reference			
coinfection**	Yes	2/33 (6)	0.7 (0.2-3.2)	0.654		
Viral co-infection***	No	14/194 (7)	Reference			
	Yes	4/36 (11)	1.6 (0.4-5.6)	0.608		
Duration of symptoms	≤7 days	6/109 (6)	Reference			
prior to admission	>7 days	12/120 (10)	1.9 (0.7-5.3)	0.213	1.6 (0.6-4.7)	0.355
Duration of	<3	4/54 (7)	Reference			
hospitalisation (days)	3-7	9/96 (9)	1.3 (0.4-4.4)	0.682		
	>7	4/76 (5)	0.7 (0.2-2.9)	0.618		

OR – Odds ratio, CI – confidence interval, HIV – human immunodeficiency virus

*Asthma, other chronic lung disease, chronic heart disease (valvular heart disease, coronary artery disease, or heart failure excluding hypertension), liver disease (cirrhosis or liver failure), renal disease (nephrotic syndrome, chronic renal failure), immunocompromising conditions excluding HIV infection (organ transplant, immunosuppressive therapy, immunoglobulin deficiency, malignancy), neurological disease (cerebrovascular accident, spinal cord injury, seizures, neuromuscular conditions) or pregnancy. Comorbidities were considered absent in cases for which the medical records stated that the patient had no underlying medical condition or when there was no direct reference to that condition.

**Positive on *lytA* PCR

***Co-infection with at least one of parainfluenza virus 1, 2 and 3; respiratory syncytial virus; enterovirus; human metapneumovirus; adenovirus; rhinovirus

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RESEARCH ARTICLE Open Access

Influenza virus infection is associated with increased risk of death amongst patients hospitalized with confirmed pulmonary tuberculosis in South Africa, 2010–2011

Sibongile Walaza[1,2*], Stefano Tempia[3,4], Halima Dawood[6], Ebrahim Variava[8,9], Jocelyn Moyes[1,2], Adam L Cohen[3,4], Nicole Wolter[1], Michelle Groome[5], Claire von Mollendorf[1,2], Kathleen Kahn[7], Marthi Pretorius[1], Marietjie Venter[1,10], Shabir A Madhi[1,5] and Cheryl Cohen[1,2*]

Abstract

Background: Data on the association between influenza and tuberculosis are limited. We describe the characteristics of patients with laboratory-confirmed tuberculosis, laboratory-confirmed influenza and tuberculosis-influenza co-infection.

Methods: Patients hospitalized with severe respiratory illness (acute and chronic) were enrolled prospectively in four provinces in South Africa. Naso/oropharyngeal specimens were tested for influenza virus by real time reverse transcriptase polymerase chain reaction. Tuberculosis testing was conducted as part of clinical management.

Results: From June 2010 through December 2011, 8032 patients were enrolled and influenza testing was conducted on 7863 (98%). Influenza virus was detected in 765 (10%) patients. Among 2959 patients with tuberculosis and influenza results, 2227 (75%) were negative for both pathogens, 423 (14%) were positive for tuberculosis alone, 275 (9%) were positive for influenza alone and 34 (1%) had influenza and tuberculosis co-infection. On multivariable analysis amongst individuals with symptoms for ≥7 days, tuberculosis influenza co-infection was associated with increased risk of death, (adjusted relative risk ratio (aRRR) (6.1, 95% confidence interval (CI) 1.6-23.4), as compared to tuberculosis only infection. This association was not observed in individuals with symptoms for <7 days (aRRR.0.8, 95% CI 0.1-7.0).

Conclusion: Tuberculosis and influenza co-infection compared to tuberculosis single infection was associated with increased risk of death in individuals with symptoms ≥7 days. The potential public health impact of influenza vaccination among persons with laboratory-confirmed tuberculosis should be explored.

Keywords: Influenza, Tuberculosis, Co-infection, South Africa

Background

Despite the availability of efficacious treatment, tuberculosis remains the second most common cause of infectious disease–related deaths worldwide, after human immunodeficiency virus (HIV) and acquired immune deficiency syndrome (AIDS) [1]. In 2010, there were an estimated 8.8 million incident cases of tuberculosis globally [2]. HIV infection is an important risk factor for tuberculosis, and HIV-infected individuals have a 20 times greater risk of developing tuberculosis as compared to HIV-uninfected individuals [3]. In South Africa, there were an estimated 390,000 incident cases of tuberculosis in 2011 and 65% were co-infected with HIV [1].

Similarly, despite the availability of effective vaccines, influenza virus infection causes substantial annual morbidity and mortality worldwide and in South Africa [4-6]. Globally, it is estimated that influenza epidemics result in three to five million cases of severe illness, and about

* Correspondence: sibongilew@nicd.ac.za; cherylc@nicd.ac.za
[1]Centre for Respiratory Disease and Meningitis, National Institute for Communicable Diseases (NICD) of the National Health Laboratory Service (NHLS), Private Bag X4, Sandringham, 2131 Johannesburg, Gauteng, South Africa
[2]School of Public Health, Faculty of Health Science, University of the Witwatersrand, Johannesburg, South Africa
Full list of author information is available at the end of the article

250,000-500,000 deaths annually [4]. In 2010 an estimated 12 million episodes of severe and 3 million episodes of very severe acute lower respiratory tract infection resulted in hospital admissions in children <5 years worldwide [5]. A number of risk factors are described for severe outcomes among patients with influenza; these include extremes of age, chronic underlying diseases, pregnancy and immune-suppression [6]. HIV-infected individuals have an increased risk of influenza-associated hospitalisations and mortality [7].

While chronic lung diseases are a known risk factor for severe outcome due to influenza infection, there are few published data on the association between influenza and tuberculosis. Review of data from the 1918 influenza pandemic suggests that individuals with tuberculosis had a disproportionately higher mortality than the general population [8,9] and that underlying tuberculosis infection may have contributed to the elevated mortality observed in young adults [8,10]. There is little information about the interaction between seasonal or pandemic influenza and tuberculosis in recent years. In South Africa, 10% of cases that died during the 2009 A(H1N1)pdm09 pandemic were co-infected with tuberculosis [11]. In South Korea, concurrent tuberculosis and influenza A(H1N1) pdm09 infection was reported in 7 immunocompetent patients, none resulting in fatal outcome [12].

We describe the epidemiological and clinical characteristics of patients admitted with laboratory-confirmed tuberculosis, influenza and tuberculosis-influenza co-infection among patients hospitalized with severe respiratory illness (SRI) in South Africa, a country with a high prevalence of HIV.

Methods

Study design

We conducted prospective hospital-based active surveillance for Severe Acute Respiratory Illness (SARI) surveillance at 6 public hospitals in 4 surveillance sites situated across the country: Chris Hani Baragwanath Academic Hospital (CHBAH), Gauteng Province; Edendale Hospital, KwaZulu-Natal Province; the two hospitals of the Klerksdorp–Tshepong Hospital Complex (KTHC), Northwest Province; and Mapulaneng and Matikwana Hospitals, Mpumalanga Province [13]. At each sentinel hospital trained surveillance officers screened all admitted patients for surveillance inclusion criteria from Monday to Friday, except at CHBAH where adult patients were screened on 2 of every 5 days per week because of large patient numbers.

