**Risk Factors for Influenza-Associated Severe Acute Respiratory Illness Hospitalization in South Africa, 2012-2015 (Supplementary Material)**

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**METHODS**

**Definitions**

For this study underlying medical conditions included: (i) asplenia or sickle cell anemia; (ii) chronic illness, including chronic lung, renal, liver or cardiac disease, diabetes mellitus and asthma; (iii) other immunocompromising conditions (excluding HIV), including organ transplant, primary immunodeficiency, immunotherapy and malignancy; (iv) neurological disorders; (v) burns; and (vi) obesity. 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. Pregnancy was evaluated independently as a potential risk factor among women of childbearing age and malnutrition and prematurity were evaluated as potential risk factors among children aged <5 years. Malnutrition was classified as weight-for-age Z score less than -2 adjusting for prematurity (World Health Organization child growth standards 2009) and/or nutritional edema. Prematurity was classified as birth before 37 weeks of gestation as reported on the road-to-health card (vaccination card). Obesity was defined as body mass index ≥30.

HIV results were obtained from a combination of two sources: (i) patient clinical records when available and (ii) for consenting patients, an anonymized linked dried blood spot was tested at National Institute for Communicable Diseases (NICD) of the National Health Laboratory Service, Johannesburg, South Africa. When both results were available, the NICD result was used. CD4+ T-cells counts were determined by flow cytometry [[[1]](#endnote-1)]. Patients were categorized into two immunosuppression categories: (i) no or mild immunosuppression (CD4+ T-lymphocytes ≥200/mm3 or equivalent age-appropriate CD4+ percentage for children aged <5 years), or (ii) severe immunosuppression (CD4+ T-lymphocytes <200/mm3 or equivalent age-appropriate CD4+ percentage for children aged <5 years) [[[2]](#endnote-2)].

**Laboratory procedures**

Respiratory specimens (i.e., nasopharyngeal aspirates for children <5 years of age and combined nasopharyngeal and oropharyngeal swabs from persons ≥5 years of age) were collected from all enrolled patients, placed in universal transport medium, stored at 4-8°C and transported to NICD within 72 hours of collection for testing. Specimens were tested for the presence of 10 respiratory viruses (influenza A and B viruses; parainfluenza virus types 1, 2 and 3; respiratory syncytial virus; adenovirus; rhinovirus; human metapneumovirus; and enterovirus) using a multiplex real-time reverse transcriptase polymerase chain reaction assay [[[3]](#endnote-3)]. Influenza A-positive samples were subtyped.

Respiratory specimens were also tested for the detection of *Streptococcus pneumoniae*, *Haemophilus influenzae* type b, *Bordetella pertussis*, *Mycoplasma pneumoniae,* *Chlamydophila pneumoniae* and *Legionella* spp.

*S. pneumoniae* was detected using a single-target (*lytA*) quantitative real-time PCR assay [[[4]](#endnote-4)]. The 25 μl PCR reaction contained 1x TaqMan gene expression mastermix (Applied Biosystems, Foster City, CA, USA), 200 nM each of forward, reverse primer and probe (5’FAM) and 2.5 μl of extracted DNA. Samples with a cycle threshold (Ct) value of >40 were recorded as negative. For *lytA*-positive specimens a standard curve was prepared using serially diluted DNA extracts from a known quantity (confirmed spectrophotometrically) of *S. pneumoniae* ATCC49619 and used to calculate pneumococcal colonization density (DNA copies/ml).

*H. influenzae* was detected using previously described methods [[[5]](#endnote-5),[[6]](#endnote-6)], modified as a triplex real-time PCR assay targeting *IgA1*, *bexA* and a gene specific for *H. influenzae* serotype b (Hib). The 25 μl PCR reaction contained 1x TaqMan gene expression mastermix (Applied Biosystems), 2 μl of extracted DNA, and:

* 400 nM Iga1-F (5’-CAAAATTGCCAAGATTAAATGCTT-3’),
* 400 nM Iga1-R (5’-TGCTCGCCATACTGCACAA-3’),
* 400 nM Hib-F (5’-TGTTCGCCATAACTTCATCTTAGC-3’) and
* 400 nM Hib-R (5’-CTTACGCTTCTATCTCGGTGATTAATAA-3’),
* 2400 nM of bexA-F (5’-CTGAATTRGGYGATTATCTTTATGA-3’) and
* 2400 nM bexA-R (5’-ACAATCAAAYTCAACHGAAAGHGA-3’),
* 100 nM of Iga1-probe (5’-NED-CCTGCGGTTAAACC-3’) and
* 100 nM Hib probe (5’-VIC-CACAAAACTTCTCATTCTTCGAGCCTA-3’), and
* 250 nM of bexA probe (5’-FAM-AGGGATGAAAGCYCGRCTTGCAT-3’).

Samples with a Ct-value of >40 were recorded as negative.

