Radiological predictors of PCP in HIV-positive adults in South Africa: a matched case-control study

Submission of dissertation for candidate Nicola K Wills[1*] at the University of Cape Town for fulfilment of Mmed (Internal Medicine) Part III. Supervisor: Professor Sean Wasserman.

Co-authors: Jared Tavares[2], Qonita Said-Hartley[3], Sean Wasserman[4, 5, 6]

1 Department of Medicine, University of Cape Town, South Africa

2 Department of Statistics, University of Cape Town, South Africa

3 Department of Radiology, University of Cape Town, South Africa

4 Institute for Infection and Immunity, St George's, University of London, UK

5 Centre for Infectious Diseases Research in Africa, Institute of Infectious Disease and Molecular Medicine, University of Cape Town, South Africa.

6 MRC Centre for Medical Mycology, Faculty of Health and Life Sciences, University of Exeter, UK

*Correspondence: Nicola K Wills, Department of Medicine, University of Cape Town, Cape Town, South Africa. Groote Schuur Hospital, Main Road, Observatory, 7935 (nicolakwills@outlook.com)

Acknowledgements, format and contributions

Format of thesis

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Some cases and controls included this study were identified from a prior retrospective cohort study[1] that explored the outcomes of HIV-associated PCP at Groote Schuur Hospital from 2004 – 2015.

Author contributions

Conception and writing of protocol: NW, SW. Record screening, data extraction: NW. Data analysis and interpretation: JT, NW, SW. Radiograph interpretation: QSH. Drafting of manuscript: NW. Critical review of the manuscript: all authors.

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Name: Nicola Wills

Student number: WLLNIC020

Signature: Signed by candidate

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Abbreviations

AIC – Akaike information criterion

aOR – adjusted odd's ratio

AECOPD – acute exacerbation of chronic obstructive lung disease

ART – antiretroviral therapy

AUC – area under the curve

bpm – breaths per minute

CAP – community acquired pneumonia

CDC – Centers for Disease Control and Prevention

CI – confidence interval

CTPA – computer tomography pulmonary angiography

CMV – Cytomegalovirus

CTX – cotrimoxazole

CXR – chest X-ray

EBV – Ebstein-Barr virus

ICU – intensive care unit

ILD – interstitial lung disease

IQR – interquartile range

MC&S – microscopy, culture and sensitivity

NHLS – National Heath Laboratory Service

OR – odds ratio

Pa02 – partial pressure of oxygen in arterial blood

PF – Pa02:Fi02 ratio (partial pressure of oxygen in arterial blood: inspired oxygen concentration ratio)

PCP – pneumocystis pneumonia

PCR – polymerase chain reaction

PE – pulmonary embolism

PTB – pulmonary tuberculosis

PTX – pneumothorax

RA – room air

ROC – Receiver Operating Characteristic

RR – respiratory rate

SE – standard error

Sp02 - pulse oximetry saturation

TB – tuberculosis

VIF - Variance Inflation Factor

VL – viral load

VP – viral pneumonia

WHO – World Health Organisation

Keywords

HIV, PCP, *Pneumocystis jirovecii*, chest X-ray, prediction rule

Running title

Chest X-ray features of HIV-associated PCP in adults

Key points

In this case-control study, diffuse ground-glass CXR changes correlated with PCP diagnosis and severe PCP. Reticulonodular changes or pleural effusion suggested non-PCP pathology. Clinical prediction models, incorporating respiratory rate or hypoxia with select CXR features, showed good accuracy for predicting PCP.

Abstract

Background

Definition of chest X-ray (CXR) features associated with laboratory-confirmed pneumocystis pneumonia (PCP) among HIV-positive adults is needed to improve diagnosis in high-burden settings.

Methods

We conducted a case-control study involving HIV-positive adults with laboratory-confirmed PCP and a matched cohort with non-PCP respiratory presentations at regional hospitals in Cape Town, South Africa (2012 – 2020). The primary objective was to identify CXR features associated with confirmed PCP diagnosis and severe PCP (defined by hypoxia, ICU referral/admission, and/or in-hospital death). We explored the performance of logistic regression models, incorporating selected clinical and CXR predictors, for PCP diagnosis and severe PCP.

Results

Records from 104 adults (52 PCP cases and 52 non-PCP controls) were included. Diffuse versus patchy ground glass opacification was associated with increased odds of PCP diagnosis (adjusted odd's ratio (aOR) 6.2, 95% confidence interval (CI) 1.6 – 28.9, p = 0.01) and severe PCP (aOR 4.5, 95%CI 1.6 – 14.4, p = 0.008). Consolidation was associated with severe PCP (aOR 3.3, 95%CI 1.2 – 11.0, p =0.03) as was increasing ground glass zone involvement (aOR 2.1 for each one-unit increase in involved zone; 95% CI, 1.4 – 3.2, p = 0.0004). Models incorporating hypoxia (hypoxia model) or tachypnoea (respiratory rate model) with diffuse ground glass opacities, absence of pleural effusion or reticular/reticulonodular changes on CXR performed well in predicting PCP (area under the receiver operating characteristic curve 0.828 (hypoxia model) and 0.857 (respiratory rate model)).

Conclusions

CXR evaluation alongside bedside clinical information offers good accuracy for discriminating definite PCP from other HIV-associated respiratory diseases.

Introduction

Pneumocystis pneumonia (PCP), caused by the ubiquitous fungus *Pneumocystis jirovecii*, is a common and severe HIV-associated opportunistic infection that carries an estimated case-fatality rate of 19% amongst HIV-positive adults in sub-Saharan Africa[2]. The lack of validated clinical definitions of PCP, frequent co-infections, and limited access to costly and invasive laboratory diagnostics complicates PCP diagnosis and may contribute to its poor treatment outcomes in high burden settings. Chest X-ray (CXR) offers a potentially cost-effective[3], widely available and non-invasive diagnostic tool to more rapidly identify patients with PCP in resource-limited healthcare settings. However, CXR features that are predictive of laboratory-confirmed PCP, as opposed to other common causes of respiratory presentations among HIV-positive adults, have not been rigorously evaluated, limiting the utility of CXR for clinical decision-making. A meta-analysis of CXR patterns associated with presumptive PCP in HIV-positive adults in low and middle income countries published in 2013 highlighted the potential diagnostic value of CXR. However in that review, clinical diagnostic definitions with low specificity were employed, and an analysis of the diagnostic predictive value of individual CXR features was not performed[4].

CXR features that are associated with severe HIV-associated PCP would be of interest for early stratification of patients who may require escalated care, but have also not been well described. Furthermore, whilst clinical prediction models for PCP diagnosis have been explored in South African[5] and other settings[6–8], these have not incorporated specific PCP-associated CXR features alongside objective clinical determinants, which may improve diagnostic performance.

We aimed to better define CXR features that discriminate HIV-associated PCP from other common respiratory presentations in a high burden setting, and to assess the performance of a prediction model incorporating significant radiological features and bedside clinical parameters for PCP diagnosis and severity.

Methods

Design

We conducted a case-control study, extracting clinical and radiological data from medical records of HIV-positive adults (≥ 18 years) admitted with respiratory disease and undergoing *Pneumocystis jirovecii* respiratory sample testing at Cape Town Metro hospitals between 2012 – 2020. Primary objectives were to explore CXR features associated with PCP diagnosis and severe PCP (as defined by marked hypoxia, ICU referral or admission, or in-hospital death). As secondary objectives, we

explored the performance of a model, incorporating *a priori* and identified clinical and radiological features, to predict HIV-associated PCP diagnosis and severity.

Population and data sources

Definite PCP (cases) and non-PCP respiratory disease (controls) was assigned using pre-specified diagnostic criteria, adapted from CDC and WHO guidelines (Table 1). PCP cases were matched to controls based on most recent CD4 count (in windows of < 100 cells/mm^3, 100 – 199 cells/mm^3, and ≥ 200cells/mm^3) and hospital admission within the same 12-month period. Potential cases and controls were identified from a prior retrospective cohort study[1] and by screening all requests for *Pneumocystis jirovecii* laboratory (microscopy or PCR) testing, on any respiratory sample, submitted to the National Health Laboratory Service (NHLS) from Cape Town Metro Hospitals from June 2015 – October 2020. These hospitals include tertiary (Groote Schuur Hospital), regional (New Somerset Hospital) and district (Mitchells Plain Hospital, Heideveld Emergency Centre, Victoria Hospital Wynberg) level care. Demographic and clinical data for included records (including laboratory testing data, HIV and ART history, co-morbidities and index hospitalisation treatment and outcome) were collected on a password-protected REDCap electronic data tool[9] hosted at the University of Cape Town (Supplementary material, appendix I), with access restricted to study authors only.

Definitions

Hypoxia was defined as (1) pulse oximetry (Sp02) < 90% on room air, (2) arterial partial pressure of oxygen (Pa02) of < 7.8 kPa on room air, or (3) ratio of arterial partial pressure of oxygen to fraction of inspired oxygen (Pa02: Fi02, PF ratio) ≤300 mmHg [10,11] on admission. Severe PCP was defined as (1) severe admission hypoxia (PF ratio < 100 mmHg), (2) patients referred or admitted to ICU (3) in-hospital death. PF ratio was imputed for patients with only SpO2 (i.e., without PaO2) data (available at: https://opencriticalcare.org/imputed-pao2-calculator/).

HIV-positive status required a documented positive HIV-enzyme linked immunosorbent assay (ELISA) or any detectable viral load result prior to or during admission. Patients were recorded to have a smoking history if currently smoking, a five pack-year history or more, or abstained for less than two years. Chronic lung disease was defined as post-pulmonary tuberculosis or other reported structural lung disease, including history of smoking or polysubstance induced chronic obstructive pulmonary disease (COPD).

Additional respiratory or systemic pathologies detected on investigation and treated during hospital admission were based on clinician assessment. These included confirmed diagnoses (laboratory-isolated pathogen with pathogen-specific treatment administered, histology-confirmed malignancy or radiologically confirmed pulmonary embolus or pneumothorax in symptomatic patient) or empiric diagnoses (clinical diagnosis assigned and treatment administered by managing clinician(s) in absence of laboratory or radiological confirmation).

Table 1. Definitions for pneumocystis pneumonia (PCP) and non-PCP respiratory disease.

Diagnostic category	Definition
Definite PCP (PCP case)	1. Microscopy-detected *Pneumocystis jirovecii* in any respiratory sample from an HIV-positive adult presenting with any respiratory symptoms, or 2. PCR-detected *Pneumocystis jirovecii* in any respiratory sample from an HIV positive adult and meeting criteria for probable PCP
Probable PCP [12,13]	In adults without microscopy or PCR-detected *Pneumocystis jirovecii*: 1. Clinical syndrome of (1) exertional dyspnoea or non-productive cough, and (2) onset within the last 3 months, and (3) tachypnoea PLUS evidence of diffuse bilateral infiltrates on CXR OR 2. Decision by treating clinicians to initiate empiric treatment for PCP
Non-PCP respiratory disease (non-PCP control)	In adults presenting with any respiratory symptom (including cough or dyspnoea with/without chest pain) with negative laboratory tests for *Pneumocystis jirovecii* and not meeting criteria for probable PCP, with: 1. Alternative aetiology found (laboratory-confirmed) AND/OR 2. PCP-specific treatment not received

CXR – chest X-ray, PCP – pneumocystis pneumonia, PCR – polymerase chain reaction, WHO – World Health Organisation

Radiology review

Admission CXRs were retrieved electronically and reviewed by a specialist radiologist (QSH), blinded to all clinical and laboratory data. CXRs were systematically analysed using a standardised assessment tool (Supplementary material, appendix II) adapted from the Chest Radiograph Reading and Recording system [14], incorporating features identified in the literature to have discriminatory value in distinguishing PCP from non-PCP respiratory disease [15–22], with use of descriptive terminology[23] to enable future interpretation by non-specialist readers, broadening study generalisability.

Analysis

Demographic, admission clinical and radiological characteristics in cases and controls were compared through generating proportions as well as crude and adjusted odds ratios (OR) using logistic regression for categorical variables and using the Chi-squared test for significance testing.

Medians and median differences were generated for continuous variables, with use of the Wilcoxon rank-sum for significance testing.

An exploratory radiographic severity score was developed based on evidence from studies using similar scores and correlating pattern of parenchymal abnormality and extent of disease on CXR with PCP prognosis[24–27], with allocation of points as per table 2.

Table 2. Radiographic severity score: point allocation.

Point allocation	Chest X-ray feature
(A) **Parenchymal pattern**	
1	Grade 1: no parenchymal abnormality
2	Grade 2: reticular or reticulonodular changes
3	Grade 3: ground glass opacification or consolidation
(B) **Lung zone involvement with parenchymal changes (extent of disease)**	
1 - 6	1 point per zone of involvement with any parenchymal changes
(C) **Diffuse involvement**	
0 or 1	1 point if diffuse descriptor used for any reticular, reticulonodular, ground glass or consolidation pattern.

For the development of the PCP diagnosis prediction models, based on 52 PCP events, we selected five *a priori,* clinically relevant, candidate variables[28]:hypoxia (Sp02 < 90% in room air, Pa02 < 7.8 kPa, or PF ratio ≤ 300 mmHg) or elevated respiratory rate (≥ 30 breaths per minute), ground glass opacification (diffuse or patchy), consolidation, reticular or reticulonodular changes and/or pleural effusion on CXR[5–8,21].

For the severe PCP model, we explored parenchymal changes (reticular or reticulonodular changes, ground glass opacification and consolidation), hypoxia or elevated respiratory rate, and either radiographic severity score or total zones of involvement as candidate variables. We explored a second prognostic model with selection of ground glass changes, consolidation, respiratory co-diagnosis, and total zones of involvement as candidate variables.

Log transformation of continuous variables with testing of restricted cubic splines to improve model fit, where linear relationship with severe PCP was not displayed, was employed. A backward stepwise approach using the Akaike information criterion (AIC) as the stopping rule was used to select the most predictive and significant variables for the reduced binary logistic regression model[29]. Model validation was performed with the Houwelingen-Le Cessie heuristic shrinkage estimate and partial residual plots were visually assessed as well as conducting VIF (Variance Inflation Factor) assessment for collinearity, with further internal validation using 200 boot strap re-

samples. Discriminatory performance of the reduced models was assessed using the area under the receiver operating curve (AUC) or equivalent c (concordance) index. Analyses were performed in RStudio (version 4.3.1).

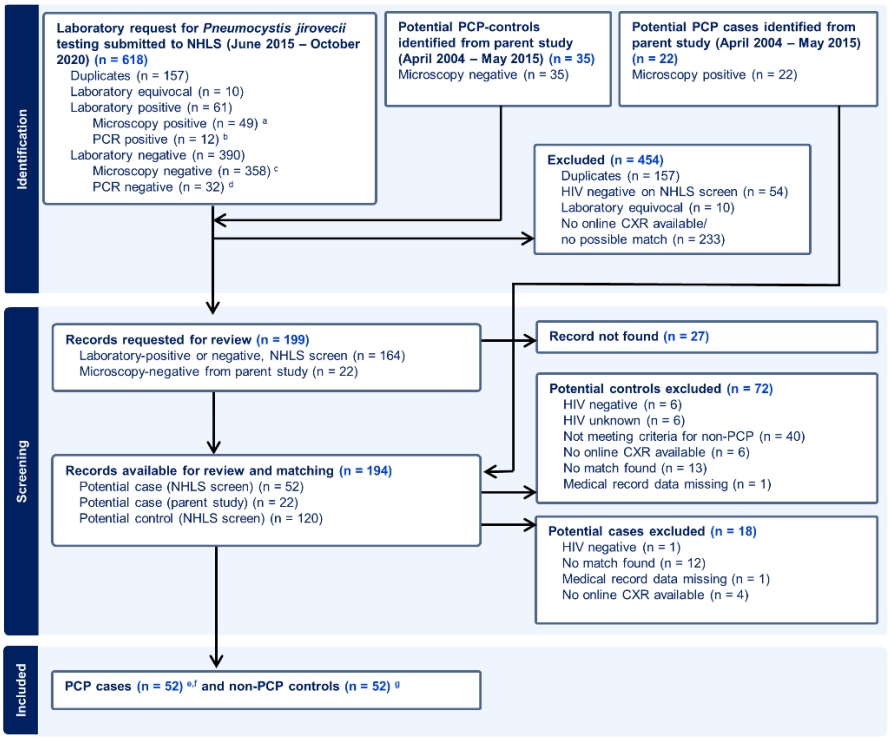
Ethical considerations

This study was approved by the University of Cape Town Human Research Ethics Committee (ref 522/2019; parent study ref 548/2015), which waived the requirement for informed consent. Hospital approval was received from facilities and the National Health Research Database (NHRD). Data retrieval from NHLS was approved via the Academic Affairs and Research Management System (AARMS).

Results

52 cases with definite PCP and 52 controls with non-PCP respiratory disease were included (Figure 1).

Figure 1. Flow of records from screening through to final inclusion of 52 adults with PCP matched to 52 adults with non-PCP respiratory disease.