Case definitions

A case of SARI was defined as a hospitalised individual with onset of illness within seven days of admission meeting age-specific clinical inclusion criteria. We included children aged two days through <3 months with physician-diagnosed sepsis or acute lower respiratory tract infection (ALRI), children aged three months through <5 years with physician-diagnosed ALRI (including, for example bronchitis, bronchiolitis, pneumonia and pleural effusion) and patients aged ≥5 years meeting a modified World Health Organization (WHO) case definition for SARI including sudden onset of fever (>38°C) or reported fever, cough or sore throat, and shortness of breath or difficulty breathing [14]. In addition expanded case definitions were applied at three of the hospitals in two of the surveillance sites, (Edendale Hospital and KTHC) to include individuals meeting the above case definition but with symptoms for >7 days and individuals with suspected or confirmed tuberculosis; these patients were defined as having severe chronic respiratory illness (SCRI). For this analysis severe respiratory illness (SRI) was used to refer to all enrolled patients i.e. patients admitted with an acute or chronic respiratory illness.

The South African National Department of Health tuberculosis case definitions were modified to define suspected tuberculosis [15]. A suspected tuberculosis case in children <12 years was defined as: i) any child presenting with a history of exposure to an infectious tuberculosis case or with a positive tuberculin skin test and symptoms of tuberculosis (chronic cough, weight loss and fever) with or without an abnormal chest x-ray suggestive of tuberculosis, or ii) admitted with a physician diagnosis of tuberculosis or suspected tuberculosis, or iii) initiated on tuberculosis treatment on admission; or iv) was on tuberculosis treatment for <2 months at the time of admission. A suspected tuberculosis case in individuals ≥12 years of age was defined as: i) any patient presenting with night sweats, chronic cough, weight loss or haemoptysis lasting for >2 weeks, or ii) had a physician diagnosis of tuberculosis or suspected tuberculosis, or iii) was initiated on tuberculosis treatment on admission, or iv) was on tuberculosis treatment for <2 months at the time of admission.

Patients who were on tuberculosis treatment for ≥2 months at the time of admission were not enrolled into the surveillance programme as cases of suspected or confirmed tuberculosis (because we assumed that after two months of treatment tuberculosis was no longer the reason for their clinical presentation). These patients were, however, enrolled if they met the criteria for SARI. The tuberculosis status of the enrolled patients was based on results of tuberculosis testing at current admission.

For this analysis, a laboratory-confirmed tuberculosis case was defined as an individual with a positive result for *Mycobacterium tuberculosis* on microscopy, culture or polymerase chain reaction (PCR) by GeneXpert MTB/RIF test (Cepheid, Sunnyvale, California) from the current hospital admission or from a specimen taken within two weeks preceding or following the admission. An influenza

case was defined as an individual with a positive PCR test for influenza. An influenza-tuberculosis co-infection case met criteria for both laboratory-confirmed tuberculosis and influenza during the same admission.

Data collection

A standardized questionnaire was used to collect demographic and clinical data, medical history of the patient and in-hospital outcome. Hospital and intensive care unit (ICU) admission and collection of specimens for bacterial culture, tuberculosis testing and CD4+ T-cell counts were performed according to attending-physician discretion.

Sample collection and processing

Respiratory specimens (oropharyngeal and nasopharyngeal swabs for patients ≥5 years of age or nasopharyngeal aspirates for children <5 years of age), were collected and placed in 4 ml virus transport medium. Whole blood samples were collected in EDTA-containing vacutainer tubes within 24 hours of hospital admission for the detection of *Streptococcus pneumoniae* and HIV infection.

After collection, respiratory and blood samples were kept at 4°C at the sentinel site, and transported on ice at least twice per week to the National Institute for Communicable Diseases of the National Health Laboratory Services (NICD-NHLS) for testing.

Detection of respiratory viruses and *S. pneumoniae*

Respiratory samples were tested by multiplex real-time reverse-transcription polymerase chain reaction (PCR) (rRT-PCR) for influenza type A and B, adenovirus, enterovirus, rhinovirus, human metapneumovirus, respiratory syncytial virus and parainfluenza virus types 1–3 [16]. The influenza A positive specimens were subtyped using the Centers for Disease Control and Prevention (CDC) rRT-PCR protocol for detection and characterization of influenza. DNA was extracted from whole blood specimens using the MagNA Pure LC 2.0 instrument and DNA Isolation kit III for bacteria (Roche, Mannheim, Germany) and were tested for the *S. pneumoniae lytA* gene by a quantitative real-time PCR [17].

Determination of HIV infection

HIV status data were obtained from two data sources. Some patients had HIV testing requested by admitting physicians as part of clinical care. This included HIV enzyme-linked immunosorbent assay (ELISA) testing with confirmation by ELISA on a second specimen for patients ≥18 months of age and qualitative HIV PCR testing for confirmation of HIV-infection status in children <18 months of age. In addition, for consenting patients, linked anonymous HIV PCR testing for children <18 months of age or ELISA for patients ≥18 months of age was performed using a dried blood spot or whole blood specimen at the NICD-NHLS laboratory.

Determination of tuberculosis infection

Testing for *M. tuberculosis*, including microscopy, culture, GeneXpert MTB/RIF test, or a combination of these, was undertaken at the discretion of the attending-physician and performed at the laboratory serving the hospital where the patient presented. Specimens for tuberculosis testing were examined by light microscopy for the presence of acid fast bacilli. Tuberculosis culture was performed using the BACTEC MGIT automated culture system (Becton Dickinson, Franklin Lakes, New Jersey). Tuberculosis PCR was performed using the Xpert MTB/RIF system (Cepheid, Sunnyvale, California). The GeneXpert MTB/RIF is an automated sample processing and nucleic acid amplification test for detection of *M. tuberculosis* and is able to detect resistance to rifampicin. The National Department of Health tuberculosis treatment guidelines recommended treatment for patients with positive smears and that patients suspected of tuberculosis should have two specimens tested on microscopy and if these were negative then a 3rd sample should be tested for smear and culture [15].