*Bordetella* spp. were detected using a multitarget real-time PCR assay targeting IS481, *B. parapertussis* IS1001 (pIS1001), *B. holmesii* IS1001 (hIS1001) in a triplex PCR reaction and pertussis toxin subunit S1 (*ptxS1*) in a singleplex PCR reaction [[[7]](#endnote-7)]. 25 µl reactions were performed using TaqMan gene expression mastermix (Applied Biosystems), 4 µl of extracted DNA, and primers and probes as previously described. Samples with a Ct-value of >45 were recorded as negative.

*M. pneumoniae, C. pneumoniae* and *Legionella* spp. were detected using a multiplex real-time PCR assay [[[8]](#endnote-8)]. The genes targeted were CARDS Tx (*M. pneumoniae*), *argR* (*C. pneumoniae*) and *ssrA* (*Legionella* spp.). In addition, the human *RNaseP* gene was included as an internal control. 25 µl reactions were performed using PerfeCTa multiplex qPCR supermix (Quanta Biosciences, Gaitherburg, MD, US), 6.5 µl of extracted DNA, and primers and probes as previously described. Samples with a Ct-value of >45 were recorded as negative.

In addition, blood samples and induced sputa were collected from patients with SARI and tested for the detection of *S. pneumoniae* (*lytA*) and *Mycobacterium tuberculosis*, respectively. For this analysis a laboratory-confirmed tuberculosis case was defined as an individual with a positive result for *M. tuberculosis* on microscopy, culture or PCR from the current hospital admission. A tuberculosis negative case was defined as a patient who tested negative for tuberculosis on microscopy, PCR, culture or on a combination of these. Smear microscopy was performed using fluoresceine auramine staining for acid fast bacilli (AFB), liquid media using BD Bactec MGIT 960 was used for culture and tuberculosis PCR was performed using the Xpert® MTB/RIF system (Cepheid, Sunnyvale, California). Positive cultures were identified as *M. tuberculosis* complex using Ziehl-Neelsen staining and antigen testing.

**RESULTS**

**Table S1: Demographic and clinical characteristics of influenza-positive pregnant women, Klerksdorp and Pietermaritzburg, South Africa, May 2012 – April 2015.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Characteristics** | **Case 1** | **Case 2** | **Case 3** | **Case 4** | **Case 5** |
| **Case** | SARI | SARI | ILI | ILI | ILI |
| **Age (in years)** | 25 | 27 | 25 | 27 | 29 |
| **Year** | 2012 | 2013 | 2013 | 2013 | 2014 |
| **Duration of symptoms (in days)** | 3 | 1 | 2 | 2 | 4 |
| **Temperature on admission/consultation** | 39.1 | 38.9 | 38.7 | 37.3 | 38.4 |
| **History of fever** | Yes | Yes | Yes | Yes | Yes |
| **Respiratory rate/min.** | 38 | 35 | 22 | 24 | 26 |
| **Cough** | Yes | Yes | Yes | Yes | Yes |
| **Shortness of breath/difficulty breathing** | Yes | Yes | No | No | No |
| **Cigarette smoking (current)** | No | No | No | No | No |
| **Cigarette smoking (previous)** | No | No | No | No | No |
| **Alcohol consumption** | No | No | No | No | No |
| **Underling medical conditionsa** | No | No | No | No | No |
| **HIV** | Pos | Neg | Neg | Pos | Neg |
| **CD4+ T-cell count/mm3** | 515 (Normal) | N/A | N/A |  | N/A |
| **Receiving HAART treatment** | Yes | N/A | N/A | Yes | N/A |
| **Receiving tuberculosis treatment** | No | No | No | No | No |
| **Antibiotics 24H before adm./consul.** | No | No | No | No | No |
| **Influenza type/subtype** | B | B | A(H1N1)pdm09 | A(H3N2) | A(H3N2) |
| **Respiratory syncytial virus** | Neg | Neg | Neg | Neg | Neg |
| **Human metapneumovirus** | Neg | Neg | Neg | Neg | Neg |
| **Parainfluenza virus (types 1-3)** | Neg | Neg | Neg | Neg | Neg |
| **Rhinovirus** | Pos | Neg | Neg | Neg | Neg |
| **Enterovirus** | Neg | Neg | Neg | Neg | Neg |
| **Adenovirus** | Neg | Neg | Neg | Pos | Pos |
| **Tuberculosis** | Neg | Neg | Not tested | Not tested | Not tested |
| **Invasive pneumococcal diseases** | Neg | Neg | Not tested | Not tested | Not tested |
| ***Haemophylus influenzae* type B** | Neg | Neg | Neg | Neg | Neg |
| ***Bordetella pertussis*** | Neg | Neg | Neg | Neg | Neg |
| ***Mycoplasma pneumoniae*** | Neg | Neg | Neg | Neg | Neg |
| ***Chlamydophila pneumoniae*** | Neg | Neg | Neg | Neg | Neg |
| **Oxygen saturation (24 hours from adm.)** | 87 | 89 | N/A | N/A | N/A |
| **Oxygen therapy during admission** | Yes | Yes | N/A | N/A | N/A |
| **Mechanical ventilation** | No | No | N/A | N/A | N/A |
| **Admission to intensive care unit** | No | No | N/A | N/A | N/A |
| **Started on tuberculosis treatment** | No | No | N/A | N/A | N/A |
| **Length of hospitalization (in days)** | 5 | 8 | N/A | N/A | N/A |
| **Admission diagnosis** | Pneumonia | Pneumonia | N/A | N/A | N/A |
| **Discharge diagnosis** | Pneumonia | Pneumonia | N/A | N/A | N/A |
| **In-hospital outcome** | Discharged | Discharged | N/A | N/A | N/A |