[a] Tertiary (n = 18), district/regional (n = 31), [b] tertiary (n = 9), district/regional (n = 3), [c] tertiary (n = 170), district/regional (n = 188), [d] tertiary (n = 29), district/regional (n = 3). [e] All received treatment for PCP by managing clinician(s), [f] tertiary (n = 32), district/regional (n = 20), [g] tertiary (n = 34), district/regional (n = 18). CXR – chest X-ray, NHLS – National Heath Laboratory Service, PCP – Pneumocystis pneumonia.

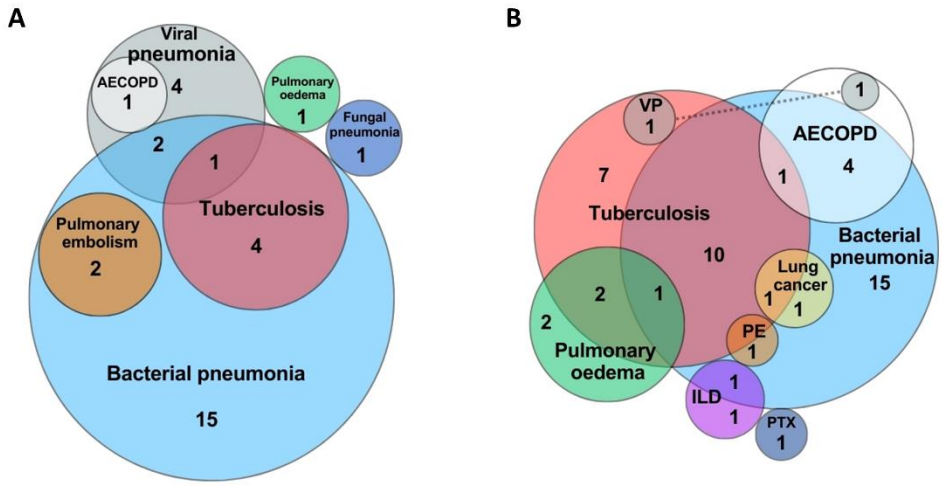
A higher proportion of cases compared to controls were newly diagnosed with HIV on admission (38% versus 21%). Cases had more respiratory distress on admission compared with controls, with higher median respiratory rate (median 34 versus 28 breaths per minute) and more frequent hypoxia (44% versus 28%). ICU referral or admission, as well as in-hospital mortality, was similar between the two groups (Table 3). Confirmed and empiric respiratory co-diagnoses are outlined in figure 2. Within cases, frequent co-diagnoses included bacterial pneumonia (46%), viral pneumonia (15%) and pulmonary or disseminated tuberculosis (10%). Within controls, primary diagnoses included bacterial pneumonia (67%), pulmonary or disseminated pulmonary TB (46%), acute exacerbation of COPD (12%), pulmonary oedema (10%) and viral pneumonia (4%).

Table 3. Comparison of demographic, admission and outcome characteristics of HIV-positive adults with PCP (cases) versus non-PCP respiratory disease (controls).

Variable			PCP, n = 52	Non-PCP, n= 52	P value
Demographics and HIV history	Female gender, n (%)		36 (69)	31 (60)	0.30
	Age, median years (IQR)		34.5 (29 – 42)	38 (31 – 42)	0.34
	Newly diagnosed with HIV on admission, n (%)		20 (38.4) [a]	11 (21.2)	0.05
	Cotrimoxazole prophylaxis use on admission	Yes, n (%)	4 (7.7)	5 (9.6)	0.8
		No, n (%)	36 (69.2)	37 (71.2)	0.8
		Not reported, n (%)	12 (23.1)	10 (19.2)	0.6
	ART history	ART naïve, n (%) or	27 (52.9) [a]	21 (41.1) [a]	
		Currently on ART, n (%)	10 (19.6) [a]	8 (15.7) [a]	0.9
		Interrupted ART, n (%)	14 (27.5) [a]	22 (43.1) [a]	0.1
		VL undetectable, n (%)	1 (1.9%)	3 (5.8%)	0.33
		Median VL, copies/mL (IQR)	125672 (18872 – 341875)[b]	53335 (8782 – 206702) [b]	0.40
	Median CD4 count, cells/mm^3 (range)(IQR)		21 (2 – 405) (10 – 47)	40 (4 – 461) (20 – 79)	0.01
	Other co-morbidities	Chronic lung disease, n (%)	6 (11.5)	20 (38.5)	0.003
		Previous pulmonary TB, n (%)	17 (34) [c]	26 (51) [a]	0.09
Admission details	Level of care	District/regional, n (%)	18 (34.6)	18 (34.6)	
		Tertiary, n (%)	34 (65.4)	34 (65.4)	1
	Admission respiratory rate, median bpm (IQR)		34 (28 – 38)	28 (22 – 32)	0.003
	Hypoxia on admission [d], n (%)		44 (88) [c]	28 (53.8)	0.0003
	Admission PF ratio, median mmHg (IQR) [e]		218.5 (164 - 269)	296 (199.5 – 346.5)	0.007
	Haemoglobin, median g/dL (IQR)		11.0 (9.7 – 12.4)	10.4 (8.7 – 11.7)	0.08
	White cell count, median x 10^9 cells/L (IQR)		8.4 (5.7 – 12.5)	7.3 (4.9 – 13.6)	0.33
Outcomes	ICU referral, n (%)		18 (34.6)	21 (40.4)	0.54
	ICU admission, n (%)		16 (30.8)	20 (38.5)	0.4
	Mechanically ventilated, n (%)		16 (30.8)	20 (38.5)	0.4
	Inotrope support, n (%)		6 (11.5)	13 (25)	0.08
	In-hospital death, n (%)		19 (36.5)	16 (30.8)	0.53
	ICU death (%)		7 (43.8) [f]	12 (60) [f]	0.43

Denominator: n = 52 unless specified. [a] denominator = 51, [b] denominator = 14 (adults with available and detectable recent VL) [c] denominator = 50, [d] Sp02 < 90% (RA) or Pa02 < 7.8 kPa (RA) or PF ratio ≤ 300 mmHg, [e] imputed PF ratio: to allow standardised assessment of PF ratio trend, an imputed PF ratio was calculated for patients with only Sp02 available on oxygen or room air (n = 39) (available at: https://opencriticalcare.org/imputed-pao2-calculator/), [f] denominator = adults admitted to ICU. ART – antiretroviral therapy, bpm – breaths per minute, ICU – intensive care unit, IQR – interquartile range, PCP – Pneumocystis pneumonia, Pa02 – partial pressure of oxygen in arterial blood, PF – Pa02:Fi02 ratio (partial pressure of oxygen in arterial blood: inspired oxygen concentration ratio), RA – room air, Sp02 - pulse oximetry saturation, VL – viral load.

Figure 2. Venn diagram: Confirmed and/or empiric co-diagnoses among cases (A) and controls (B).



Pulmonary/disseminated TB. Laboratory confirmed A: n = 0, B: n = 13. Empiric diagnosis A: n = 5, B: n = 11
Bacterial pneumonia. Laboratory confirmed A: n = 1, B: n = 8. Empiric diagnosis A: n = 23, B: n = 27
Viral pneumonia. Laboratory confirmed A: n = 4, B: n = 1. Empiric diagnosis A: n = 4, B: n = 1
Pulmonary oedema. Cardiac failure A & B: n = 0. Renal failure A: n = 1, B: n = 6.
AECOPD – acute exacerbation of chronic obstructive lung disease, CTPA – computer tomography pulmonary angiography, ILD – interstitial lung disease, PE – pulmonary embolism confirmed on CTPA, PTX – pneumothorax (spontaneous), TB – tuberculosis, VP – viral pneumonia.

CXR features associated with PCP

Admission CXR quality was assessed as optimal for 69% of radiographs; suboptimal CXR quality was mainly due to poor lung expansion or inadequate inspiration (20%) and/or poor patient positioning (14%) (supplementary table S1).

One case and one control patient had a normal CXR. Three control patients (5.8%), and no cases, had a pneumothorax on admission CXR (5 patients overall subsequently developed pneumothorax as related to mechanical ventilation or a procedure, 3 of whom had PCP). Cystic lesions were only seen in 2 control patients. Parenchymal calcification was seen in 7 (13.5%) control patients and in none of the PCP cases (supplementary table S2).

Diffuse ground glass opacification was associated with significantly increased odds of PCP on adjusted analysis (aOR 6.2, 95% confidence interval (CI) 1.6 – 28.9, p = 0.01). Consolidation was frequently seen on CXR in both cases and controls, but patchy compared to diffuse consolidation was associated with increased odds of PCP (aOR 5.8, 95% CI 1.1 – 45.7, p = 0.05, table S3 and figure 3). In contrast, pleural effusion was associated with decreased odds of PCP (aOR 0.1, 95% CI 0.0 – 0.4, p = 0.01), as were reticular or reticulonodular abnormalities, cavitation and central

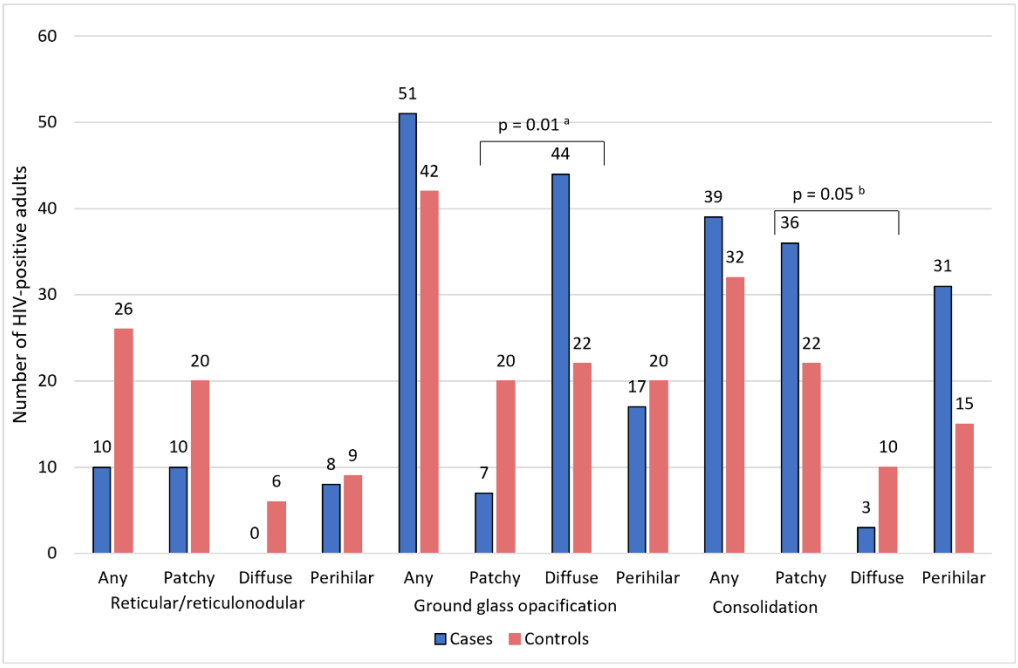
lymphadenopathy, although these were not statistically significant (Table 4). There was no difference in the median radiographic severity score in cases (median 10, range 3 – 12) compared to controls (median 10, range 1 - 12), with no relationship on adjusted analysis (OR 1.3 for one-unit score increase, 95%CI 0.9 – 2.2, p = 0.2). Selected examples of typical CXRs with notable features from cases with PCP in this series are shown in figure 4 and figure S1.

Table 4. Chest X-ray (CXR) changes in HIV-positive adults with PCP (cases) compared to non-PCP respiratory disease (controls).

CXR feature		PCP (n = 52), n (%)	Non-PCP (n = 52), n (%)	Crude OR (95% CI)	Adjusted OR [a] (95% CI)	P value
Parenchymal change	Reticular ± nodular	10 (19.2)	26 (50)	0.2 (0.1 – 0.6)	0.4 (0.1 – 1.4)	0.1
	Reticulonodular	6 (11.5)	21 (40.4)	0.2 (0.1 – 0.5)	0.2 (0.0 – 1.1)	0.1
	Diffuse ground glass opacities	44 (86.3)	22 (52.4)	5.7 (2.2 – 16.5)	6.2 (1.6 – 28.9)	0.01
	Patchy ground glass opacities	7 (13.7)	20 (47.6)	0.2 (0.1 -0.5)	0.2 (0.0 – 0.6)	0.01
	Consolidation	39 (75)	32 (61.5)	1.9 (0.8 – 4.4)	1.9 (0.4 – 8.0)	0.4
	Cavitation	3 (5.8)	9 (17.3)	0.3 (0.1 – 1.1)	1.4 (0.1 – 20.6)	0.8
Other	Pleural effusion	2 (3.8)	14 (26.9)	0.1 (0.0 – 0.4)	0.1 (0.0 – 0.4)	0.01
	Central lymphadenopathy	5 (9.6)	14 (26.9)	0.3 (0.1 – 0.8)	0.4 (0.1 – 1.7)	0.2

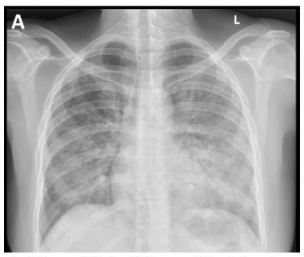
[a] Adjusted for chronic lung disease and PF ratio. CI – confidence interval, CXR – chest X-ray, OR – odds ratio, PCP – Pneumocystis pneumonia, PF - Pa02:Fi02 ratio (partial pressure of oxygen in arterial blood: inspired oxygen concentration ratio).

Figure 3. Distribution of chest X-ray (CXR) parenchymal changes in cases and controls.

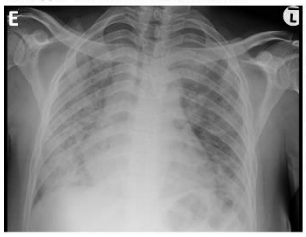


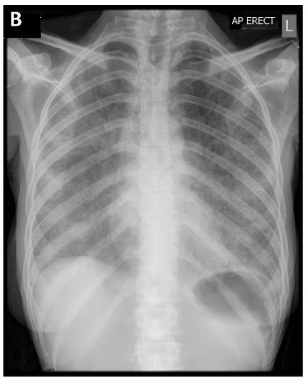
[a] Diffuse versus patchy ground glass opacification: aOR 6.2, 95% confidence interval (CI) 1.6 – 28.9, p = 0.01), [b] patchy versus diffuse consolidation: aOR 5.8, 95% CI 1.1 – 45.7, p = 0.05 (table S3)

Figure 4 (A-E). Selected chest X-rays (CXRs) from HIV-positive adults with PCP.

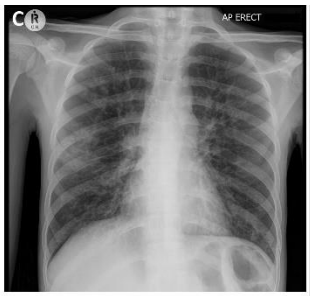


A. 35 year old lady, CD4 count 11 cells/mm³. Admitted with RR 38 bpm, PF of 226 mmHg and CXR showing ground glass opacification (seen in 98% of PCP cases, table 4), diffusely distributed, involving all six zones with perihilar involvement. Perihilar sparing was more common (in 67% of PCP cases) in this series (table S4). Consolidation, seen in 75% of PCP cases in this series (table S4), is seen (patchy left upper and lower zone) on this film.

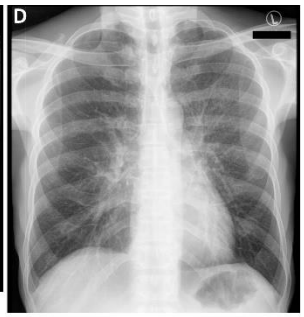




B. 29 year old lady, CD4 count 29 cells/mm³, pre-ART. Admitted with RR 40 bpm and PF 190 mmHg and demised after 16 days of inpatient care with treatment for bacterial pneumonia (*Pseudomonas aeruginosa* on admission sputum MC&S) alongside PCP. CXR shows diffuse ground glass involvement, with the typical peripheral rather than perihilar predominance, across all six lung zones. Patchy consolidation in the right and left lower zones is seen on this film; patchy consolidation predominantly involved the lower zones in PCP cases in this series (table S4).



E. 21 year old man, CD4 count 21 cells/mm³, VL 32852 copies/mL, interrupted second line ART; two prior episodes of PTB. Admitted with RR 48 bpm and PF 247 mmHg. Alongside patchy ground glass opacification in the right middle zone, atypically for PCP, there are patchy, perihilar reticular changes with fine nodularity (<1mm) in all 6 zones, with bilateral hilar and mediastinal LAD. Reticular and nodular CXR changes were seen in the minority of PCP cases in this series (19% and 12% respectively) with central adenopathy in 10% (vs 27% of non-PCP cases); 20% of PCP cases with reticular changes had a laboratory-confirmed co-diagnosis. Reticular or nodular changes were frequent in non-PCP cases (50% and 40% respectively) (table 4 and S4).