Data analysis

To identify factors associated with tuberculosis testing, tuberculosis positivity and tuberculosis-influenza co-infection we included both potential determinants for, as well as outcomes or characteristics of the primary endpoints of the analysis. Univariate comparisons were performed using logistic or multinomial regression. In addition, we implemented three multivariable models to identify factors associated with: (i) tuberculosis testing among enrolled patients; (ii) tuberculosis positivity among enrolled patients tested for tuberculosis; and (iii) tuberculosis single infection compared to influenza single infection or tuberculosis-influenza co-infection. The tuberculosis testing and positivity models were implemented using stepwise forward selection logistic regression. Multinomial regression was used for the comparison of tuberculosis only, influenza only and tuberculosis-influenza co-infected groups and this analysis was performed separately in individuals with symptoms for <7 days and ≥7 days. Multinomial regression allows modelling of outcome variables with >2 categories and relates the probability of being in category j to the probability of being in a baseline category. A complete set of coefficients are estimated for each of the j levels being compared with the baseline and the effect of each predictor in the model is measured as relative risk ratio (RRR). For this analysis, we used the tuberculosis single infection group as the baseline category and compared it with the influenza single infection and tuberculosis-influenza co-infection

groups and we restricted the analysis to laboratory confirmed cases for both influenza and tuberculosis. The general form of the multivariable multinomial model with dichotomous predictors was as follows:

$$\ln\frac{p(Y_j)}{p(Y_1)} = \beta_0^{(j)} + \beta_1^{(j)}X_1 + \ldots + \beta_n^{(j)}X_n + \varepsilon \quad (1)$$

Where $p(Y_1)$ is the probability of the outcome variable in the base category (tuberculosis single infection) and $p(Y_j)$ is the probability of the outcome variable in the other categories; j represents the two other categories ($j = 2$: influenza single infection; and $j = 3$: tuberculosis-influenza coinfection) that are compared to the base category; $\beta_0^{(j)}$ is the model constant for category j; $\beta_1^{(j)}$ to $\beta_n^{(j)}$ are coefficients associated with predictors X_1 to X_n in category j; and ε is the error term.

The model in equation 1 was fitted to any enrolled case with available tuberculosis and influenza results irrespective of duration of symptoms as well as separately among individuals with acute (duration of symptoms <7 days) and chronic (duration of symptoms ≥7 days) clinical presentation to specifically assess the clinical characteristics of individuals within these two groups. In order to allow for direct comparison of the final multivariable models for acute and chronic patients we elected to retain any variable which was significant at 0.05 in either one of these models in the final multivariable model. Covariates with a p-value <0.2 at the univariate analysis were assessed for significance at the multivariable analysis and statistical significance was assessed at $p < 0.05$ for all models. Statistically significant variables were retained in the multivariable models. HIV status was retained in the multinomial models a priori as this is a potential confounder of the interaction between influenza and tuberculosis. Two-way interactions were assessed by inclusion of product terms for all variables remaining in the final additive models. The statistical analysis was implemented using STATA® version 12 (StataCorp, Texas, USA).

Ethics

Ethical approval for the study was obtained from the University of the Witwatersrand, Human Research Ethics committee (reference M081042) and the University of KwaZulu-Natal Biomedical Research Ethics committee (reference BF157/08). The United States Centers for Disease Control and Prevention deemed this surveillance not to be research and therefore this project did not need human subjects review.

Results

Patient enrolment and testing

From June 2010 through December 2011, we enrolled 8032 patients, and influenza results were obtained from 7863 (98%) cases (Figure 1). Of these 4077 (52%) were females and 3291 (42%) were children ≤5 years. Influenza

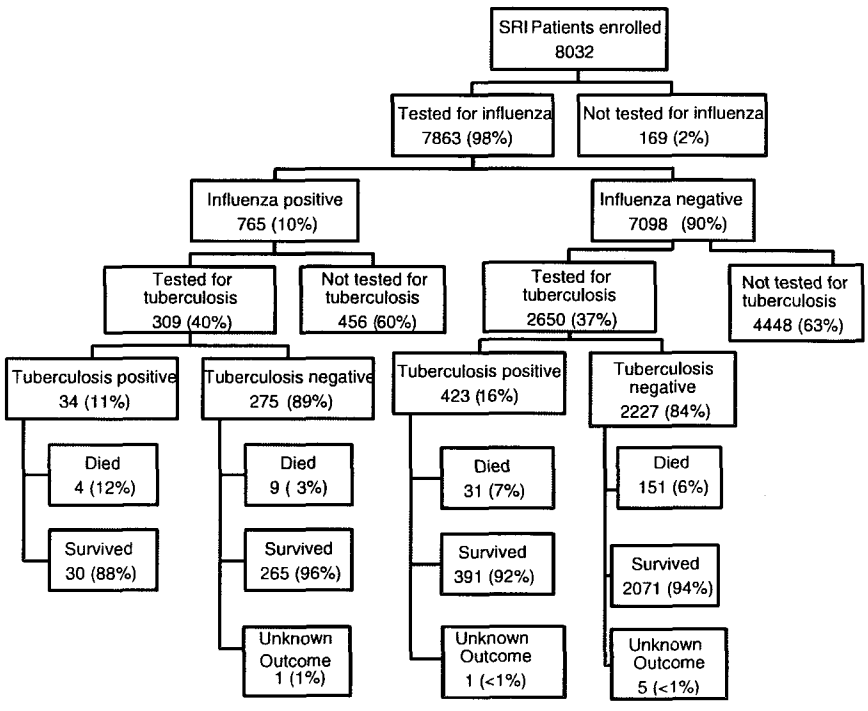

Figure 1 Flow chart of patients enrolled into the severe respiratory illness (acute and non acute) surveillance, South Africa 2009-2011.

virus was detected in 765 (10%) patients. An influenza subtype was characterized from 764 (99.8%) influenza positive samples, including 219 (29%) influenza A(H1N1)pdm09, 195 (25%) A(H3N2) and 350 (46%) influenza B. Among patients with available influenza results, 2959 (38%) were tested for tuberculosis during admission. Of these, 69% (2029) and 31% (930) met the SARI and SCRI case definitions, respectively. HIV results were available for 2739 (93%) of 2959 patients with available tuberculosis results and 1877 (69%) were HIV-infected. Among influenza tested patients with available data <1% (6/7863) received oseltamivir and <1% (4/7857) were vaccinated against influenza. Two percent (179/7863) of enrolled patients with results for influenza and 2% (49/2959) of those tested for influenza and tuberculosis were already receiving tuberculosis treatment at time of current admission.

Factors associated with tuberculosis testing on admission

Among patients with available influenza results, on multivariable analysis factors associated with tuberculosis testing were age ≥5 years, with the highest proportion tested (56%, 1378/2473) in the 25–44 age group (adjusted odds ratio (aOR) 3.5, 95% CI 3.0-4.2) as compared to age <5 years, HIV infection (aOR 1.5, 95% CI 1.3- 1.7), symptom duration ≥7 days (aOR 1.3, 95% CI 1.1-1.5), hospitalisation duration >7 days (aOR 1.3, 95% CI 1.1- 1.4), requiring oxygen therapy (aOR 1.2, 95% CI 1.0-1.3) and antibiotic (aOR 1.9, 95% CI 1.4-2.5) or tuberculosis (aOR 1.2, 95% CI 1.0-1.4) treatment started during current admission (Table 1). The proportion of patients tested for tuberculosis was similar for patients testing influenza-positive and influenza-negative and for those who died in-hospital and those who survived.