Abbreviations: ILI: influenza-like illness; SARI: severe acute respiratory illness; HIV: human immunodeficiency virus.

a Evaluated underlying medical conditions included: asplenia, including asplenia or sickle cell anemia; chronic illness, including chronic lung, renal, liver or cardiac disease, diabetes mellitus and asthma; other immunocompromising conditions (excluding HIV), including organ transplant, primary immunodeficiency, immunotherapy and malignancy; neurological disorders; burns and obesity.

**Table S2: Demographic and clinical characteristics of influenza-positive SARI cases that died, Klerksdorp and Pietermaritzburg, South Africa, May 2012 – April 2015.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Characteristics** | **Case 1** | **Case 2** | **Case 3** | **Case 4** |
| **Case** | SARI | SARI | SARI | SARI |
| **Age** | 62 years | 79 years | 71 years | 3 months |
| **Sex** | Male | Female | Female | Female |
| **Year** | 2012 | 2012 | 2013 | 2013 |
| **Duration of symptoms (in days)** | 3 | 2 | 2 | 2 |
| **Temperature on admission/consultation** | 37.5 | 36.8 | 37.6 | 37.8 |
| **History of fever** | Yes | Yes | Yes | Yes |
| **Respiratory rate/min.** | 36 | 26 | 32 | 26 |
| **Cough** | Yes | Yes | Yes | Yes |
| **Shortness of breath/difficulty breathing** | Yes | Yes | Yes | Yes |
| **Cigarette smoking (current)** | No | No | No | No |
| **Cigarette smoking (previous)** | No | No | No | No |
| **Alcohol consumption** | No | No | No | No |
| **Underling medical conditionsa** | No | Diabetes | No | Malnutrition |
| **HIV** | Pos | Neg | Neg | Neg |
| **CD4+ T-cell count/mm3** | 145 (Severe) | N/A | N/A |  |
| **Receiving HAART treatment** | Yes | N/A | N/A | Yes |
| **Receiving tuberculosis treatment** | No | No | No | No |
| **Antibiotics 24H before adm./consul.** | No | No | No | No |
| **Influenza type/subtype** | A(H3N2) | A(H3N2) | A(H1N1)pdm09 | A(H3N2) |
| **Respiratory syncytial virus** | Neg | Neg | Neg | Neg |
| **Human metapneumovirus** | Neg | Neg | Neg | Neg |
| **Parainfluenza virus (types 1-3)** | Neg | Neg | Neg | Neg |
| **Rhinovirus** | Neg | Neg | Neg | Neg |
| **Enterovirus** | Neg | Neg | Neg | Neg |
| **Adenovirus** | Neg | Neg | Neg | Neg |
| **Tuberculosis** | Neg | Neg | Neg | Not tested |
| **Invasive pneumococcal diseases** | Neg | Neg | Neg | Neg |
| ***Haemophylus influenzae* type B** | Neg | Neg | Neg | Neg |
| ***Bordetella pertussis*** | Neg | Neg | Neg | Neg |
| ***Mycoplasma pneumoniae*** | Neg | Neg | Neg | Neg |
| ***Chlamydophila pneumoniae*** | Neg | Neg | Neg | Neg |
| **Oxygen saturation (24 hours from adm.)** | 87 | 89 | 92 | 90 |
| **Oxygen therapy during admission** | Yes | Yes | Yes | Yes |
| **Mechanical ventilation** | No | No | No | No |
| **Admission to intensive care unit** | No | No | No | No |
| **Started on tuberculosis treatment** | No | No | No | No |
| **Length of hospitalization (in days)** | 10 | 18 | 1 | 3 |
| **Admission diagnosis** | Pneumonia | Pneumonia | Pneumonia | Pneumonia |
| **Discharge diagnosis** | Pneumonia | Pneumonia | Pneumonia | Pneumonia |

Abbreviations: ILI: influenza-like illness; SARI: severe acute respiratory illness; HIV: human immunodeficiency virus.

a Evaluated underlying medical conditions included: asplenia, including asplenia or sickle cell anemia; chronic illness, including chronic lung, renal, liver or cardiac disease, diabetes mellitus and asthma; other immunocompromising conditions (excluding HIV), including organ transplant, primary immunodeficiency, immunotherapy and malignancy; neurological disorders; burns; obesity; pregnancy; malnutrition and prematurity.

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