D. 51 year old man, CD4 count 48 cells/mm³, smoker and pre-ART. Admitted with RR 28 bpm and PF 380 mmHg. Diffuse ground glass opacification, sparing only the right upper zone, seen on admission CXR. Discharged after 30 days of inpatient care.

E. 33 year old lady, CD4 count 14 cells/mm³, interrupted ART, demised after 15 days of inpatient PCP treatment. Admission CXR shows diffuse ground glass changes, sparing only the right lower zone where patchy perihilar consolidation is seen (extending to right middle, left upper and left lower zones). In this series, ground glass changes involved > 4 and 6 lung zones in 92% and 67% of PCP cases respectively (table S4). Diffuse ground glass changes correlated with severe PCP in this series (table 4).

ART – antiretroviral therapy, bpm – breaths per minute, CAP – community acquired pneumonia, CMV – Cytomegalovirus, CTX – cotrimoxazole, EBV – Ebstein-Barr virus, MC&S – microscopy, culture and sensitivity, PF - Pa02:Fi02 ratio (partial pressure of oxygen in arterial blood: inspired oxygen concentration ratio), PTB – pulmonary tuberculosis, RR – respiratory rate. Inpatient treatment: high dose cotrimoxazole with steroid therapy, unless otherwise specified. No co-diagnoses in above cases unless specified.

Clinical prediction model for PCP diagnosis

Variables selected *a priori* for inclusion in the diagnostic model, selected based on reported clinical relevance, were hypoxia (Sp02 < 90% in room air, Pa02 < 7.8 kPa, or PF ratio ≤ 300 mmHg) or elevated respiratory rate (≥ 30 breaths per minute), ground glass opacification (diffuse or patchy), consolidation, reticular or reticulonodular changes and/or pleural effusion on CXR[5–8,21]. After backward stepwise selection using the AIC as the stopping rule, the following variables were significant, and included in the reduced binary regression models: (1) for the hypoxia model: hypoxia, diffuse or patchy ground glass opacification, and pleural effusion (table 5), and (2) for the respiratory rate model: respiratory rate ≥ 30 breaths per minute, diffuse or patchy ground glass opacification, pleural effusion and reticular or reticulonodular changes (table 6). Both models showed good calibration – the Houwelingen-Le Cessie heuristic shrinkage estimate for the hypoxia model and respiratory rate model was 0.14 and 0.13 respectively, suggesting only a marginal (14% and 13% respectively) decrease in performance can be expected with model testing on new data). Partial residual plots showed no collinearity with variance inflation factors (VIF) close to 1 (indicating marginal inter-variable correlation, and therefore predictor regression coefficients are likely reliable and not inflated due to interactions with other predictors)(Figure 5 A and B). Regression coefficients for the full (all candidates) and reduced models (selected candidates) are show in table 5 (hypoxia model) and table 6 (respiratory rate model).

Table 5. Multivariable model of predictors of PCP – hypoxia model.

Variable	Full model		Reduced model		
	Regression coefficient (SE)	P value	Regression coefficient (SE)	OR (95% CI)	P value
Hypoxia [a]	0.8 (0.5)	0.1	1.0 (0.5)	2.8 (1.1 – 7.5)	0.03
Diffuse ground glass opacification	2.9 (1.1)	0.01	3.0 (1.1)	20.6 (2.3 – 183.8)	0.006
Patchy ground glass opacification	1.2 (1.2)	0.3	1.2 (1.2)	3.3 (0.3 – 32.9)	0.3
Pleural effusion	-2.6 (0.9)	0.003	-2.6 (0.9)	0.1 (0.0 – 0.4)	0.002
Reticular ± nodular changes [b]	-1.0 (0.5)	0.1	-	-	-
Consolidation [b]	-0.1 (0.6)	0.9	-	-	-

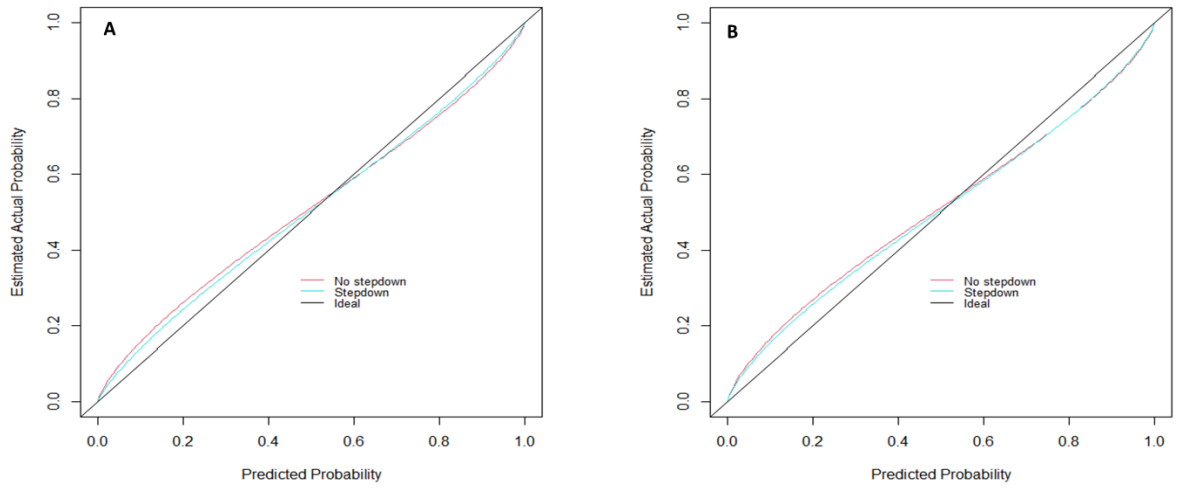
[a] Sp02 < 90% (RA) or Pa02 < 7.8 kPa (RA) or PF ratio ≤ 300 mmHg, [b] dropped from model on backwards stepwise regression using the Akaike information criterion (AIC) as a stopping rule. OR – odds ratio, PCP – Pneumocystis pneumonia, RA – room air, Sp02 – pulse oximetry saturation, Pa02 – partial pressure of oxygen in arterial blood, PF – Pa02:Fi02 ratio (partial pressure of oxygen in arterial blood: inspired oxygen concentration ratio), SE – standard error. C index: full model = 0.843, reduced model = 0.828.

Table 6. Multivariable model of predictors of PCP – respiratory rate model.

Variable	Full model		Reduced model		
	Regression coefficient (SE)	P value	Regression coefficient (SE)	OR (95% CI)	P value
Respiratory rate ≥ 30 bpm	1.3 (0.5)	0.02	1.3 (0.5)	3.5 (1.3 – 9.8)	0.02
Diffuse ground glass opacification	2.6 (1.1)	0.02	2.6 (1.1)	13.6 (1.5 – 123.5)	0.02
Patchy ground glass opacification	0.8 (1.2)	0.5	0.8 (1.2)	2.1 (0.2 – 2.1)	0.5
Pleural effusion	-2.7 (0.9)	0.002	-2.7 (0.9)	0.1 (0.0 – 0.4)	0.001
Reticular ± nodular changes	-1.1 (0.6)	0.04	-1.1 (0.5)	0.3 (0.1 – 0.9)	0.04
Consolidation [a]	-0.03 (0.6)	1.0	-	-	-

[a] Dropped from model on backwards stepwise regression using the Akaike information criterion (AIC)) as a stopping rule. Bpm – breaths per minute, OR – odds ratio, PCP – Pneumocystis pneumonia, RR – respiratory rate, SE – standard error. C index: full model = 0.859, reduced model = 0.857.

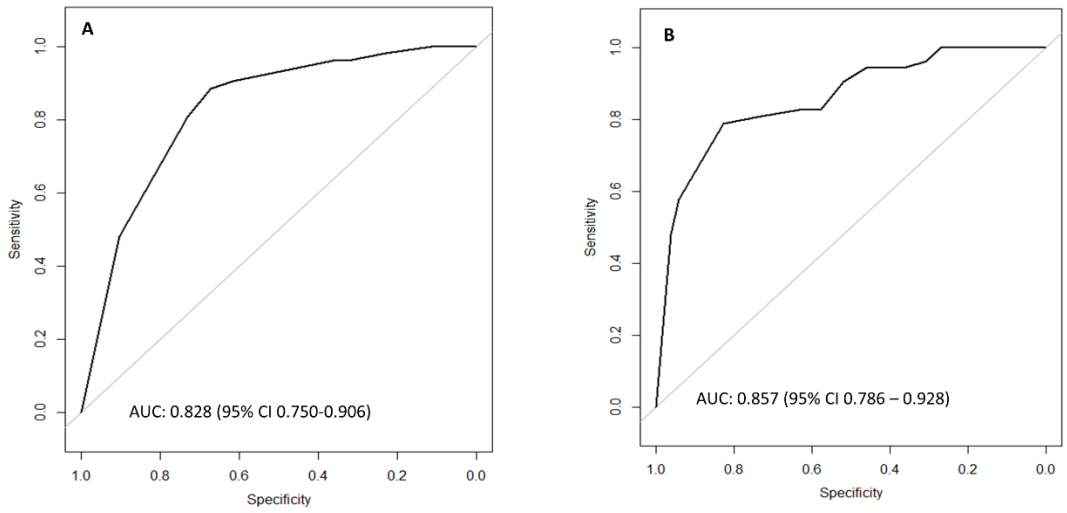
Figure 5 (A and B). Calibration curve of the multivariable logistic regression hypoxia model (A) and respiratory rate model (B), comparing actual probability to model-predicted probability (for the full (no stepdown) and reduced (stepdown) models), for the diagnosis of PCP in HIV-positive adults.



No stepdown: full model (all candidate variables). Step down: reduced model (backward stepwise selection of variables using the AIC as the stopping rule). VIF values for stepdown (reduced) model: (A) hypoxia = 1.0, diffuse ground glass = 4.7, patchy ground glass = 4.6, pleural effusion = 1.0; (B) RR ≥ 30 bpm = 1.1, diffuse ground glass = 4.4, patchy ground glass = 4.5, pleural effusion = 1.1, reticular/reticulonodular = 1.0. VIF values close to 1 indicate marginal inter-variable correlation. bpm – breaths per minute, RR – respiratory rate, VIF - Variance Inflation Factor.

The area under the curve (AUC) of the receiver operating characteristic (ROC) was 0.828 (95% CI 0.750-0.906) (Figure 6A) for the hypoxia model and 0.857 (95% CI 0.786 – 0.928) (Figure 6B) for the respiratory model (using DeLong method on bootstrap validation samples). Additional probability and validation plots are included in supplementary material, figure S2 and S3.

Figure 6 (A and B). Receiver operating characteristic (ROC) and area under the curve (AUC) for the (A) hypoxia and (B) respiratory rate models.



AUC – area under the curve, CI – confidence interval

CXR changes associated with severe PCP

29 cases met criteria for severe PCP disease (PF ratio < 100 mmHg (n = 3), ICU referral or admission (n = 18) or in-hospital death (n = 19)). Median PF ratio was lower among patients with severe versus non-severe PCP (189 mmHg (IQR 129 – 192) versus 247 mmHg (IQR 208 – 270)).

Diffuse, compared to patchy, ground glass opacification was associated with increased odds of severe PCP (aOR 4.5, 95% 1.6 – 14.4, p = 0.008) as was upper zone involvement (aOR 5.9, 95% CI 2.0 – 20.7, p = 0.003). The odds of severe PCP increased with increasing ground glass zone involvement (aOR 2.1 for each one-unit increase in involved zone; 95% CI, 1.4 – 3.2, p = 0.0004). Consolidation was strongly associated with severe PCP (aOR 3.3, 95% CI 1.2 – 11.0, p = 0.03), with higher odds for severe disease with patchy rather than diffuse involvement (aOR 5.5, 95% CI 1.2 - 39.3, p = 0.04) and perihilar sparing (aOR 3.9, 95% CI 1.2 – 13.5, p = 0.04). Increasing zones of involvement with consolidation did not correlate with severe PCP (Table 7, full details in supplementary table S4 and figures S4 A-C). Increasing radiographic score was not predictive of severe PCP (aOR 1.08, 95% CI 0.8 – 1.4, p = 0.6 for each point increase).

Table 7. Chest X-ray (CXR) features in adults with severe compared to non-severe PCP.

CXR feature			Severe PCP [a] (n = 29), n (%)	Non-severe PCP (n = 23), n (%)	Crude OR (95% CI)	Adjusted OR [b] (95% CI)	P value
Ground glass	Any		29 (100)	22 (95.7)	12.1 (2.2 – 227.4)	5.1 (0.8 – 99.6)	0.1
	Diffuse		26 (89.7)	18 (81.8)	5.7 (2.2 – 16.5)	4.5 (1.6 – 14.4)	0.008
	Perihilar		7 (24.1)	10 (45.5)	0.6 (0.2 – 1.3)	0.5 (0.2 – 1.1)	0.08
	Number of zones involved [c]	< 2	1 (3.4)	1 (4.5)			
		2 – 4	3 (10.3)	3 (13.6)	0.2 (0.02 – 2.2)	0.2 (0.0 – 2.7)	0.2
		> 4	25 (86.2)	18 (81.8)	3.1 (0.3 – 27.6)	2.1 (0.2 – 28.2)	0.5
Consolidati	Any		25 (86.2)	14 (60.9)	1.9 (0.8 – 4.4)	3.3 (1.2 – 11.0)	0.03
	Patchy		22 (75.8)	14 (60.9)	5.5 (1.5 – 26.3)	5.5 (1.2 - 39.3)	0.04
	Perihilar		19 (65.5)	12 (52.2)	4.4 (1.6 – 13.0)	3.9 (1.2 – 13.5)	0.02

[a] No difference in associations when using in-hospital death (n = 19) as a single outcome, rather than composite outcome (PF ratio < 100 mmHg, ICU referral or admission, or in-hospital death), as definition for severe PCP. [b] adjusted for chronic lung disease and respiratory co-diagnosis. [c] For every one zone increase in ground glass involvement adjusted OR 2.1 (1.4 – 3.2), p = 0.0004. CXR – chest X-ray, OR – odds ratio, PCP – Pneumocystis pneumonia.

Clinical prediction model for severe PCP

None of the selected candidate variables for the first model (reticular/reticulonodular changes, ground glass opacification, consolidation, with either elevated respiratory rate or hypoxia, and either radiographic severity score or total zones of involvement) or second model (with candidate variables for the first model, but omitting reticular or reticulonodular and including respiratory co-diagnosis) met the model inclusion threshold using the AIC stopping rule.

Discussion and conclusions

In this matched case-control study involving 104 HIV-positive adults in South Africa, diffuse ground glass changes, predominantly with perihilar sparing and involving more than four lung zones, were significantly associated with HIV-associated PCP. Pleural effusion had a strong negative correlation with PCP, and alongside reticulonodular changes, was seen with higher frequency in non-PCP disease. Two regression models, incorporating either hypoxia or elevated respiratory rate, with diffuse ground glass changes, absence of pleural effusion or absence of reticular/reticulonodular changes, performed well in discriminating PCP from non-PCP respiratory disease in this population.

The hazy shadowing of ground glass changes seen in PCP are a reflection of the exuberant host inflammatory response that is triggered by *Pneumocystis jirovecii* attachment to alveolar pneumocytes and extracellular matrix proteins[30], with resultant interstitial thickening, partial alveolar exudative filling, air displacement and/or alveolar collapse[23]. The neutrophil- and $CD8^+$-

driven immune reaction that incites lung injury and contributes to respiratory failure in PCP[31] occurs paradoxically in patients with advanced immunodeficiencies – with depleted $CD4^+$ reserve leading to unbalanced and unregulated $CD8^+$ cytotoxicity[32]. In this study, a significant proportion of adults with PCP were profoundly immunosuppressed (77% of adults had CD4 count < 50 cells/mm^3); concordantly, hypoxia was seen in 88% of PCP cases.

Reticular changes, inferring net-like interlobular septal thickening that may coalesce into nodules but characteristically spare the airspace[23], as well as pleural disease, were not associated with PCP in our study as in others[6,33–35] and strongly suggest an alternative non-PCP diagnosis. This is consistent with pathology induced by tropism of *Pneumocystis jirovecii* for alveolar epithelium (airspace opacification). No patients with PCP had pneumothorax or cystic changes on admission CXR in this study. Previous commentaries, largely skewed by retrospective reviews of all cases of HIV-associated pneumothorax[36,37] and historical reports linking pneumothorax to pentamidine prophylaxis failure with progressive upper zone fibrocystic disease[38], may over-represented true and contemporary rates of pneumothorax amongst adults with PCP. Three patients in our PCP cohort developed ventilation-associated pneumothorax, a complication attributed to the pathological reduction in alveolar surfactant seen as a consequence of the *Pneumocystis jirovecii* immune response, that reduces lung compliance and increases risk of alveolar rupture[39]. Central lymphadenopathy correlated more strongly with non-PCP respiratory disease in our study, and is a well described radiographic feature in many alternative HIV-associated pathologies including tuberculosis, fungal infections and lymphoma[40,41].