Factors associated with laboratory-diagnosed tuberculosis

Among 2959 patients with available tuberculosis and influenza results, 15% (457/2959) tested positive for tuberculosis on the current admission and 2% (10/457) of these were receiving tuberculosis treatment at time of current admission. The highest percentage testing tuberculosis positive was in the 25–44 years age group (18%, 249/1378) (Table 2). Of the patients testing positive for tuberculosis, 332 (73%) were positive on microscopy only. The tuberculosis detection rate was 10% among those with symptoms for <7 days and 25% among those with symptoms for ≥7 days. Of the 457 laboratory-confirmed tuberculosis cases, 198 (43%) and 259 (57%) met the SARI and SCRI case definitions respectively. On multivariable analysis, patients with laboratory-diagnosed tuberculosis were less likely to be co-infected with *S. pneumoniae* (aOR 0.5, 95% CI 0.3-0.9) and to have received oxygen therapy (aOR 0.7, 95% CI 0.5- 0.9). They were more likely to be admitted at KTHC as compared to CHBAH (aOR 1.9, 95% CI 1.4-2.6) and to be started on tuberculosis treatment at current admission (aOR 4.6, 95% CI 3.5-6.0) (Table 2).

HIV results were available for 93% (424/457) of tuberculosis-positive and 92% (2315/2502) of tuberculosis-negative individuals. Of these, 335 (79%) of tuberculosis-positive and 1542 (67%) of tuberculosis-negative individuals were HIV infected. Amongst tuberculosis-positive patients tested for HIV, age-specific HIV prevalence were 36% (15/42), 79% (35/44), 95% (222/234), 65% (58/89), 33% (5/15) in the <5 years, 5–24 years, 25–44 years, 45–64 years and ≥65 years age groups, respectively. Amongst tuberculosis-negative patients tested for HIV, age-specific HIV prevalence were 19% (78/417), 71% (147/206), 89% (966/1081), 65% (325/502) and 24% (26/109) in the <5 years, 5–24 years, 25–44 years, 45–64 years and ≥65 years age groups, respectively.

Factors associated with tuberculosis and influenza infection

Among the 2959 patients with available tuberculosis and influenza results, 2227 (75%) were negative for both pathogens, 423 (14%) were positive for tuberculosis alone, 275 (9%) were positive for influenza alone and 34 (1%) had influenza and tuberculosis co-infection. Tuberculosis treatment was being received at the time of admission for 2% (n = 10) of patients positive for tuberculosis only and <1% (n = 2) of patients positive for influenza only. None of the patients with influenza and tuberculosis co-infection were on tuberculosis treatment at the time of admission.

On multivariable analysis adjusting for age group, patients infected with influenza only compared to patients with tuberculosis only were less likely to be HIV infected (adjusted relative risk ratio (aRRR) 0.6, 95% CI 0.4-0.9), to present with duration of symptoms ≥7 days (aRRR 0.3, 95% CI 0.2-0.4), to be admitted for longer than 7 days (aRRR 0.6, 95% CI 0.4-0.9) while they were more likely to be started on oxygen therapy (aRRR 1.9, 95% CI 1.3-2.8) (Table 3). Patients co-infected with tuberculosis and influenza compared to patients with tuberculosis only were less likely to present with symptoms ≥7 days (aRRR 0.3, 95% CI 0.1-0.7), but were at increased risk of death (aRRR 3.1, 95% CI 1.1-10.1).

Of the 720 patients testing positive for influenza and/or tuberculosis, 384 (53%) presented with symptom duration <7 days. Of these, 166 (43%) were positive for tuberculosis alone, 194 (50%) were positive for influenza alone and 24 (6%) had tuberculosis-influenza co-infection. Of the 336/720 (47%) patients testing positive for influenza and/or tuberculosis with symptom duration ≥7 days, 246 (73%) were positive for tuberculosis alone, 81 (24%) were positive for influenza alone and 9 (3%) had tuberculosis-influenza co-infection.

Table 1 Demographic and clinical characteristics of patients admitted with severe respiratory illness (acute and chronic), by tuberculosis testing status at four sites in South Africa, June 2010- December 2011

Characteristic		Tested for tuberculosis n/N (%)	Unadjusted odds ratio (95% CI)	P value	Adjusted odds ratios (95% CI)	P value
Age group (years)	0-4	571/3291 (17)	Reference		Reference	
	5-24	270 /587 (46)	4.05 (3.4-4.9)	<0.001	3.3 (2.6-4.1)	<0.001
	25-44	1378/2473 (56)	5.99 (5.3-6.8)	<0.001	3.5 (3.0-4.2)	<0.001
	45-64	611/1197 (51)	4.96 (4.3-5.7)	<0.001	3.2 (2.7-3.8)	<0.001
	≥65	129/315 (39)	3.30 (2.6-4.2)	<0.001	2.6 (2.0-3.4)	<0.001
Sex	Female	1664/4077 (41)	Reference			
	Male	1295/3786 (34)	0.8 (0.7-0.8)	<0.001		
Site	Chris Hani Baragwanath	1618/4437 (36)	Reference		Reference	
	Mapulaneng & Matikwana	144/749 (19)	0.4 (0.3-0.5)	<0.001	0.4 (0.3-0.5)	<0.001
	Edendale	385/1156 (33)	0.9 (0.8-0.99)	0.046	0.7 (0.6-0.8)	<0.001
	Klerksdorp/Tshepong	812/1521 (53)	1.99 (1.8-2.2)	<0.001	1.1 (0.95-1.3)	0.152
Influenza	Negative	2650/7098 (37)	Reference			
	Positive	309/765 (40)	1.1 (0.9-1.3)	0.097		
HIV status	Negative	862/3410 (25)	Reference		Reference	
	Positive	1877/3523 (53)	3.4 (3.0-3.7)	<0.001	1.5 (1.3-1.7)	<0.001
Underlying medical condition*	No	2652/7041 (38)	Reference			
	Yes	307/821 (37)	0.98 (0.85-1.14)	0.879		
Duration of symptoms prior to admission (days)	<7	1884/5957(32)	Reference		Reference	
	≥7	1012/1765 (57)	2.9 (2.6-3.2)	<0.001	1.3 (1.1-1.5)	0.003
Concurrent invasive bacterial infection**	No	653/1832 (36)	Reference			
	Yes	24/46(52)	1.96 (1.1- 3.5)	0.023		
Pneumococcal	No	2435/6284 (39)	Reference			
Infection***	Yes	214/434 (49)	1.5 (1.3-1.9)	<0.001		
Receiving tuberculosis treatment at time of admission	No	158/292 (54)	Reference	<0.001		
	Yes	49/179 (27)	0.3 (0.2-0.5)			
Started on treatment for tuberculosis	No	2301/6716 (34)	Reference		Reference	
	Yes	647/1108 (58)	2.7 (2.4-3.1)	<0.001	1.2 (1.0-1.4)	0.036