Severe PCP was seen in more than half (56%) of PCP cases. Poor outcomes in adults with PCP are thought to be, in part, a consequence of dysregulated host immune response, in keeping with the mortality benefit demonstrated with addition of corticosteroids in treating severe PCP[42], and the correlation between poor outcomes and more extensive CXR involvement[24,25,27], higher serological indices of inflammation and hypoxia[1,25]. In keeping with these observations, diffuse ground glass opacification was associated with severe PCP in our study. There was a shift to increasing zone (particularly with ground glass opacities) and diffuse CXR involvement in severe disease. Consolidation was a frequent feature in both PCP cases (75%) and in non-PCP controls (62%). In keeping with prior studies showing radiological progression from interstitial to alveolar infiltrates correlating with worsening clinical PCP severity [24,25], consolidation had predictive value for severe PCP disease in our study.

Prior studies have examined a combination of clinical and radiological variables for PCP prediction[6–8] but have had limited power and/or discriminatory value and therefore not easily translated into tools that can enhance clinical decision making at the bedside. A clinical prediction rule developed by Maartens et al[5], based on 29 microscopy-confirmed PCP cases with imputation to a total of 56 events, incorporated CXR changes (possible or likely PCP), low haemoglobin and either elevated respiratory rate or low pulse oximetry saturation for predicting PCP; both models performed well with receiver operative characteristic AUCs of at least 0.8. In our study, we interrogated specific and descriptive CXR features that offer discriminatory value for predicting PCP versus other common HIV-associated respiratory diseases, to develop prediction models. Using either presence of hypoxia or elevated respiratory rate, together with selected CXR features, these models had robust internal validation and good discriminatory performance (both with AUC greater than 0.82). In contrast to the Maartens study, we did not find a correlation between haemoglobin and PCP diagnosis.

Despite our use of a broad definition for PCP severity and high representation of severe cases in our cohort, none of the selected candidate variables for severe PCP were sufficiently predictive and we were not able to generate a prognostic model for PCP. Our cross-sectional analysis of admission CXRs did not capture CXR evolution over time and after exposure to PCP-directed therapy; there is some evidence linking CXR progression over time, rather than baseline features, to need for incremental mechanical ventilation support and poor outcome in ICU settings[43]. A study from China, enrolling 1001 adults with HIV-associated PCP and a 17% in-hospital mortality rate, found a six-variable predictive model incorporating elevated LDH, hypoxia, ICU admission, anaemia, low CD4 count and development of post-admission pneumothorax to offer good discriminatory value for predicting in-hospital death (AUC 0.9)[44]. Other studies have found increasing age[45], hypoxia[1,24,45,46], LDH[1,47], concomitant comorbidity or coinfection[1,45,48,49], high SOFA (or APACHE scores[49,50], acidosis[43] and incremental ventilatory support requirement[43,49] be associated with poor outcome. This wide between-study variability in markers of severe PCP highlight the challenges with developing generalisable and reproducible prognostic rules for PCP, and a may indicate differing clinical phenotypes and gaps in our current understanding of the pathophysiology of severe disease and therefore its clinical or radiological correlates.

Our study has some limitations. By virtue of its retrospective design, our findings are limited by the reliance on laboratory specimen submission, accuracy of medical records and quality of radiographs. Selection bias may have been introduced since patients with suggestive clinical or radiological features of PCP may have received empiric treatment without pursuing laboratory confirmation,

with possibly higher representation of those with less typical presentations. Furthermore, critically ill (and non-ventilated) adults, unable to produce sputum or undergo invasive respiratory sampling, who may represent a distinct clinical and radiological phenotype, would not be represented in this study. Whilst adults with negative *Pneumocystis jirovecii* laboratory testing, but meeting the criteria for probable PCP, were excluded to strengthen the confidence in the definite PCP versus non-PCP comparative analysis, inclusion of this subgroup may have added power to a predictive model for any (definite or probable) PCP or severe PCP. Our predictive model performed well on internal validation but requires evaluation in a separate cohort to confirm external validity. Although clinician-assigned co-diagnoses were captured and adjusted for in the severe PCP analysis (a recent study showed increased mortality with pulmonary TB co-infection)[1], this could not be done in the PCP diagnosis models as the control group required an alternate primary, non-PCP, diagnosis. Lastly, due to the well-described inflammatory responses and radiological differences between HIV-associated and HIV-negative PCP[51,52], our results may have limited applicability to immunosuppressed adults without HIV.

In conclusion, our study identified CXR changes that correlate with laboratory-confirmed PCP and that can be utilised together with objective, easily-obtainable clinical information for accurate and prompt PCP recognition. These findings may be used to train evolving artificial intelligence (AI)-assisted CXR reading software[53,54], offering potential value in settings where access to specialist radiologist services are limited. Other non-sputum-based diagnostics, such as serum (1-3)-β-D-Glucan which has good sensitivity for PCP diagnosis[55], may further enhance performance of diagnostic algorithms incorporating clinical and radiological predictors, and should be explored in high-burden settings where access to sputum-based diagnostics for PCP is limited.

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Potential conflicts of interest

The authors have no conflicts of interest to declare.

Code repository

Code repository (Rstudio version 4.3.1) available at: https://github.com/Jared-T/PCP_MMED_code/tree/main.

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Appendices

Appendix A: Supplementary material

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Table S1. Quality assessment, by specialist radiologist, of admission chest-X ray (CXR)

CXR quality		PCP, n (%) (n = 52)	Non-PCP respiratory disease, n (%) (n = 52)	Total (n = 104)
Optimal		33 (63.5)	39 (75)	72 (69.2)
Suboptimal		19 (36.5)	13 (25)	32 (30.8)
Reason for suboptimal quality	Too dark/too light	2 (3.8)	0	2 (1.9)
	Poor position	8 (15.4)	7 (13.4)	15 (14.4)
	Artefact	1 (1.9)	0	1 (1.0)
	Other, of which	14 (26.9)	9 (17.3)	23 (22.1)
	Poor lung expansion or suboptimal inspiration	14 (26.9)	7 (13.5)	21 (20.2)
	Rotation	2 (3.8)	2 (3.8)	4 (3.8)
	AP sitting with poor expansion	0	1 (1.9)	1 (1.0)
	Poor lung expansion, mobile film	1 (1.9)	0	1 (1.0)
	AP sitting, poor expansion	0	1 (1.9)	1 (1.0)

AP – anterior posterior film, CXR – chest X-ray, PCP – Pneumocystis pneumonia

Table S2. Chest X-ray (CXR) features in adults with PCP (cases) and non-PCP respiratory disease (controls).

CXR feature				PCP, n (%) (n = 52)	Non-PCP, n (%) (n = 52)
Parenchymal abnormality	Reticular pattern ± nodularity	**Any reticular pattern ± nodularity**		**n = 10 (19.2)**	**n = 26 (50)**
		Nodules present	Any	6 (60.0)	21 (80.8)
			Fine (nodules < 1mm)	2 (33.3)	6 (28.6)
			Coarse (nodules ≥1mm)	4 (66.7)	15 (71.4)
		Extent	Patchy	10 (50)	20 (76.9)
			Diffuse	0	6
		Number of zones involved	<2	0	0
			2-4	7 (70)	15 (57.7)
			>4	3 (30)	11 (42.3)
		Distribution	Any upper zone	5 (50)	19 (73.1)
			Any mid zone	9 (90)	25 (96.2)
			Any lower zone	9 (90)	23 (88.5)
		Perihilar distribution	Yes	8 (80)	9 (34.6)
			No	2 (20)	17 (65.4)
		Calcifications	Yes	0	5 (19.2)
			No	10 (100)	21 (80.8)
	Ground glass opacities	**Any ground glass opacities**		**n = 51 (98.1)**	**n = 42 (80.8)**
		Extent	Patchy	7 (13.7)	20 (47.6)
			Diffuse	44 (82.3)	22 (52.3)
		Number of zones involved	<2	2 (3.9)	2 (4.8)
			2-4	6 (11.8)	26 (50.0)
			>4	43 (84.3)	14 (33.3)
		Distribution	Upper zone	46 (90.2)	26 (61.9)
			Mid zone	50 (98.0)	34 (81.0)
			Lower zone	47 (92.2)	39 (92.9)
		Perihilar distribution	Yes	17 (33.3)	20 (47.6)
			No	34 (66.6)	22 (52.4)
		Calcifications	Yes	0	1 (2.4)
			No	51 (100)	41 (97.6)
	Consolidation	**Any consolidation**		**n = 39 (75)**	**n = 32 (61.5)**
		Extent	Patchy	36 (92.3)	22 (68.8)
			Diffuse	3 (7.7)	10 (31.2)
		Number of zones involved	<2	0	4 (12.5)
			2-4	36 (92.3)	25 (78.1)
			>4	3 (7.7)	3 (9.4)
		Distribution	Upper zone	12 (30.8)	12 (37.5)
			Mid zone	25 (64.1)	23 (71.9)
			Lower zone	37 (94.9)	27 (84.4)
		Perihilar distribution	Yes	31 (79.5)	15 (46.9)
			No	8 (20.5)	17 (53.1)
		Calcifications	Yes	0	1 (3.1)
			No	39 (100)	31 (96.9)
	Cystic lesions	**Any cystic lesions**		**0 (0)**	**2 (3.8)**
	Cavities	**Any cavitation**		**n = 3 (5.8)**	**n = 9 (17.3)**
		Size	< 5cm	3 (100)	6 (66.7)

			>/=5cm	0	3 (33.3)
	Other	**Atelectasis, n (%)**		**1 (1.9)**	**1 (1.9)**
		Lobar collapse, n (%)		**0**	**1 (1.9)**
		Any calcification, n (%)		**0**	**7 (13.5)**
Pleural abnormality	**Any pleural abnormality**			**5 (9.6)**	**23 (44.2)**
	Calcification/plaque			0 (0)	0 (0)
	Pleural effusion			2 (3.8)	14 (26.9)
	Apical cap			3 (5.8)	6 (11.5)
	Pneumothorax			0 (0)	3 (5.8)
Other	Mediastinal shift			2 (3.8)	4 (7.7)
	Central Lymphadenopathy			5 (9.6)	14 (26.9)
	Bullae			0 (0)	1 (1.9)
	Bronchiectasis			0 (0)	1 (1.9)
	Suspected malignancy			0 (0)	0 (0)
	Hyperinflation			0 (0)	1 (1.9)
	Mycetoma			0 (0)	1 (1.9)
	Volume loss			0 (0)	3 (5.8)
	Cardiac enlargement			3 (5.8)	5 (9.6)
	Bronchial wall thickening			1 (1.9)	0 (0)
Total number of zones involved with any changes				6 (IQR 6 – 6, range 3 – 6)	6 (IQR 4 – 6, range 0 – 6)

CXR – chest x-ray, IQR – interquartile range, PCP – Pneumocystis pneumonia

Table S3. Distribution of parenchymal changes on CXR in patients with PCP (cases) versus non-PCP respiratory disease (controls).

CXR feature		PCP (n = 52), n (%)	Non-PCP (n = 52), n (%)	Crude OR (95% CI)	Adjusted OR [a] (95% CI)	P value
Reticular ± nodules	Any	10 (19.2)	26 (50)	0.2 (0.1 – 0.6)	0.4 (0.1 – 1.4)	0.1
	Diffuse	0	6 (23.1)	1.7e-08	2.3e-08	1.0
	Patchy	10 (100)	20 (76.9)	5.8e+07	4.3e+07	1.0
	Perihilar	8 (80)	9 (34.6)	7.6 (1.5 – 57.7)	3.2 (0.9 – 12.2)	0.08
Ground glass	Any	51 (98.1)	42 (80.8)	12.1 (2.2 – 227.4)	5.6e+06	0.99
	Diffuse	44 (86.3)	22 (52.4)	5.7 (2.2 – 16.5)	6.2 (1.6 – 28.9)	0.01
	Patchy	7 (13.7)	20 (47.6)	0.2 (0.1 -0.5)	0.2 (0.0 – 0.6)	0.01
	Perihilar	17 (33.3)	20 (47.6)	0.6 (0.2 – 1.3)	1.0 (0.3 – 3.0)	0.98
Consolidation	Any	39 (75)	32 (61.5)	1.8 (0.8 – 4.4)	1.9 (0.4 – 8.0)	0.4
	Diffuse	3 (7.7)	10 (31.2)	0.2 (0.0 – 0.7)	0.2 (0.0 – 0.9)	0.05
	Patchy	36 (92.3)	22 (68.8)	5.5 (1.5 – 26.3)	5.8 (1.1 – 45.7)	0.05
	Perihilar	31 (79.5)	15 (46.9)	4.4 (1.6 – 13.0)	3.2 (0.9 – 12.3)	0.08

[a] adjusted for chronic lung disease and PF.

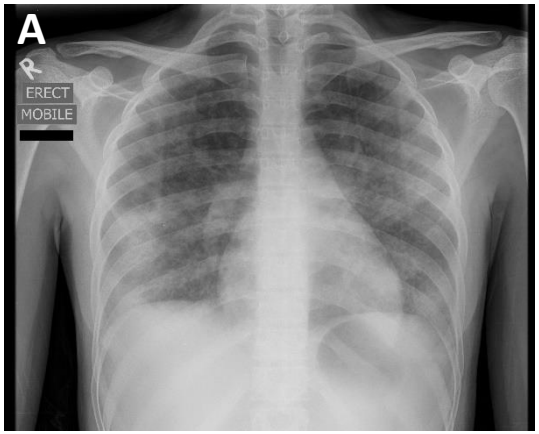
CI – confidence interval, CXR – chest X-ray, PF – $Pa0_2$:$Fi0_2$ ratio (Partial pressure of oxygen in arterial blood: inspired oxygen concentration ratio, OR – odd's ratio, PCP – Pneumocystis pneumonia

Table S4. Chest X-ray (CXR) features in adults with severe compared to non-severe PCP.

CXR feature			Severe PCP [a] (n = 29), n (%)	Non-severe PCP (n = 23), n (%)	Crude OR (95% CI)	Adjusted OR [b] (95% CI)	P value
Reticular ± nodules			**1 (3.4)**	**9 (39.1)**	**0.2 (0.1 – 0.6)**	**0.2 (0.1 – 0.7)**	**0.01**
Ground glass	**Any (n = 51)**		**29 (100)**	**22 (95.7)**	**12.1 (2.2 – 227.4)**	**5.1 (0.8 – 99.6)**	**0.1**
	Patchy		3 (10.3)	4 (18.2)	0.2 (0.1 – 0.5)	0.2 (0.1 – 0.6)	0.008
	Diffuse		26 (89.7)	18 (81.8)	5.7 (2.2 – 16.5)	4.5 (1.6 – 14.4)	0.008
	Perihilar		7 (24.1)	10 (45.5)	0.6 (0.2 – 1.3)	0.5 (0.2 – 1.1)	0.08
	Number of zones involved [c]	< 2	1 (3.4)	1 (4.5)			
		2 – 4	3 (10.3)	3 (13.6)	0.2 (0.02 – 2.2)	0.2 (0.0 – 2.7)	0.2
		> 4	25 (86.2)	18 (81.8)	3.1 (0.3 – 27.6)	2.1 (0.2 – 28.2)	0.5
	Any upper zone involvement [d]		28 (96.6)	18 (78.3)	7.7 (3.0 – 22.8)	5.9 (2.0 – 20.7)	0.003
	Any mid-zone involvement		28 (96.6)	22 (95.7)	13.2 (3.5 – 86.7)	6.4 (1.6 – 43.5)	0.02
	Any lower zone involvement		26 (89.7)	21 (91.3)	3.1 (1.1 – 10.5)	2.0 (0.6 – 8.1)	0.3
Consolidation	**Any (n = 39)**		**25 (86.2)**	**14 (60.9)**	**1.9 (0.8 – 4.4)**	**3.3 (1.2 – 11.0)**	**0.03**
	Patchy		22 (88)	14 (100)	5.5 (1.5 – 26.3)	5.5 (1.2 - 39.3)	0.04
	Diffuse		3 (12)	0	0.2 (0.04 – 0.7)	0.2 (0.03 – 0.8)	0.04
	Perihilar		19 (76)	12 (85.7)	4.4 (1.6 – 13.0)	3.9 (1.2 – 13.5)	0.02
	Number of zones involved	< 2	0	0			
		2 – 4	22 (88.0)	14 (100)	2.3e+07	6.9e+07	0.99
		> 4	3 (12.0)	0	1.6e+07	3.7e+07	0.99
	Any upper zone involvement [d]		10 (34.5)	2 (8.7)	1.0 (0.4 – 2.5)	1.0 (0.3 – 2.8)	0.96
	Any mid-zone involvement		17 (58.6)	8 (34.8)	1.2 (0.5 – 1.6)	1.6 (0.6 – 4.0)	0.3
	Any lower zone involvement		24 (82.8)	13 (56.5)	2.3 (1.0 – 5.2)	3.8 (1.4 – 11.8)	0.01
Other	Cavitation		2 (6.9)	1 (4.3)	0.3 (0.1 – 1.1)	0.9 (0.2 – 3.9)	0.8
	Central lymphadenopathy		2 (6.9)	3 (13)	0.3 (0.1 – 0.8)	0.4 (0.1 – 1.2)	0.1
Radiographic severity score, median (range)(IQR) [e]			10 (8 – 10) (10 – 10)	10 (3 – 12) (10 – 11)	-	-	0.6
Total zones, median (range)(IQR) [f]			6 (3 – 6) (6 – 6)	6 (3 – 6) (6 – 6)	-	-	0.07