Table 1 Demographic and clinical characteristics of patients admitted with severe respiratory illness (acute and chronic), by tuberculosis testing status at four sites in South Africa, June 2010- December 2011 *(Continued)*

Antibiotics prescribed	No	77/332 (23)	Reference		Reference	
	Yes	2877/7515 (38)	2.1 (1.6-2.7)	<0.001	1.9 (1.4-2.5)	<0.001
Oxygen therapy	No	1869/5145 (36)	Reference			
	Yes	1086/2698 (40)	1.2 (1.1-1.3)	0.001	1.2 (1.0-1.3)	0.018
ICU admission	No	2936/7772 (38)	Reference			
	Yes	15/65 (23)	0.5 (0.3-0.9)	0.017		
Duration of hospitalisation (days)	≤7	1913/5666 (34)	Reference		Reference	
	>7	1029/2143 (48)	2.9 (2.6-3.2)	<0.001	1.2 (1.1-1.4)	0.002
Died	No	2757/7321 (38)	Reference	Reference		
	Yes	195/509 (38)	1.02 (0.9-1.2)	0.769		

HIV – human immunodeficiency virus; ICU– intensive care unit, TB-Tuberculosis.

*Underlying conditions included any of the following: Asthma, other chronic lung disease, chronic heart disease (valvular heart disease, coronary artery disease, or heart failure excluding hypertension), liver disease (cirrhosis or liver failure), renal disease (nephrotic syndrome, chronic renal failure), diabetes mellitus, immunocompromising conditions excluding HIV infection (organ transplant, immunosuppressive therapy, immunoglobulin deficiency, malignancy), neurological disease (cerebrovascular accident, spinal cord injury, seizures, neuromuscular conditions) or pregnancy. Comorbidities were considered absent in cases for which the medical records stated that the patient had no underlying medical condition or when there was no direct reference to that condition.

**Concurrent invasive bacterial infections were defined as a bacterial pathogen isolated from blood, cerebrospinal fluid or another sterile site from a specimen taken within 48 hours of hospitalisation; organisms viewed as likely contaminants were excluded.

***Pneumococcal co-infection (lyt A PCR positive for *Streptococcus pneumoniae* on blood specimen).

Other variables evaluated but not presented in the table because they were not significant on univariate analysis were, Oseltamivir treatment prescribed and smoking and alcohol intake for patients ≥12 years.

Table 2 Demographic and clinical characteristics associated with laboratory-confirmed tuberculosis among patients admitted with severe respiratory illness (acute and chronic) that were tested for tuberculosis and influenza at four sites in South Africa, June 2010- December 2011

Characteristic		Laboratory confirmed tuberculosis n/N (%)	Unadjusted odds ratio (95% CI)	P value	Adjusted odds ratios (95% CI)	P value
Age group (years)	0-4	55/571 (10)	Reference		Reference	
	5-24	47/270 (17)	1.97 (1.3-3.0)	0.001	1.36 (0.8-2.3)	0.233
	25-44	249/1378 (18)	2.1 (1.5-2.8)	<0.001	1.37 (0.9-2.06)	0.120
	45-64	90/611 (15)	1.6 (1.1-2.3)	0.008	1.11 (0.7-1.7)	0.628
	65+	16/129 (12)	1.3 (0.7-2.4)	0.348	0.94 (0.5-1.9)	0.872
Site	Chris Hani Baragwanath	141/1618 (9)	Reference		Reference	
	Mapulaneng & Matikwana	23/144 (16)	1.99 (1.2-3.2)	0.005	1.4 (0.8-2.3)	0.228
	Edendale	49/385 (13)	1.5 (1.1-2.2)	0.016	0.9 (0.6-1.4)	0.668
	Klerksdorp/Tshepong	244/812 (30)	4.5 (3.6-5.6)	<0.001	1.9 (1.4-2.6)	<0.001
Influenza status	Negative	423/2650 (16)	Reference			
	Positive	34/309 (11)	0.7 (0.5-0.9)	0.023		
HIV status	Negative	89/862 (10)	Reference			
	Positive	335/1877 (18)	1.9 (1.5-2.4)	<0.001		
Underlying medical condition*	No	426/2652 (16)	Reference			
	Yes	31/307 (10)	0.6 (0.4-0.9)	0.007		
Duration of symptoms prior to admission	<7	190/1884 (10)	Reference			
	≥7	255/1012 (25)	3.0 (2.4-3.7)	<0.001		
Pneumococcal co-infection on PCR**	No	400/2435 (16)	Reference		Reference	
	Yes	18/214 (8)	0.5 (0.3-0.8)	0.003	0.5 (0.3-0.9)	0.016
Oxygen therapy	No	337/1869 (18)	Reference		Reference	
	Yes	120/1086 (11)	0.6 (0.4-0.7)	<0.001	0.7 (0.5- 0.9)	0.007
Receiving TB treatment at time of admission	No	25/158 (16)	Reference			
	Yes	10/49 (20)	1.4 (0.6-3.1)	0.456		
Started on TB treatment	No	205/2301 (9)	Reference		Reference	
	Yes	251/647 (39)	6.5 (5.2-8.0)	<0.001	4.6 (3.5-6.0)	<0.001
Duration of hospitalisation (days)	≤7	279/1913 (14)	Reference			
	>7	174/1029 (17)	1.2 (0.96-1.6)	0.096		
Season	Summer	57/366 (15)	Reference			
	Autumn	78/449 (17)	1.1 (0.8-1.6)	0.492		
	Winter	178/1027 (17)	1.1 (0.8-1.6)	0.441		
	Spring	144/1117 (13)	0.8 (0.6-1.1)	0.194		
Died	No	421/2757 (15)	Reference			
	Yes	35/195 (18)	1.2 (0.8-1.8)	0.318		

HIV – human immunodeficiency virus; ICU– Intensive Care Unit, TB-Tuberculosis, CI – confidence interval.
Analysis includes 2029 individuals with severe acute respiratory illness and 930 with severe chronic respiratory illness.
*Underlying conditions included any of the following: Asthma, other chronic lung disease, chronic heart disease (valvular heart disease, coronary artery disease, or heart failure excluding hypertension), liver disease (cirrhosis or liver failure), renal disease (nephrotic syndrome, chronic renal failure), diabetes mellitus, immunocompromising conditions excluding HIV infection (organ transplant, immunosuppressive therapy, immunoglobulin deficiency, malignancy), neurological disease (cerebrovascular accident, spinal cord injury, seizures, neuromuscular conditions) or pregnancy. Comorbidities were considered absent in cases for which the medical records stated that the patient had no underlying medical condition or when there was no direct reference to that condition.
**_lyt A_ PCR positive for *Streptococcus pneumoniae* on blood specimen.
Other variables that were evaluated but not presented in the table because they were not significant on univariate analysis were sex, influenza type, concurrent bacterial infection, antibiotic prescribed on admission, admission to intensive care unit, and smoking and alcohol intake for patients ≥12 years.