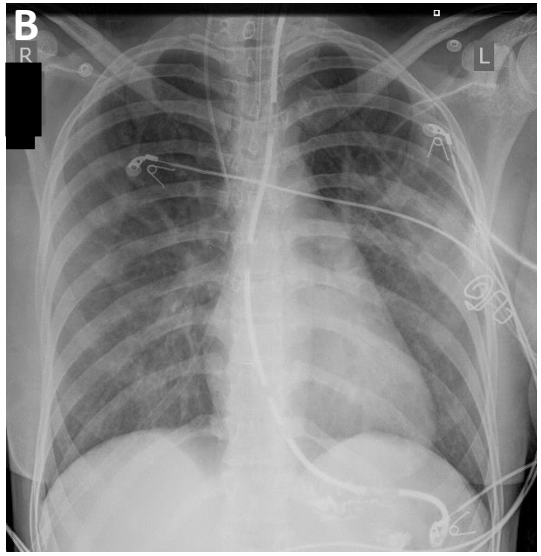
[a] No difference in associations found with radiological predictors when using in-hospital death (n = 19), rather than composite outcome (PF ratio < 100, ICU admission or in-hospital death), as definition for severe PCP. [b] adjusted for chronic lung disease and respiratory co-diagnosis. [c] For every one zone increase in ground glass involvement adjusted OR 2.1 (1.4 – 3.2) p = 0.0004. [d] proportions (%) do not add up to 100% as some records with > 1 zone involved. Comparison of relevant zone involvement versus no involvement [e] Total zones involved with ground glass changes: severe PCP median 6 zones (IQR 5 – 6, range 1 – 6), non-severe PCP median 6 zones ((IQR 5 – 6, range 1 – 6), p = 0.9 on univariate analysis. 6 zone involvement with ground glass changes seen in 66% of severe PCP cases (n = 19) and 68% of non-severe PCP cases (n = 15). [f] Total zones: for every increase in one zone of involved with any parenchymal changes, adjusted odds of severe PCP increased by 57% (OR 1.57; 95% 1.01 – 2.7, p = 0.07). Total zones ≥ 4 involved with any parenchymal changes: severe PCP n = 28 (96.6%), non-severe PCP n = 22 (95.6%). CXR – chest x-ray, IQR – interquartile range, OR – odds ratio, PCP – Pneumocystis pneumonia

Figure S1 (A-E). Selected chest X-rays (CXRs) from HIV-positive adults with PCP.

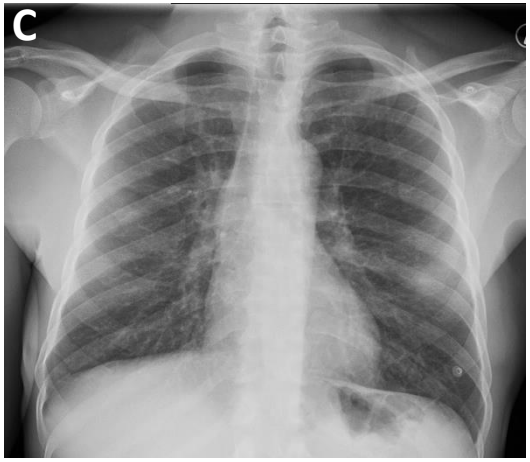


A. 29 year old lady, CD4 count 5 cells/mm^3, interrupted ART, admitted with RR 30bpm and PF 274mmHg. Her admission CXR shows typical diffuse ground glass changes across all 6 zones with a peripheral predominance, and patchy consolidation involving the right and left middle and lower zones. She was discharged on day 6 of inpatient PCP care[a].

ART – antiretroviral therapy, bpm – breaths per minute, CMV – Cytomegalovirus, CTX – cotrimoxazole, EBV – Ebstein-Barr virus, PF - Pa02:Fi02 ratio (partial pressure of oxygen in arterial blood: inspired oxygen concentration ratio), PCP – Pneumocystis pneumonia, RR – respiratory rate. Inpatient treatment: high dose cotrimoxazole with steroid therapy, unless otherwise specified. No co-diagnoses in above cases unless specified.

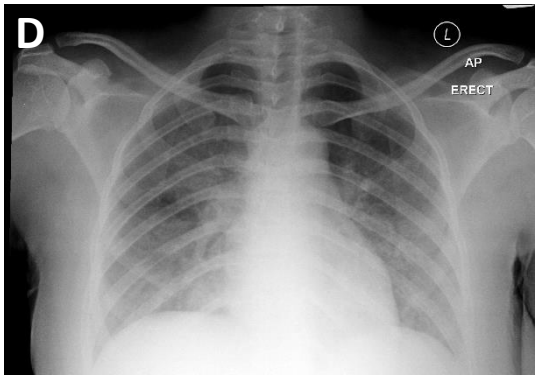


B. 24 year old lady, CD4 count 25 cells/mm^3, newly diagnosed with HIV, admitted with RR 28 bpm and PF 111mmHg. Tracheal aspirate in ICU, alongside *Pneumocystis jirovecii*, tested positive for CMV (VL log 5.9), EBV (VL log 5.5) and parainfluenza virus. On CXR, there is diffuse ground glass opacification with a perihilar predominance. Radiological features of viral pneumonia often overlap with those of PCP[56]. In this series, ground glass opacification was seen in four cases of PCP with concomitant laboratory-confirmed viral pneumonia and in the single laboratory-confirmed viral pneumonia in the non-PCP group, without any discriminating features in terms of ground glass extent, distribution or other radiological patterns between PCP plus viral, exclusive PCP and exclusive viral pneumonia diagnoses.



C. 56 year old man, newly diagnosed with HIV, CD4 count 13 cells/mm^3 admitted with RR 36 and PF 236 mmHg. Bronchoscopy also positive for CMV (log 4.3); the patient improved to discharge from the general ward with ganciclovir and PCP care. CXR shows patchy, rather than diffuse, ground glass opacification; patchy ground glass changes in this series were a more frequent in non-PCP (48%) compared to PCP (14%) cases (table 4).

E. 40 year old lady, newly diagnosed with HIV, with a history of prior PTB, CD4 count 2 cells/mm^3. Admitted with RR 40 bpm and PF 269 mmHg and blood culture positive for *Cryptococcus neoformans.* CXR shows diffuse ground glass perihilar changes, sparing only the left upper zone, with patchy consolidation in the right and left lower zones. Atypical changes are also seen: perihilar reticular pattern, with coarse nodularity (≥1mm) in right and left middle and right lower zone with left apical cap.



D. 29 year old lady, CD4 118 cells/ mm^3, viral load 265 copies/mL on second line ART. Admitted with RR 36 bpm, PF 180 mmHg. Alongside diffuse ground glass opacification with patchy consolidation, right hilar adenopathy is seen on CXR. Adenopathy was infrequent in PCP cases in this series (10% of cases of PCP (80% of which received empiric treatment for bacterial pneumonia) versus 27% of cases of non-PCP, table 4).

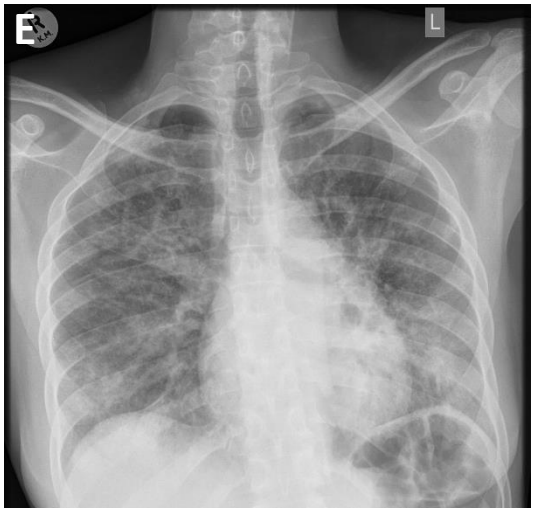
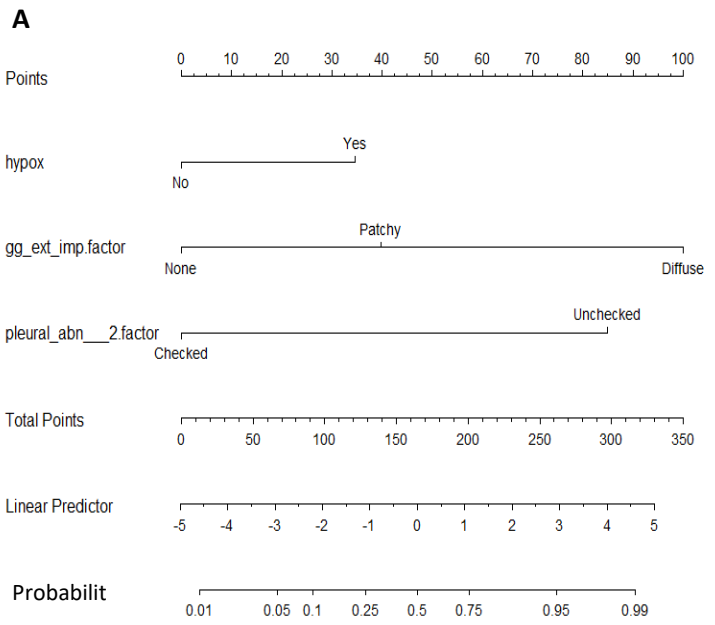
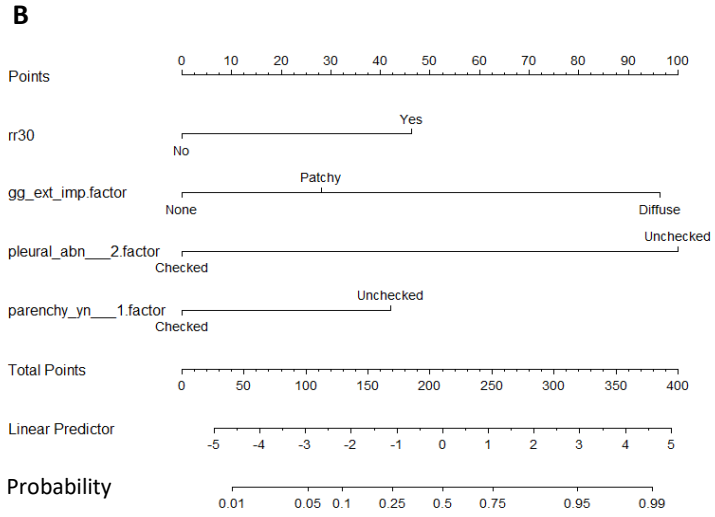


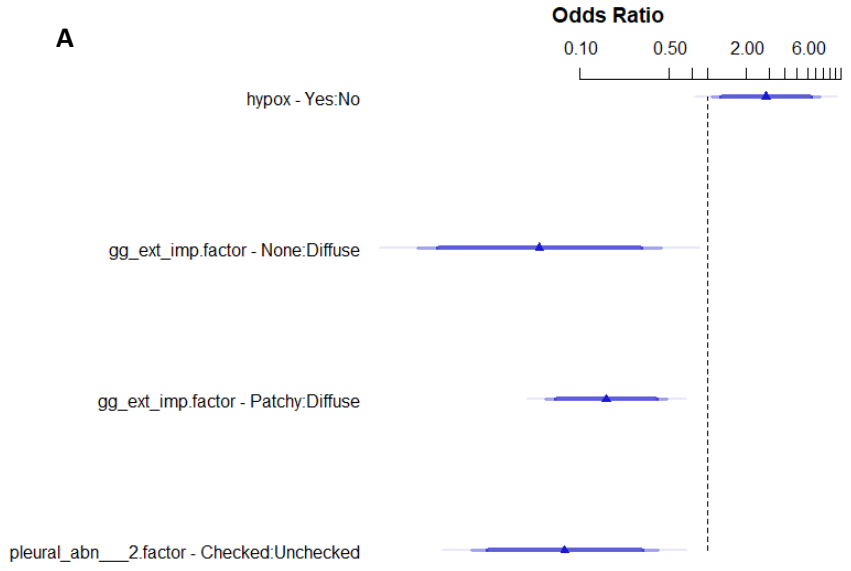
Figure S2 (A and B). Normogram showing the predicted log-odds and probabilities for PCP with the step-down model, for the (A) hypoxia model and (B) respiratory rate model.

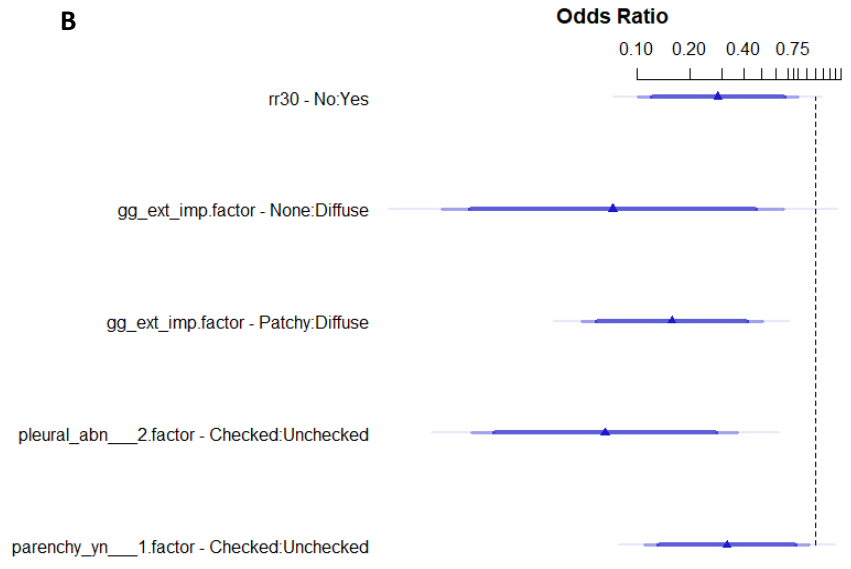




gg_ext_imp.factor – ground glass extent (no imputation required), hypox – hypoxia, pleural_abn__2 – pleural effusion, parenchy_yn__1.factor – reticular or reticulonodular changes, PCP – Pneumocystis pneumonia, rr30 – respiratory rate > 30 breaths/minute

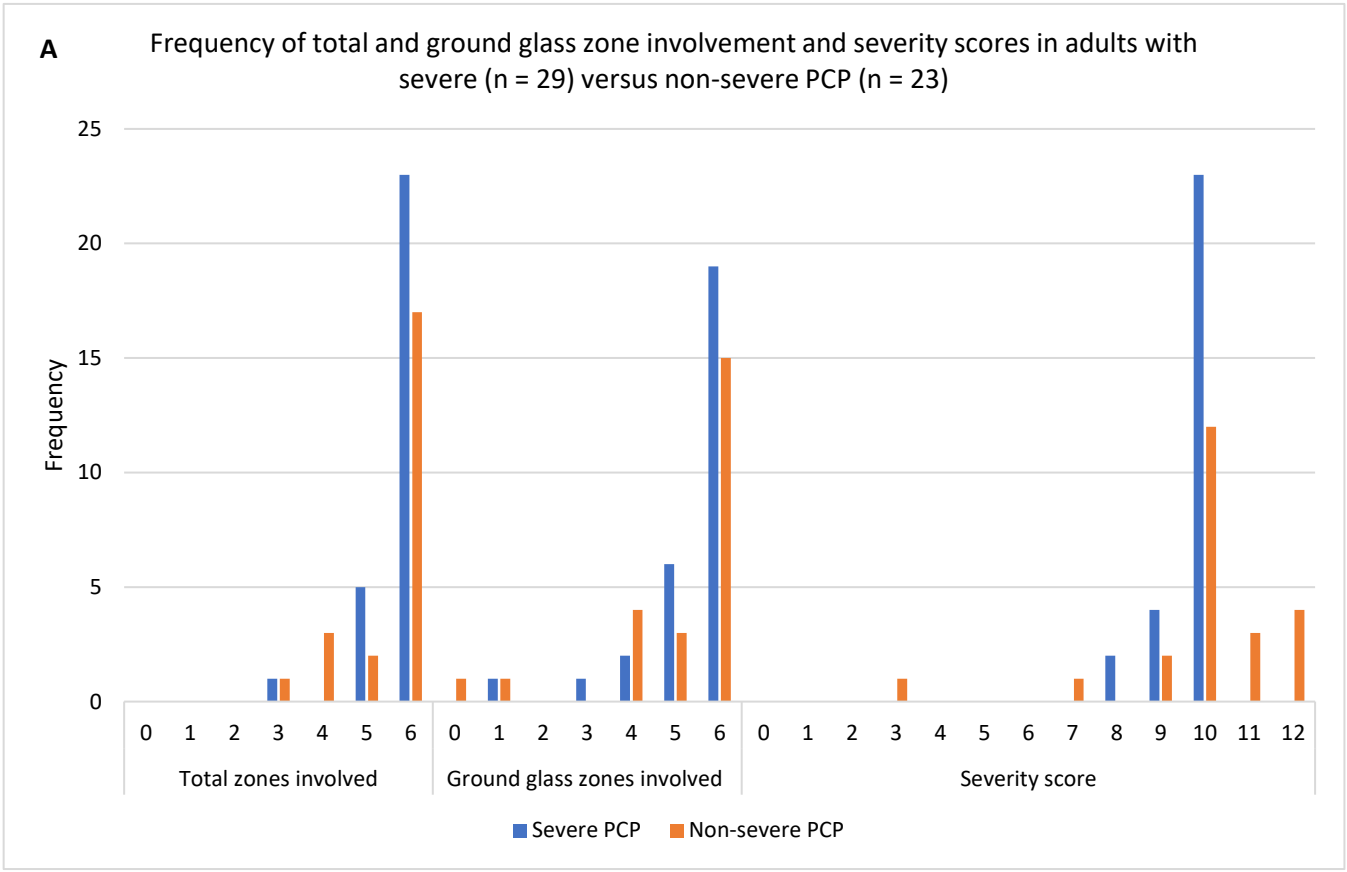
Figure S3 (A and B). Odds ratios with interquartile range for predictors for the (A) hypoxia model and (B) respiratory rate model. Numbers at the left are upper quartile: lower quartile or current: reference group, with bars representing with 0.9, 0.95 and 0.99 confidence limits. The intervals are drawn on the log odds ratio scale and labelled on the odds ratio scale, and ranges are on the original scale.





gg_ext_imp.factor – ground glass extent (no imputation required), hypox – hypoxia, pleural_abn__2 – pleural effusion, parenchy_yn__1.factor – reticular or reticulonodular changes, rr30 – respiratory rate > 30 breaths/minute

Figure S4 (A-C). Comparison of HIV-positive adults with severe (n = 29) versus non-severe PCP (n = 23) with regards to (A) the frequency of total and ground glass zones of involvement, and severity scores, (B) Pa02:Fi02 ratio, and (C) Pa02:Fi02 ratio stratified by total zones of involvement with any chest X-ray changes.