Table 3 Factors associated with influenza infection and tuberculosis-influenza co-infection compared with tuberculosis infection only in patients admitted with severe respiratory illness (acute and chronic) and tested for tuberculosis and influenza at four sites in South Africa, June 2010- December 2011

Variables		Laboratory confirmed tuberculosis Reference[a]	Laboratory confirmed influenza			Laboratory confirmed tuberculosis-influenza co-infection		
			n/N (%)	RRR[b]	ARRR[c] (95% CI)	n/N (%)	RRR[b]	ARRR[c] (95% CI)
Age group (years)	0-4	47/423 (11)	46/275 (10)	Reference	Reference	8/34 (22)	Reference	Reference
	5-24	44/423 (10)	28/275 (11)	0.6 (0.3-1.2)	1.3 (0.6-2.9)	3/34 (9)	0.4 (0.1-1.6)	0.5 (0.1-2.5)
	25-44	231/423 (55)	121/275 (44)	0.5 (0.3-0.8)	1.5 (0.8-2.8)	18/34 (53)	0.5 (0.2-1.2)	0.5 (0.2-1.7)
	45-64	86/423 (20)	57/275 (21)	0.7 (0.4-1.1)	1.3 (0.7-2.5)	4/34 (12)	0.3 (0.1-0.9)	0.3 (0.1-2.5)
	65+	15/423 (3)	23/275 (8)	1.6 (0.7-3.4)	3.9 (1.6-9.8)	1/34 (3)	0.3 (0.04-3.4)	9.53e-07
Sex	Female	247/423 (58)	176/275 (64)	Reference		20/34 (59)	Reference	
	Male	176/176 (42)	99/275 (36)	0.8 (0.6-1.1)		14/34 (41)	1.0 (0.5-1.9)	
Site	Chris Hani Baragwanath	124/423 (29)	169/275 (61)	Reference		17/34 (50)	Reference	
	Mapulaneng & Matikwana	21/423 (5)	11/275 (4)	0.4 (0.2-0.8)		2/34 (6)	0.7 (0.1-3.2)	
	Edendale	48/423 (11)	22/275 (8)	0.3 (0.2-0.6)		1/34 (3)	0.2 (0.01-1.2)	
	Klerksdorp/ Tshepong	230/423 (54)	73/275 (27)	0.2 (0.2-0.3)		14/34 (41)	0.4 (0.2-0.9)	
HIV status	Negative	82/392 (21)	99/257 (39)	Reference	Reference	7/32 (22)	Reference	Reference
	Positive	310/392 (79)	158/257 (61	0.4 (0.3-0.6)	0.6 (0.4-0.9)	25/32 (78)	0.9 (0.4-2.3)	1.6 (0.5-4.9)
Underlying medical conditions[d]	No	394/423 (93)	240/275 (87)	Reference		32/34 (94)	Reference	
	Yes	29/423 (7)	35/275 (13)	1.98 (1.2-3.3)		2/34 (6)	0.8 (0.2-3.7)	
Duration of symptoms prior to admission	<7	166/412 (40)	194/275 (71)	Reference	Reference	24/33 (73)	Reference	Reference
	≥7	246/412 (60)	81/275 (29)	0.3 (0.2-0.4)	0.4 (0.2-0.4)	9/33 (27)	0.3 (0.1-0.6)	0.3 (0.1-0.7)
Pneumoccocal co-infection on PCR[e]	No	371/388 (96)	233/246 (95)	Reference	Reference	29/30 (97)		
	Yes	17/388 (4)	13/246 (5)	1.2 (0.6-2.6)		1/30 (3)	0.8 (0.1-5.8)	
Oxygen therapy	No	313/423 (74)	158/275 (57)	Reference	Reference	24/34 (71)	Reference	Reference
	Yes	110/423 (26)	117/275 (43)	2.1 (1.5-2.9)	1.9 (1.3-2.8)	10/34 (29)	1.2 (0.5-2.6)	0.97 (0.4-2.3)
Tuberculosis treatment	No	185/422 (44)	243/274 (89)	Reference		20/34 (59)	Reference	
	Yes	237/422 (56)	31/274 (11)	0.1 (0.1-0.2)		14/34 (41)	0.5 (0.3-1.1)	
Hospital duration (days)	≤	257/420 (61)	198/274 (72)	Reference		22/33 (67)	Reference	Reference
	>7	163/420 (39)	76/274 (28)	0.6 (0.4-0.8)	0.6 (0.4-0.9)	11/33 (33)	0.8 (0.4-1.7)	0.7 (0.3-1.5)
Died	No	391/422 (73)	265/274	Reference	Reference	30/34 (88)	Reference	Reference
	Yes	31/422 (7)	9/274	0.4 (0.2-0.9)	0.5 (0.2-1.1)	4/34 (12)	1.7 (0.6-5.1)	3.2 (1.1-10.0)

[a]Tuberculosis only group used as reference for multinomial model analysis (i.e. comparing influenza only to tuberculosis only and tuberculosis–influenza co-infection to tuberculosis only group).
[b]Unadjusted relative risk ratio (RRR) at multivariable analysis.
[c]Adjusted relative risk ratio (ARRR) at multivariable analysis.
[d]Underlying conditions included any of the following: Asthma, other chronic lung disease, chronic heart disease (valvular heart disease, coronary artery disease, or heart failure excluding hypertension), liver disease (cirrhosis or liver failure), renal disease (nephrotic syndrome, chronic renal failure), diabetes mellitus, immunocompromising conditions excluding HIV infection (organ transplant, immunosuppressive therapy, immunoglobulindeficiency, malignancy), neurological disease (cerebrovascular accident, spinal cord injury, seizures, neuromuscular conditions) or pregnancy. Comorbidities were considered absent in cases for which the medical records stated that the patient had no underlying medical condition or when there was no direct reference to that condition.
[e]*lyt* A PCR positive for *Streptococcus pneumoniae* on blood specimen.

The median duration of symptoms was 2 (range 0–6) and 14 (7–195) days in patients presenting with symptoms <7 days and ≥7 days respectively.