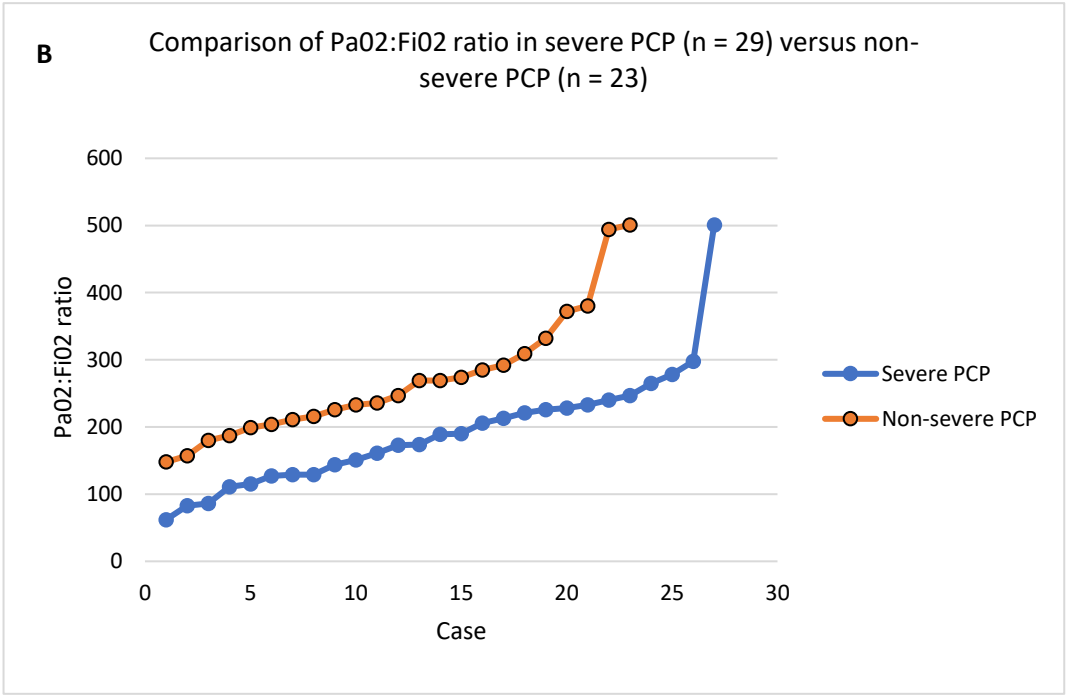
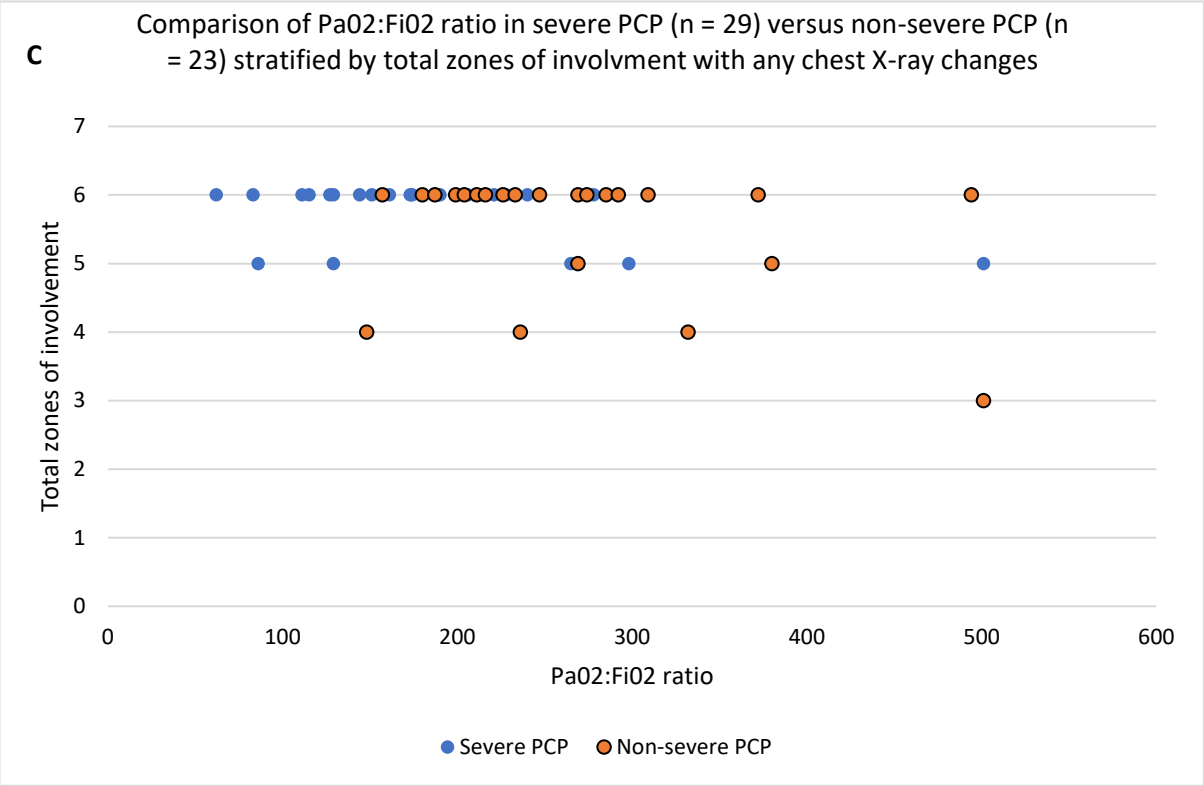


Figure S4 (A-C) (Cont.) Comparison of HIV-positive adults with severe (n = 29) versus non-severe PCP (n = 23) with regards to (A) the frequency of total and ground glass zone involvement, and severity scores, (B) Pa02:Fi02 ratio, and (C) Pa02:Fi02 ratio stratified by total zones of involvement with any chest X-ray changes.



PCP – Pneumocystis pneumonia, PF - Pa02:Fi02 ratio (partial pressure of oxygen in arterial blood: inspired oxygen concentration ratio).

Appendix I: REDCap Data Capture Form[9]

Appendix I: REDCap Data Capture Form

Record ID

Record ID	128
Exclude = yes (1), Include = No (0)	⊗ Yes ◯ No (excl = 2)

Identifying and testing data

Newly screened patient?	◯ No - case or control from parent study database ◯ Yes - new case/control derived from NHLS screen
Patient name	_____
Folder number	_____
Date of birth	_____
Sex	◯ Male ◯ Female ◯ Not reported
Date of laboratory request	_____
Hospital	_____
Requesting ward	◯ ICU ◯ High care unit ◯ General ward/C15/other ◯ unknown/not recorded
Respiratory sample type	◯ Oral wash ◯ Induced sputum ◯ Expectorated sputum ◯ Bronchoalveolar lavage ◯ Transbronchial biopsy ◯ Endotracheal aspirate ◯ Other ◯ Sputum - not noted if expectorated or induced
Pneumocystis microscopy result	◯ Positive ◯ Negative ◯ Equivocal

HIV/ART history

Timing of HIV diagnosis?	◯ During admission (new diagnosis) ◯ Prior to admission ◯ Unknown/not recorded
Trimethoprim/sulfamethoxazole prophylaxis use on admission	◯ Yes ◯ No ◯ Unknown/not reported (Use of TMP/SMZ prophylaxis at the time of index admission with suspected PCP)
Any prior exposure to TMP/SMZ prophylaxis?	◯ Yes ◯ No ◯ Unknown/not reported
ART use at the time of admission	◯ Currently taking ART ◯ Known to have defaulted ART ◯ Known to have no prior exposure to ART ◯ Not currently on ART, prior ART exposure unknown ◯ Unknown/not reported
CD4 count	_____ (Most recent CD4 count within time of admission)
Date of CD4 count	_____
Most recent viral load	_____ (Most recent VL within time of admission)
Date of viral load	_____ (If no viral load/unknown/preART: write 01-01-9999)

Admission clinical characteristics

Date of admission	_____
Admission respiratory rate	_____ (breaths per minute)
Admission WCC	_____
Admission albumin	_____
Admission laboratory haemoglobin (g/dL)	_____
Admission LDH (U/L)	_____ (If LDH not done or lab error, note: not done/lab error)
Admission PaO2 (please indicate if on RA or on oxygen, and what percent)	_____ (Insert first PaO2 available on/after admission (kPa))
Recorded oxygen saturation (pulse oximetry) on admission (please indicate if on RA or on oxygen, and what percent)	_____
Pa02 (RA)	_____
Sats (RA)	_____
PF	_____
Imputed PF (if sats on oxygen/RA only data available)	_____

Co-morbidities and treatment details

Previous PCP/currently on treatment for PCP?	◯ Yes ◯ No ◯ Unknown/not reported
Previous pulmonary or disseminated tuberculosis?	◯ Yes ◯ No ◯ Unknown/not reported
On TB treatment at the time of admission?	◯ Yes ◯ No ◯ Unknown/not reported
Previous pulmonary/disseminated cryptococcosis?	◯ Yes ◯ No ◯ Unknown/not reported
On cryptococcal treatment at the time of admission?	◯ Yes ◯ No ◯ Unknown/not reported
Details of any other pre-existing cardiac, respiratory or other disease/comorbidity?	_____ (Write "nil" if no other comorbidities outside that already captured.)
Underlying chronic or structural lung disease? (Previous PTB with SLD, other underlying SLD, current smoker or >= 5 pack year history)	◯ Yes ◯ No
Other concurrent new (from index admission) laboratory or histologically-confirmed respiratory diagnosis	☐ PTB ☐ CAP ☐ Kaposi's sarcoma ☐ Pulmonary/disseminated cryptococcosis ☐ Other ☐ None ☐ COVIDPCR positive
Sputum or other respiratory specimen sent for TB testing?	◯ Yes: GeneXpert/GeneXpert Ultra ◯ Yes: TB smear and culture for AFBs ◯ No ◯ Unknown/not reported
Second sputum or other respiratory specimen sent for TB testing?	◯ Yes: GeneXpert/GeneXpert Ultra ◯ Yes: TB smear and culture for AFBs ◯ No ◯ Unknown/not reported
ULAM testing done?	◯ Yes - ULAM positive ◯ Yes - ULAM negative ◯ Not done/no record of testing

Sputum or other respiratory specimen sent for MC&S?	◯ Yes ◯ No ◯ Unknown/not reported
Result of covid testing	◯ Positive ◯ Negative ◯ Equivocal ◯ Not done
Laboratory-confirmed diagnosis 1	◯ Pulmonary or disseminated TB ◯ Community acquired pneumonia ◯ Hospital acquired pneumonia; developed during course of admission ◯ Covid pneumonia ◯ Other viral pneumonia ◯ Disseminated fungal infection with respiratory involvement ◯ Lung malignancy ◯ Other ◯ None ◯ Biopsy suggestive of interstitial lung disease ◯ Hospital acquired pneumonia; diagnosed on admission (at time of CXR) ◯ CTPA confirmed PE(s)
Laboratory confirmed diagnosis 2	◯ Pulmonary or disseminated TB ◯ Community acquired pneumonia ◯ Hospital acquired pneumonia; developed during course of admission ◯ Covid pneumonia ◯ Other viral pneumonia ◯ Disseminated fungal infection with respiratory involvement ◯ Lung malignancy ◯ Other ◯ None ◯ Biopsy suggestive of interstitial lung disease ◯ Hospital acquired pneumonia; diagnosed on admission (at time of CXR) ◯ CTPA confirmed PE(s)
Laboratory confirmed diagnosis 3	◯ Pulmonary or disseminated TB ◯ Community acquired pneumonia ◯ Hospital acquired pneumonia; developed during course of admission ◯ Covid pneumonia ◯ Other viral pneumonia ◯ Disseminated fungal infection with respiratory involvement ◯ Lung malignancy ◯ Other ◯ None ◯ Biopsy suggestive of interstitial lung disease ◯ Hospital acquired pneumonia; diagnosed on admission (at time of CXR) ◯ CTPA confirmed PE(s)

Other suspected co-diagnosis	☐ Community-acquired pneumonia ☐ Pulmonary or disseminated tuberculosis ☐ Congestive cardiac failure ☐ Kaposi's sarcoma ☐ Other lung malignancy ☐ Other presumed bacterial infection ☐ Other ☐ None ☐ Unknown/not reported
Treatment received for other suspected respiratory/cardiac diagnosis	☐ Antibiotics for suspected bacterial pneumonia ☐ Antibiotics given, unclear indication ☐ Tamiflu/other antiviral treatment for suspected viral LRTI ☐ Antibiotics for other non-respiratory bacterial infection ☐ Anti-failure treatment for cardiac failure ☐ Treatment for Kaposi's sarcoma ☐ Treatment for other suspected lung malignancy ☐ No other additional medication given ☐ Other ☐ Unknown/not reported ☐ Empiric TB treatment - specify below if pulmonary/disseminated/unknown ☐ Treatment for suspected (swab negative) covid pneumonia
Empiric diagnosis 1	◯ Pulmonary or disseminated TB ◯ Community acquired pneumonia ◯ Hospital acquired pneumonia; developed during course of admission ◯ Covid pneumonia ◯ Other viral pneumonia ◯ Disseminated fungal infection with respiratory involvement ◯ Lung malignancy ◯ Cardiac failure ◯ Renal failure ◯ Other ◯ Antibiotics administered; unclear indication ◯ Antifungal treatment administered; unclear indication ◯ Antiviral treatment administered; unclear indication ◯ Unknown/not reported ◯ None ◯ Interstitial lung disease ◯ Non-specific acute exacerbation asthma or COPD ◯ Antibiotics for non-respiratory/cardiac bacterial infection (eg abdominal) ◯ Antibiotics for other respiratory/cardiac infection ◯ Pneumothorax on admission (spontaneous) ◯ Pneumothorax secondary to ventilation/procedure related ◯ Hospital acquired pneumonia; diagnosed on admission (at time of CXR) ◯ TB IRIS

Empiric diagnosis 2	◯ Pulmonary or disseminated TB ◯ Community acquired pneumonia ◯ Hospital acquired pneumonia; developed during course of admission ◯ Covid pneumonia ◯ Other viral pneumonia ◯ Disseminated fungal infection with respiratory involvement ◯ Lung malignancy ◯ Cardiac failure ◯ Renal failure ◯ Other ◯ Antibiotics administered; unclear indication ◯ Antifungal treatment administered; unclear indication ◯ Antiviral treatment administered; unclear indication ◯ Unknown/not reported ◯ None ◯ Interstitial lung disease ◯ Non-specific acute exacerbation asthma or COPD ◯ Antibiotics for non-respiratory/cardiac bacterial infection (eg abdominal) ◯ Antibiotics for other respiratory/cardiac infection ◯ Pneumothorax on admission (spontaneous) ◯ Pneumothorax secondary to ventilation/procedure related ◯ Hospital acquired pneumonia; diagnosed on admission (at time of CXR) ◯ TB IRIS