On multivariable analysis, among patients presenting with symptoms <7 days, patients with influenza only compared to patients with tuberculosis only were less likely to be admitted for >7 days (aRRR 0.4, 95% CI 0.3-0.7). No differences were found in patients co-infected with tuberculosis and influenza compared to patients with tuberculosis only and there was no increased risk of death

Table 4 Factors associated with influenza infection and tuberculosis-influenza co-infection compared with tuberculosis infection only among patients presenting with symptoms duration <7 days admitted with severe respiratory illness and tested for tuberculosis and influenza at four sites in South Africa, June 2010-2011

Variables		Laboratory confirmed tuberculosis Reference[a]	Laboratory confirmed influenza			Laboratory confirmed tuberculosis-influenza co-infection		
			n/N (%)	ARRR[b]	P-value	n/N (%)	ARRR[b]	P-value
Duration of symptoms	<7	100/165 (61)	148/194 (76)	Reference		6/9 (67)	Reference	
	≥7 days	65/165 (39)	46/194 (24)	0.4 (0.3-0.7)	0.001	3/9 (33)	0.5 (0.2-1.5)	0.240
HIV status	Negative	46/148 (31)	74/179 (41)	Reference		5/22 (23)	Reference	
	Positive	102/148 (69)	105/ 179 (59)	0.8 (0.5-1.2)	0.280	17/22 (77)	1.6 (0.5-4.7)	0.404
Oxygen therapy	No	101/166 (61)	108/194 (56)	Reference		17/24 (71)	Reference	
	Yes	65/166 (39)	86/194 (44)	1.4 (0.9-2.3)	0.141	7/24 (29)	0.7 (0.3-2.0)	0.560
Died	No	157/166 (95)	191/194 (98)	0.5(0.2-1.5)		24/24 (96)	Reference	
	Yes	9/166 (5)	3/194 (2)	0.2 (0.04-1.2)	0.081	1/24 (4)	0.98 (0.1-8.6)	0.986

HIV – human immunodeficiency virus.
[a]Tuberculosis only group used as reference for multinomial model analysis (i.e. comparing influenza only to tuberculosis only and tuberculosis–influenza co-infection to tuberculosis only group).
[b]Adjusted relative risk ratio (ARRR) at multivariable analysis.

in influenza tuberculosis co-infected individuals (aRRR 0.8, 95% CI 0.1-8.6) (Table 4).

On multivariable analysis among patients presenting with symptoms ≥7 days, patients with influenza only compared to tuberculosis only were less likely to be HIV-infected (aRRR 0.4, 95% CI 0.2-0.7) and more likely to receive oxygen (aRRR 1.7, 95% CI 1.7-5.4). Patients co-infected with tuberculosis and influenza as compared to patients with tuberculosis only were at increased risk of death (aRRR 5.5, 95% CI 1.2-25.3) (Table 5).

Discussion

We describe an increased risk of death associated with influenza co-infection among patients with symptoms ≥7 days hospitalized with laboratory-confirmed tuberculosis in South Africa. Mortality was not increased in patients co-infected with influenza and tuberculosis presenting with a more recent symptom onset. This suggests that influenza infection may contribute to a proportion of the mortality burden amongst patients with tuberculosis, in particular amongst those with a longer duration of symptoms. Because influenza illness is considered to be an acute infection it is possible that it is not considered as a possible diagnosis in patients with longer duration of symptoms. Both influenza and tuberculosis should be considered and tested for among patients hospitalized with pneumonia in settings with high tuberculosis prevalence such as South Africa. Based on the findings of this study our surveillance programme has been amended at two sites to include systematic testing for tuberculosis

Table 5 Factors associated with influenza infection and tuberculosis-influenza co-infection compared with tuberculosis infection only among patients admitted with severe respiratory illness presenting with symptoms duration ≥7 days and tested for tuberculosis and influenza at four sites in South Africa, June 2010- December 2011

Variables		Laboratory confirmed tuberculosis Reference[a]	Laboratory confirmed influenza			Laboratory confirmed tuberculosis-influenza co-infection		
			n/N (%)	ARRR[b]	P-value	n/N (%)	ARRR[b]	P-value
Hospital duration	<7	150/244 (61)	50/80 (62.5)	Reference		6/9 (67)	Reference	
	≥7 days	94/244 (39)	30/80 (37.7)	0.99 (0.6-1.7)	0.991	3/9 (33)	0.6 (0.1-2.7)	0.520
HIV status	Negative	34/233 (83)	25/78 (32)	Reference		1/9 (11)	Reference	
	Positive	199/233 (17)	53/78 (68)	0.4 (0.2-0.7)	0.002	8/9 (89)	2.2 (0.2-20.5)	0.486
Oxygen therapy	No	203/246 (83)	50/81 (62)	Reference		6/9 (67)	Reference	
	Yes	43/246 (17)	31/81 (38)	3.0 (1.7-5.5)	<0.001	3/9 (33)	2.1 (0.5-9.3)	0.315
Died	No	224/245 (91)	74/80 (92.5)	Reference		6/9 (67)	Reference	
	Yes	21/245 (9)	6/80 (7.5)	0.54 (0.2-1.5)	0.875	3/9 (33)	5.48 (1.2-25.4)	0.029

HIV – human immunodeficiency virus.
[a]Tuberculosis only group used as reference for multinomial model analysis (i.e. comparing influenza only to tuberculosis only and tuberculosis–influenza co-infection to tuberculosis only group).
[b]Adjusted relative risk ratio (ARRR) at multivariable analysis.

in patients admitted with severe respiratory illness. In addition, we suggest that where possible tuberculosis testing should be included in SARI programmes in Sub-Saharan Africa where HIV prevalence is high.

A recent study from Thailand did not identify an increased risk of severe outcomes or mortality in patients co-infected with tuberculosis and seasonal influenza when compared to tuberculosis and influenza single infection [18]. The number of patients co-infected with influenza and tuberculosis in this study were, however, small, and patients with a more chronic presentation were not enrolled in the surveillance system. Similar to the findings of our study, ecological and record review studies have reported elevated mortality among tuberculosis-infected individuals during previous influenza pandemics [9,10,19] and 10% of A(H1N1)pdm09 laboratory-confirmed deaths in South Africa in 2009, were co-infected with tuberculosis [11].

In patients presenting with longer duration of symptoms, tuberculosis may have caused lung damage leading to reduced lung capacity and thus impairing the ability to cope with viral infections such as influenza and potentially increasing the severity of illness associated with influenza virus infection. In addition, influenza infection impacts on antibacterial host defenses by either increased production of pro-inflammatory cytokines, causing cell damage [20], or due to inadequate pro-inflammatory chemokine response [21,22], which could increase susceptibility to tuberculosis. Lastly, immunosuppression induced by influenza virus through production of glucocorticoids [23], production of immunosuppressive cytokines [20] or, suppression of T-cell immunity [24] could trigger reactivation of latent tuberculosis and aggravate pulmonary tuberculosis.

Persons with AIDS experience a significant excess mortality due to influenza infection as compared to HIV-uninfected individuals [7,25]. The relative risk of *M. tuberculosis* infection among HIV-infected compared to -uninfected persons varies between 21–37 depending on the country's HIV prevalence [2]. In our study, a high proportion of patients with tuberculosis were also HIV-infected; however the association with increased mortality in patients co-infected with tuberculosis and influenza virus remained statistically significant even when controlling for concurrent HIV infection in the model.