Empiric diagnosis 3	◯ Pulmonary or disseminated TB ◯ Community acquired pneumonia ◯ Hospital acquired pneumonia; developed during course of admission ◯ Covid pneumonia ◯ Other viral pneumonia ◯ Disseminated fungal infection with respiratory involvement ◯ Lung malignancy ◯ Cardiac failure ◯ Renal failure ◯ Other ◯ Antibiotics administered; unclear indication ◯ Antifungal treatment administered; unclear indication ◯ Antiviral treatment administered; unclear indication ◯ Unknown/not reported ◯ None ◯ Interstitial lung disease ◯ Non-specific acute exacerbation asthma or COPD ◯ Antibiotics for non-respiratory/cardiac bacterial infection (eg abdominal) ◯ Antibiotics for other respiratory/cardiac infection ◯ Pneumothorax on admission (spontaneous) ◯ Pneumothorax secondary to ventilation/procedure related ◯ Hospital acquired pneumonia; diagnosed on admission (at time of CXR) ◯ TB IRIS
Details of suspected co-diagnoses and treatment received	_____ (Pathology found, grounds of suspected diagnoses, date treatment started, drugs used, date stopped (if prior to discharge))

Hospitalisation and outcomes

Patient referred to ICU?	◯ Yes ◯ No ◯ Unknown/not reported
Mechanically ventilated?	◯ Yes ◯ No ◯ Unknown/not reported
Inotrope/vasopressor support given?	◯ Yes ◯ No ◯ Unknown/not reported
Given CPAP?	◯ Yes ◯ No ◯ Unknown/not reported
In-hospital death	◯ Yes ◯ No ◯ Unknown/not reported

PACS Identifying data

Patient name: _____
Folder number: _____
Date of birth: _____
Hospital: _____
Date of admission: _____
Date of Pneumocystis test: _____

Radiology review

Radiologist initials	_____ (e.g QSH)
Date of radiograph review	_____
Film quality	◯ Optimal ◯ Suboptimal ◯ Unreadable
Parenchymal abnormalities seen?	☐ Reticular pattern, with/without nodules ☐ Ground glass opacity/opacities ☐ Consolidation ☐ Cystic lesion(s) ☐ Cavitation ☐ Other non-specific opacifications or changes ☐ No parenchymal abnormality seen
Pleural abnormality seen?	☐ Calcification/plaque ☐ Pleural fluid/effusion ☐ Apical cap ☐ Pneumothorax ☐ Other pleural abnormality (specify below) ☐ No pleural abnormality
Central abnormalities seen?	☐ Tracheal deviation ☐ Mediastinal shift ☐ Hilar elevation ☐ Lymphadenopathy ☐ Other ☐ No central abnormality
Any other abnormality present?	☐ Bullets/Artifact/Foreign body ☐ Suspected lung resection ☐ Sternotomy wire/clips ☐ Bullae ☐ Bronchiectasis ☐ Suspected malignancy ☐ Hyperinflation ☐ Mycetoma ☐ Volume loss ☐ Rib fracture or abnormality ☐ Spinal/vertebral abnormality ☐ Enlarged cardiac shadow ☐ Other cardiac abnormality ☐ Other abnormality, not classified elsewhere (fill in details below)
Please give further details on any other radiological changes not already mentioned or explored above. If no further comments, note "nil".	_____
Total number of zones of the lung involved (if no parenchymal pathological changes, answer "0").	_____

Appendix II: Chest X-ray review tool

1. Participant ID	☐☐☐ Gender: ☐1 Male ☐2 Female	DOB	D D M M Y Y Y Y
2. Radiologist initials	☐☐☐ Signature: _____	Reading date	D D M M Y Y Y Y
3. Film quality	☐1 Optimal ☐2 Suboptimal ☐3 Unreadable **Comments:** ☐2 Too dark/too light ☐2 Poor position ☐3 Artifact OR ☐4 Other, specify: _____		

Parenchymal abnormalities ☐1 Yes ☐2 No OR ☐1 Normal radiograph (end of form)

Reticular pattern ☐1 Yes ☐2 No	With nodules ☐1 Yes ☐2 No If yes: Type ☐1 Fine (nodular component < 1mm) ☐2 Coarse (nodules ≥ 1mm)		Extent ☐1 Patchy ☐2 Diffuse	Zones R L ☐1 U ☐4 U ☐2 M ☐5 M ☐3 L ☐6 L	Perihilar ☐1 Yes ☐2 No	Calcification ☐1 Yes ☐2 No
Ground class opacities ☐1 Yes ☐2 No			Extent ☐1 Patchy ☐2 Diffuse	Zones R L ☐1 U ☐4 U ☐2 M ☐5 M ☐3 L ☐6 L	Perihilar ☐1 Yes ☐2 No	Calcification ☐1 Yes ☐2 No
Consolidation ☐1 Yes ☐2 No			Extent ☐1 Patchy ☐2 Diffuse	Zones R L ☐1 U ☐4 U ☐2 M ☐5 M ☐3 L ☐6 L	Perihilar ☐1 Yes ☐2 No	Calcification ☐1 Yes ☐2 No
Cystic lesions ☐1 Yes ☐2 No		Size (maximum) ☐1 <1mm ☐2 1 – 5mm ☐3 > 5mm	Number ☐1 Single cyst ☐2 1 – 5 cysts ☐3 > 5	Zones R L ☐1 U ☐4 U ☐2 M ☐5 M ☐3 L ☐6 L	Perihilar ☐1 Yes ☐2 No	Calcification ☐1 Yes ☐2 No
Cavities ☐1 Yes ☐2 No		Size (maximum) ☐1 < 5cm ☐2 ≤ 5 cm	Number ☐1 Single cavitiy ☐2 1 – 5 cavities ☐3 > 5	Zones R L ☐1 U ☐4 U ☐2 M ☐5 M ☐3 L ☐6 L		Calcification ☐1 Yes ☐2 No
Other non-specific opacities ☐1 Yes ☐2 No	Type ☐1 Round ☐2 Irregular	Size (maximum) ☐1 <1mm ☐2 1 – 5mm ☐3 > 5mm	Number ☐1 Single opacity ☐2 1 – 5 opacities ☐3 > 5 Extent (if multiple) ☐1 Patchy ☐2 Diffuse	Zones R L ☐1 U ☐4 U ☐2 M ☐5 M ☐3 L ☐6 L	Perihilar ☐1 Yes ☐2 No	Calcification ☐1 Yes ☐2 No

Pleural abnormalities ☐1 Yes ☐2 No

		Chest side	Extent of lateral chest wall
Calcification/ plaque	☐1 Yes ☐2 No	☐1 R ☐2 L	☐1 <1/4 ☐2 1/4 - 1/2 ☐3 >1/2
Pleural fluid/effusion	☐1 Yes ☐2 No	☐1 R ☐2 L	☐1 <1/4 ☐2 1/4 - 1/2 ☐3 >1/2
Apical cap	☐1 Yes ☐2 No	☐1 R ☐2 L	
Pneumothorax	☐1 Yes ☐2 No	☐1 R ☐2 L	

Central abnormalities ☐1 Yes ☐2 No

		Chest side
Tracheal deviation	☐1 Yes ☐2 No	☐1 R ☐2 L
Mediastinal shift	☐1 Yes ☐2 No	
Hilar elevation	☐1 Yes ☐2 No	☐1 R ☐2 L

Lymphadenopathy ☐1Yes ☐2 No	☐1 R ☐2 L ☐1 R ☐2 L (Hilar) OR ☐1 R ☐2 L (Mediastinal)	
Other abnormalities ☐1Yes ☐2 No		
Surgical ☐1 Bullets/ Artifact/Foreign body ☐2 Suspected lung resection ☐3 Sternotomy wire/clips	Lung ☐1 Bullae ☐2 Bronchiectasis ☐3 Suspected cancer ☐4 Hyperinflation ☐5 Mycetoma ☐6 Volume loss	Skeletal ☐1 Rib fracture or abnormality ☐2 Spinal abnormality Cardiac ☐1 Enlarged ☐2 Other abnormality
Other: INVOLVED: _____ _____ _____ _____ _____		TOTAL NUMBER ZONES

Tool adapted from the Chest Radiograph Reading and Recording system [14], and designed to incorporate assessment of features identified in the literature to have discriminatory value in distinguishing PCP from non-PCP disease [15–22]. Note: "patchy" distribution selected if normal areas of parenchyma can be delineated between areas of parenchymal infiltration. R – right, L – left, U – upper zone, M – mid zone, L – lower zone.

Appendix B: Ethics approvals: Human Research Ethics Committee, Groote Schuur Hospital, District and Regional Hospital, Data retrieval from NHLS via the Academic Affairs and Research Management System (AARMS).

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room E53-46 Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6492
Email: sumayah.ariefdien@uct.ac.za
Website: www.health.uct.ac.za/fhs/research/humanethics/forms

07 October 2019

HREC REF: 522/2019

Dr S Wasserman
Division of Infectious Diseases and HIV Medicine
G16.63
NGSH

Dear Dr Wasserman

PROJECT TITLE: RADIOLOGICAL PREDICTORS OF PCP IN HIV-POSITIVE ADULTS ADMITTED TO A TERTIARY HOSPITAL IN SOUTH AFRICA: A RETROSPECTIVE COHORT STUDY (RadPredict) - SUB-STUDY LINKED TO 548/2015

Thank you for submitting your study to the Faculty of Health Sciences Human Research Ethics Committee (HREC) for review.

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

Approval is granted for one year until the 30 October 2020.

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

Please quote the HREC REF in all your correspondence.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please note that for all studies approved by the HREC, the principal investigator **must** obtain appropriate institutional approval, where necessary, before the research may occur.

Yours sincerely

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE

Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938
NHREC-registration number: REC-210208-007

This serves to confirm that the University of Cape Town Human Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use: Good Clinical Practice (ICH GCP), South African Good Clinical Practice Guidelines (DoH 2006), based on the Association of the British Pharmaceutical Industry Guidelines (ABPI), and Declaration of Helsinki (2013) guidelines. The Human Research Ethics Committee granting this approval is in compliance with the ICH Harmonised Tripartite Guidelines E6: Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) and FDA Code Federal Regulation Part 50, 56 and 312.

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

E52 Room 46 Old Main Building
Groote Schuur Hospital
Observatory 7925
Email: hrec-submissions@uct.ac.za
Website: www.health.uct.ac.za/fhs/research/humanethics/forms

16 May 2023

HREC REF: 522/2019

Prof S Wasserman
Infectious Diseases and HIV Medicine
Email: sean.wasserman@uct.ac.za

Dear Prof Wasserman

PROJECT TITLE: RADIOLOGICAL PREDICTORS OF PCP IN HIV-POSITIVE ADULTS IN SOUTH AFRICA: A RETROSPECTIVE COHORT STUDY (RadPredict) (MMed student Dr Nicola Wills)

Thank you for submitting your documentation dated 01 May 2023 to the Faculty of Health Sciences Human Research Ethics Committee (HREC) for review and approval.

The HREC acknowledges and approves the MMed student Dr Nicola Wills as part of the above-mentioned study.

The study has been formally approved and annual approval has been granted until the 30th May 2024

Please quote the HREC reference number 522/2019 in all your correspondence.

Yours sincerely

PROFESSOR MARC BLOCKMAN
CHAIRPERSON, FACULTY OF HEALTH SCIENCES HUMAN RESEARCH ETHICS COMMITTEE

UNIVERSITY OF CAPE TOWN
IYUNIVESITHI YASEKAPA • UNIVERSITEIT VAN KAAPSTAD

FACULTY OF HEALTH SCIENCES
Human Research Ethics Committee

FHS016: Annual Progress Report / Renewal

HREC office use only (FWA00001637; IRB00001938) This serves as notification of annual approval, including any documentation described below.			
☑ Approved	Annual progress report	Approved until/next renewal date	30/05/24
☐ Not approved	See attached comments		
Signature Chairperson of the HREC/ Designee		Date Signed	5/5/2023

Note: Please email this form and supporting documents (if applicable) in a combined pdf-file to hrec-enquiries@uct.ac.za.
Please clarify your plan for research-related activities during COVID-19 lockdown.
Please use the latest form found on our website:
http://www.health.uct.ac.za/fhs/research/humanethics/forms

Comments to PI from the HREC
Thank you for your Study Deviation HREC Chair Signature Date: 5/5/2023

05 MAY 2023
SCIENCE FACULTY
CITY OF CAPE TOWN

Principal Investigator to complete the following:

1. Protocol information

Date (when submitting this form)	30 APR 2023		
HREC REF Number	522/2019	Current Ethics Approval was granted until	31 November 2022
Protocol title	Radiological predictors of PCP in HIV-positive adults in South Africa: a retrospective cohort study (RadPredict)		
Protocol number (if applicable)	Protocol Version 4.0		
Are there any sub-studies linked to this study?	☑ Yes	☐ No	
If yes, could you please provide the HREC Reference number for all sub-studies? **Note:** A separate FHS016 must be submitted for each sub-study.	548/2015		

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

Patient study number ___ ___ ___ ___

References:

Tool adapted from the Chest Radiograph Reading and Recording system [1], and designed to incorporate assessment of features identified in the literature to have discriminatory value in distinguishing PCP from non-PCP disease [2–9]

[1] Dawson R, Masuka P, Edwards DJ, Bateman ED, Bekker LG, Wood R, et al. Chest radiograph reading and recording system: Evaluation for tuberculosis screening in patients with advanced HIV. *Int J Tuberc Lung Dis* 2010; 14: 52–58.

[2] Worodria W, Okot-Nwang M, Yoo S, Aisu T. Causes of lower respiratory infection in HIV-infected Ugandan adults who are sputum AFB smear-negative. *Int J Tuberc Lung Dis* 2003; 7: 117–123.

[3] Vray M, Germani Y, Chan S, Duc NH, Sar B, Sarr FD, et al. Clinical features and etiology of pneumonia in acid-fast bacillus sputum smear-negative HIV-infected patients hospitalized in Asia and Africa. *AIDS* 2008; 22: 1323–1332.

[4] Siika AM, Ayuo PO, Sidle MJE, Wools-Kaloustian K, Kimaiyo SN, Tierney WM, et al. Admission characteristics, diagnoses and outcomes of HIV-infected patients registered in an ambulatory HIV-care programme in western Kenya. *East Afr Med J* 2008; 85: 523–528.

[5] Malin AS, Gwanzura LK, Klein S, Robertson VJ, Musvaire P, Mason PR. Pneumocystis carinii pneumonia in Zimbabwe. *Lancet (London, England)* 1995; 346: 1258–1261.

[6] Aderaye G, Bruchfeld J, Olsson M, Lindquist L. Occurrence of Pneumocystis carinii in HIV-positive patients with suspected pulmonary tuberculosis in Ethiopia. *AIDS* 2003; 17: 435–440.

[7] Hartung T, Chimbayo D, Van Oosterhout J, Chikaonda T, Van Doornum G, Claas E, et al. Etiology of suspected pneumonia in adults admitted to a high-dependency unit in Blantyre, Malawi. *Am J Trop Med Hyg* 2011; 85: 105–112.

[8] Kibiki G, Beckers P, Mulder B, Arens T, Mueller A, Boeree M, et al. Aetiology and presentation of HIV/AIDS-associated pulmonary infections in patients presenting for bronchoscopy at a referral hospital in northern Tanzania. *East Afr Med J* 2007; 84: 420–428.

[9] Millar AB, Mitchell DM. AIDS and the lung: 4 - Non-invasive investigation of pulmonary disease in patients positive for the human immunodeficiency virus. *Thorax* 1990; 45: 57–61.

Principal Investigator	Professor Sean Wasserman
Department / Office Internal Mail Address	sean.wasserman@uct.ac.za

1.1 Does this protocol receive US Federal funding?	☐ Yes	☑ No
1.2 If the study receives US Federal Funding, does the annual report require full committee approval? **Note:** Any annual approvals for **Full Committee** review MUST be submitted on the monthly HREC submission dates. (Please send electronic copy for full committee review to hrec-submission@uct.ac.za)	☐ Yes	☐ No

If yes in 1.2 please complete section 1.3 below for invoicing purposes

1.3 Ethics Renewal Fee

Please **(tick ✓)**appropriate box for billing purposes:

Submission Type	***Description***	***New fee (Vat Incl.)***	***tick ✓***
Research funded solely from UCT departmental/ divisional/group budget	Annual evaluation of research progress report for re-certification	**R0,00**	☐
Non-sponsored student research for degree purposes at UCT/Other Universities & Colleges	Annual evaluation of research progress report for re-certification	**R0,00**	☑
Annual re-certification / Progress report (FHS016 Form)	Clinical Trial & International Grant Funded Research - Annual evaluation of research progress report for re-certification for Full Committee Approval	**R7000,00**	☐
Annual re-certification / Progress report (FHS016 Form)	Clinical Trial & International Grant Funded Research - Annual evaluation of research progress report for re-certification for Expedited review	**R3 710.00**	☐
Annual re-certification / Progress report (FHS016 Form)	National grant funded research - Annual evaluation of research progress report for re-certification for Full Committee Approval	**R6000.00**	☐
Annual re-certification / Progress report (FHS016 Form)	National Grant funded research for Annual evaluation of research progress report for re-certification for Expedited review	**R1 500,00**	☐

NB: Protocols funded by UCT (e.g. departmental funding / student research) and by certain grant funding organizations (e.g. MRC, NRF, CANSA,) are exempt from these charges.