Despite the high prevalence of tuberculosis in South Africa, in our study, only 37% (3018/8032) of patients admitted with SRI were tested for tuberculosis. Even amongst patients who were initiated on tuberculosis treatment during the current admission, only 58% (656/1123) were tested before initiation of treatment. A study conducted in South Africa reported tuberculosis as the most common cause (40%) of community acquired pneumonia in both HIV-infected and-uninfected patients [26]. A post mortem study in patients dying in hospital found that among patients not suspected of having tuberculosis at time of death, 42% (40/96) were culture positive for tuberculosis and that pneumonia, was the leading admission diagnosis (25%) in those not suspected of tuberculosis [27]. Only 32% of patients with symptom duration of <7 days were tested for tuberculosis, as compared to 57% in those with a chronic presentation, but amongst those tested, 10% had laboratory confirmed-tuberculosis. National guidelines for tuberculosis management advise screening for tuberculosis in patients presenting with persistent chronic symptoms (cough or fever >14 days) [28], however results from a pooled analysis of sensitivity of symptoms for active pulmonary tuberculosis, showed that in studies from sub-Saharan countries, with high burden of HIV, sensitivity of any cough was higher 62% than that of chronic cough >2 weeks, 49% [29]. Failure to test for tuberculosis could lead to delayed or missed diagnosis of tuberculosis, an increase in nosocomial tuberculosis transmission and missed opportunities to identify drug resistance when testing with the GeneXpert assay. Because of the long duration of tuberculosis treatment and possible associated adverse events, a microbiologic diagnosis of tuberculosis should be sought wherever possible [30]. The majority of tuberculosis-positive patients in our study were aged >5 years. Microbiological confirmation of tuberculosis in children is difficult [14,31-34], because childhood tuberculosis tends to be paucibacillary and smear negative. This difficulty can be further compounded by HIV co-infection.

Less than 1% of patients in our study received influenza antiviral treatment or influenza vaccination despite being recommended in national guidelines [35,36]. The association between influenza and tuberculosis seen in our study may be different in settings where influenza vaccination and antivirals are widely used. In South Africa, while there are recommendations for annual influenza vaccination, tuberculosis is not specifically listed as an underlying co-morbid illness to be targeted for vaccination [37]. Tuberculosis treatment guidelines do not routinely include influenza vaccination as part of management [15]. A randomised controlled study of trivalent inactivated influenza vaccine conducted in HIV-infected adults with CD4+ T cell counts >200 μg/ml in South Africa reported 75% (95% CI 9.2-95.6) efficacy in adults [38]. However, patients with tuberculosis co-infection or low CD4+ T cell counts were not included. Although vaccination remains the most effective method to prevent influenza infection, tuberculosis infection suppresses immune responses and thus vaccine effectiveness may be lower in this patient group. There are no published data on the effectiveness of influenza vaccination in patients with tuberculosis, particularly those co-infected with HIV (79% of individuals in our study).

Our study has limitations that warrant discussion. Firstly, we restricted our analysis to patients with tuberculosis laboratory results at current admission, and our case definition of laboratory-confirmed tuberculosis included smear-positive results. As testing for tuberculosis is not 100% sensitive, especially for extra pulmonary tuberculosis which is common in patients with HIV, we may have misclassified a proportion of tuberculosis-positive individuals as not having tuberculosis; however there is no reason to suspect that sensitivity would differ between influenza-positive and -negative patients. The majority of patients with laboratory-confirmed tuberculosis only had microscopy results and it is possible that some may have been infected with mycobacteria other than tuberculosis in a high HIV prevalence setting such as South Africa. However, current South African guidelines recommend initiation of tuberculosis treatment based on smear microscopy results [11]. Secondly we enrolled acute and chronic cases only at two enhanced sites and we didn't systematically test all enrolled cases for tuberculosis. The patients tested for tuberculosis differed in several characteristics from those not tested. These differences in enrolment and testing procedures as well as the low proportion of patients tested for tuberculosis may have introduced potential selection bias in the population included in the study. Testing all patients admitted with SRI will improve identification of tuberculosis cases and the ability to detect significant differences between groups. We did not collect data on chest X- ray findings. Availability of data on radiological changes in the lungs could have strengthened the evidence in support of our hypothesis regarding changes in the lungs resulting in severe outcome in patients with underlying tuberculosis co-infected with influenza.

Conclusion

In conclusion, we observed an increased risk of death among tuberculosis-infected patients co-infected with influenza with a symptom history of ≥7 days. The potential public health impact of influenza vaccination among persons with laboratory-confirmed tuberculosis should be explored.

Abbreviations

AIDS: Acquired immune deficiency syndrome; ALRI: Acute lower respiratory tract infection; aOR: adjusted odds ratio; aRRR: Adjusted relative risk ratio; CDC: Centers for Disease Control; CHBAH: Chris Hani Baragwanath Academic Hospital; ELISA: Enzyme-linked immunosorbent assay; HIV: Human immunodeficiency virus; KTHC: Klerksdorp Tshepong Hospital Complex; ICU: Intensive care unit; *M. tuberculosis*: *Mycobacterium tuberculosis*; NICD-NHLS: National Institute for Communicable Diseases of the National Health Laboratory Services; PCR: Polymerase chain reaction; RR: Relative risk; RRR: Relative risk ratio; SARI: Severe acute respiratory illness; SCRI: Severe chronic respiratory illness; *S. pneumoniae*: *Streptococcus. pneumoniae*; SRI: Severe respiratory illness; WHO: World Health Organization.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

SW, CC, ST, JM– contributed to conception and the study design, acquisition of data, analysis and interpretation of data, and drafting of manuscript. CMV, ALC contributed to interpretation of data, drafting of the manuscript and review of manuscript. SAM, MG, HD, EV, KK contributed to acquisition of data and review of manuscript. MV, MP and NW contributed to laboratory testing, acquisition of laboratory data and review of the manuscript. All authors read and approved the final manuscript.

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Author details

[1]Centre for Respiratory Disease and Meningitis, National Institute for Communicable Diseases (NICD) of the National Health Laboratory Service (NHLS), Private Bag X4, Sandringham, 2131 Johannesburg, Gauteng, South Africa. [2]School of Public Health, Faculty of Health Science, University of the Witwatersrand, Johannesburg, South Africa. [3]US Centres for Disease Control and Prevention, Atlanta, GA, USA. [4]US Centres for Disease Control and Prevention, Pretoria, South Africa. [5]Medical Research Council: Respiratory and Meningeal Pathogens Research Unit and Department of Science and Technology/National Research Foundation: Vaccine-Preventable Diseases, University of the Witwatersrand, Johannesburg, South Africa. [6]Pietermaritzburg Metropolitan Hospital Complex, KwaZulu- Natal, South Africa. [7]MRC/Wits Rural Public Health and Health Transition Research Unit (Agincourt), Bushbuckridge, South Africa. [8]Tshepong Hospital, North West Province, South Africa. [9]Faculty of Medicine, University of Witwatersrand, Johannesburg, South Africa. [10]Zoonosis Research Unit, Department of Medical Virology, University of Pretoria, Pretoria, South Africa.

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