Please provide details for Invoicing, either complete section 1 or 2 :

1. Invoice billing – Directly to Sponsor

Sponsor's name		Not applicable

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

Billing Address of Sponsor:	
Vat Number:	Not applicable
Contact person	
Telephone number	
Email Address	
2. **Internal Journal Billing:**	
Fund Number:	
Cost Centre Number:	Not applicable
Account Holder Name:	
Division of Account Holder:	

2. List of documentation for approval

We are requesting renewal of the current ethics/HREC approval (approved up to 31 NOV 2022)

Due to late submission of this renewal, we have attached a protocol deviation form (FHS011)

3. Protocol status (tick ✓)

☐	Open Enrolment
☐	Closed to enrolment (tick ✓)
☐	Research-related activities are ongoing
☐	Research-related activities are complete, long-term follow-up only
☐	Research-related activities are complete, data analysis only
☑	Main study is complete but sub-study research-related activities are ongoing
☐	Study is closed → Please submit a Study Closure Form (FHS010)

4. Enrolment

Number of participants enrolled to date	N/A see below
Number of participants enrolled, since last HREC Progress report (continuing review)	Not applicable

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

Additional number of participants still required	

5. Refusals

Total number of refusals (participants invited to join the study, but refused to take part)	

6. Cumulative summary of participants

Total number of participants who provided consent	
Number of participants determined to be ineligible (i.e. after screening)	
Number of participants currently active on the study	Not applicable
Number of participants completed study (without events leading to withdrawal)	
Number of participants withdrawn at participants' request (i.e. changed their mind)	
Number of participants withdrawn by PI due to toxicity or adverse events	
Number of participants withdrawn by PI for other reasons (e.g. pregnancy, poor compliance)	
Number of participants lost to follow-up. Please comment below on reasons for loss of follow-up.	
Number of participants no longer taking part for reasons not listed above. Please provide reasons below:	

7. Progress of study

Please provide a brief summary of the research to date including the overall progress and the progress since the last annual report as well as any relevant comments/issues you would like to report to the HREC:

1. Data for 104 records captured on RedCap (52 PCP cases and 52 non-PCP controls)
2. Approval obtained from National Health Research Database (NHRD) for record and radiological review from Mitchells Plain Hospital, Victoria Hospital Wynberg, Heideveld Hospital and New Somerset Hospital (NHRD approval attached)
3. Approval obtained from National Health Laboratory Service (NHLS) on the Academic Affairs and Research Management System (AARMS) to access data on requests for *Pneumocystis jirovecii* testing submitted from Groote Schuur and regional district hospitals (indicated above) from the Central Data Warehouse.

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

8. Protocol violations and exceptions (tick ✓ all that apply)

☐	No prior violations or exceptions have occurred since the original approval
☐	Prior violations or exceptions have been reported since the last review and have already been acknowledged or approved
☑	**Unreported minor violations** that have occurred since the last review, as well as significant deviations not yet reported, are attached for review See protocol deviation attached for late submission of renewal

9. Amendments (tick ✓ all that apply)

☐	No Prior amendments have been made since the original approval
☐	Prior amendments have been reported since the last review and have already been approved
☑	New protocol changes/ amendments are requested as part of this continuing review (See note below) See FHS006 attached for minor protocol amendments.

Note: If new protocol changes are being requested in this review, please complete an amendment form (FHS006).
Specific changes in the amended protocol and consent/assent forms must be **bolded**, *italicised* or tracked and all changes must include a rationale.

10. Adverse events

10.1 Please provide below or attach a narrative summary of serious adverse events and/ or unanticipated problems since the last progress report. Please indicate changes made to the protocol and informed consent document(s) as a result (if not already reported to the HREC). Please comment on whether causality to any study procedure or intervention could be established.
Nil adverse events

10.2 Have participants received appropriate treatment/ follow-up/ referral when indicated (e.g. in the case of abnormal or incidental clinical findings, distress or anxiety)?		
☐ Yes	☐ No	☑ Not applicable
If yes, please describe:		

11. Summary of Monitoring and Audit Activities (tick ✓)

11.1 Was this study monitored or audited by an external agency (e.g. SAHPRA, FDA)?		
☐ Yes	☑ No	☐ Not applicable

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

11.2 Did a Data and Safety Monitoring Board publish a report?		
☐ Yes	☐ No	☑ Not applicable

11.3 If yes, please identify the agency and attach a summary of the findings.					
Agency Name		Report attached	☐ Yes	☐ No	☐ Not applicable
		DSMB report attached	☐ Yes	☐ No	☐ Not applicable

11.4 Has there been any agency, institutional or other inquiry into non-compliance in this study, or any finding of non-compliance concerning a member of the research team?	
☐ Yes	☐ No
If yes, please explain:	

12. Level of risk (tick ✓)

12.1 In light of your experience of this research, please indicate whether the level of risk to participants has:	
☐	Increased
☐	Decreased
☑	Shown no change
If there has been a change, please explain:	

12.2 Please provide a narrative summary of recent relevant literature that may have a bearing on the level of risk.

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

13. Insurance

Please confirm that valid no fault insurance is still in place? **(tick ✓)**			
☐ Yes		☑ No	
If yes, please complete the following:			
Insurer's name:			
Policy no.		*Coverage Period:	
For UCT sponsored studies please liaise the Insurance office via fhs.sponsorship@uct.ac.za regarding the required documentation and information required obtain a renewed UCT No-fault Insurance Certificate.			

14. Statement of conflict of interest

Has there been any change in the conflict of interest status of this protocol since the original approval? (tick ✓)	
☐ Yes	☑ No
If yes, please explain and if necessary, attach a revised conflict of interest statement (Section #7 in the New Protocol Application Form FHS013):	

15. Signature

My signature certifies that the above is complete and correct.			
Signature of PI		Date	30 APR 2023

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

Form FHS011: Study deviation

HREC office use only (FWA00001637; IRB00001938)			
This serves as acknowledgement of a protocol deviation as described below.			
Chairperson of the HREC signature/ Designee		Date	5/5/2023

Note: Please note that incomplete submissions will not be reviewed.
Please email this form and supporting documents (if applicable) in a combined pdf-file to hrec-enquiries@uct.ac.za.

Please clarify your plan for research-related activities during COVID-19 lockdown

Principal Investigator to complete the following:

1. Protocol information

Date (when submitting this form)	30 APRIL 2023
HREC REF Number	522/2019
Project Title	Radiological predictors of PCP in HIV-positive adults hospitalized in South Africa: a retrospective cohort study (RadPredict), sub-study linked to 548/2015
Protocol number (if applicable)	Protocol Version 4.0
Principal Investigator	Professor Sean Wasserman
Department / Office Internal Mail Address	sean.wasserman@uct.ac.za

HUMAN RESEARCH ETHICS COMMITTEE
05 MAY 2024
HEALTH SCIENCES FACULTY
UNIVERSITY OF CAPE TOWN

2. Protocol deviation description

Please describe the deviation below, including the reason why the deviation occurred.
Late submission of annual progress/renewal (due before 30 November 2022). Data collection for HREC 522/2019 is ongoing and the risk profile remains low in this secondary analysis and retrospective review.

3. Follow-up actions

3.1 Please describe any follow-up action(s) taken or planned as a result of this deviation e.g. DSMB reporting, report to sponsor, informing participants.
Protocol deviation submitted HREC. Nil impact on participants (this is a secondary analysis and retrospective folder review)
3.2 Please describe what action(s) have or will be taken to prevent similar deviations in future.
Timeous completion of annual renewal documentation will be done in the future.

4. Principal Investigator's acknowledgement of responsibility

This signature indicates the PI has reviewed the deviation, taken appropriate follow-up action and implemented or plans to implement preventative steps where possible.			
Signature of PI		Date	30 APR 2023

Western Cape Government
Health

GROOTE SCHUUR HOSPITAL
Enquiries: Dr Bernadette Eick
e-mail: Bernadette.Eick@westerncape.gov.za

Dr Sean Wasserman
MEDICINE – INFECTIOUS DISEASES

E-mail: sean.wasserman@uct.ac.za / nicolakwills@outlook.com

Dear Dr Wasserman,

RESEARCH PROJECT: Radiological Predictors Of PCP In HIV-Positive Adults Admitted To A Tertiary Hospital In South Africa: A Retrospective Cohort Study (RadPredict) – Sub-Study Linked To 548/2015

Your recent letter to the hospital refers.

You are granted permission to proceed with your research, which is valid until **30 October 2020.**

Please note the following:

a) Your research may not interfere with normal patient care.
b) Hospital staff may not be asked to assist with the research.
c) No additional costs to the hospital should be incurred i.e. Lab, consumables or stationary. **If access to TRACK Care/NHLS is required, kindly attach our letter of approval to the application form.**
d) **No patient folders may be removed from the premises or be inaccessible.**
e) Please provide the research assistant/field worker with a copy of this letter as verification of approval.
f) Confidentiality must always be maintained.
g) Should you at any time require photographs of your subjects, please obtain the necessary indemnity forms from our Public Relations Office (E45 OMB or ext. 2187/2188).
h) Should you require additional research time beyond the stipulated expiry date, please apply for an extension.
i) Please discuss the study with the HOD before commencing.
j) Please introduce yourself to the person in charge of an area before commencing.
k) On completion of your research, please forward any recommendations/findings that can be beneficial to use to take further action that may inform redevelopment of future policy / review guidelines.
l) Kindly submit a copy of the publication or report to this office on completion of the research.
m) At no time should any posters encouraging patients to partake in research, be displayed within a clinical area.

I would like to wish you every success with the project.

Yours sincerely

DR BERNADETTE EICK
CHIEF OPERATIONAL OFFICER
Date: 8 November 2019

C.C. Mr. L. Naidoo
Dr L. Booyens
Professor N. Ntusi
Professor s. Beningfield

Western Cape Government

GROOTE SCHUUR HOSPITAL

Enquiries: Dr Bernadette Eick
E-mail : GSHResearch.Request@westerncape.gov.za

Professor Sean Wasserman
Department of Medicine: Infectious Disease

Email: sean.wasserman@uct.ac.za

Dear Professor Wasserman

RESEARCH PROJECT EXTENSION: Radiological Predictors of PCP in HIV-Positive Adults Admitted to a Tertiary Hospital in South Africa: A Retrospective Cohort Study (RadPredict)

Your recent communication to the hospital refers.

The extension of your research is approved in accordance with **UCT Ethics** clearance, until **30 May 2024**

As previously mentioned,

a) Your research may not interfere with normal patient care.
b) Hospital staff may not be asked to assist with the research.
c) No additional costs to the hospital should be incurred as indicated in your Annexure 2 i.e. Lab, consumables or stationery. **If access to TRACK Care/NHLS is required, kindly attach our letter of approval to the application form and approach Information Management to assist with data.**
d) **No patient folders may be removed from the premises or be inaccessible.**
e) Please provide the research assistant/field worker with a copy of this letter as verification of approval.
f) Confidentiality must always be maintained.
g) Once the research is complete, please submit a copy of the publication or report.
h) Please adhere to ALL COVID-19 regulations and Groote Schuur Hospital policies.
i) All Clinical Trials to be registered on Clinicom with Michelle Riley or Rowan James. michelle.riley@westerncape.gov.za / rowan.james@westerncape.gov.za

I would like to wish you every success with the project.

Yours sincerely

DR BERNADETTE EICK
CHIEF OPERATIONAL OFFICER
Date: 31 May 2023

C.C. Mr. L. Naidoo, Mr. A. Mohamed, Professor N. Ntusi, Dr N. Khumalo, Professor S. Moosa, Dr H. Aziz

STRATEGY & HEALTH SUPPORT
Health.Research@westerncape.gov.za
tel: +27 21 483 0866: fax: +27 21 483 6058
5th Floor, Norton Rose House,, 8 Riebeek Street, Cape Town, 8001
www.capegateway.gov.za)

REFERENCE: WC_202203_032
ENQUIRIES: Dr Sabela Petros

University of Cape Town
Anzio Road
Observatory
Cape Town
7925

For attention: Prof Sean Wasserman, Dr Nicola Wills, Dr Qonita Mariam Said-Hartley

Re: Radiological predictors of PCP in HIV-positive adults in South Africa: a retrospective cohort study (RadPredict)

Thank you for submitting your proposal to undertake the above-mentioned study. We are pleased to inform you that the department has granted you approval for your research.
Please contact the following people to assist you with any further enquiries in accessing the following sites:

New Somerset Hospital	**Dr Donna Stokes**	**021 402 6408**
Victoria Hospital	**Dr Graeme Dunbar**	**021 799 1211**
Mitchells Plain Hospital	**Dr Jacek Marszalek**	**021 377 4782**
	Jonathan Naude	**021 377 4760**

Kindly ensure that the following are adhered to:

1. Arrangements can be made with managers, providing that normal activities at requested facilities are not interrupted and the constraints caused by the Covid-19 epidemic above are respected and adhered to.
2. Researchers, in accessing provincial health facilities, are expressing consent to provide the department with an electronic copy of the final feedback (**Annexure 9**) within six months of completion of research. This can be submitted to the provincial Research Co-ordinator (Health.Research@westerncape.gov.za).
3. In the event where the research project goes beyond the *estimated completion* date which was submitted, researchers are expected to complete and submit a progress report (**Annexure 8**) and an updated ethics clearance letter to the provincial Research Co-ordinator (Health.Research@westerncape.gov.za).
4. The reference number above should be quoted in all future correspondence.

Yours sincerely

PROF. V ZWEIGENTHAL
DIRECTORATE: HEALTH INTELLIGENCE
DATE: 7 July 2022
CC

Academic Affairs and Research
1 Modderfontein Road, Sandringham, 2031
Tel: +27 (0)11 555 0367/0406
Email: babatyi.kgokong@nhls.ac.za
academic.research@nhls.ac.za
Web: www.nhls.ac.za

04 October 2022

Applicant: Nicola Wills
Institution: University of Cape Town
E-mail Address: nicolakwills@outlook.com
Cell: 078 106 9560

CC: Amanda Overmeyer, Adrian Brink, Sean Wasserman

Project Title: Radiological predictors of PCP in HIV-positive adults admitted to a tertiary hospital in South Africa: a retrospective cohort study (RadPredict)
Reference Number: PR2113679

Research Application Type(s):
1. Request for Data

RE: APPROVAL LETTER: REQUEST TO ACCESS NHLS RESOURCES FOR RESEARCH PURPOSES

This letter serves to advise that the application requesting permission to conduct the above-mentioned research using the listed NHLS resources has been reviewed and **"Approved"**. Please note that the approval is granted on the condition that you comply with the NHLS Research Material and Data Access Policy and requirements stated below.

1. All material and data requested shall be used as per the research protocol submitted to the NHLS and as approved by the relevant Health Research Ethics Committee (HREC) in South Africa.
2. Access to the NHLS material and/or data shall be limited to the minimum required for successful completion of the approved study and shall be made available ***without patient names and other patient identifiers (including, but not limited to, national identity numbers, hospital/clinic file numbers, addresses and telephone numbers)***.
3. Confidentiality shall be maintained at the participant and institutional level and there shall be no disclosure of personal information or confidential information.
4. Data and/or material shall not be shared with other parties unless approved by the NHLS
5. The material and/or data obtained from the NHLS shall be anonymised and not, for any reason, be used to track or recruit patients as no pre-approval/consent is obtained from patients.
6. Processes shall be discussed with the relevant NHLS departments (i.e. Corporate Data Warehouse (CDW), NHLS Laboratory Management, Operations Office, etc.) and agreed upon.
7. Any amendments to the study requirements, including the use of the material and/or data for purposes not initially disclosed to the NHLS) shall be cleared by an approved HREC and submitted to the NHLS for approval via the AARMS system – https://aarms.nhls.ac.za.
8. The NHLS shall be acknowledged as a source of material and/or data in any output, such as abstracts and journal articles, emanating from the project.
9. A final report of the research study and any published output resulting from this study shall be submitted to the NHLS via AARMS

Please note that this letter constitutes approval by the NHLS Academic Affairs and Research Office. The NHLS entities tasked with providing the material and/data may have additional requirements for access. Data related queries may be directed to NHLS CDW, email: zarina.sabat@nhls.ac.za; contact number: 011 386 6074 and sample related queries (if applicable) shall be directed to the relevant business manager.

Dr Babatyi Malope-Kgokong
National Manager: Academic Affairs and Research

Appendix C: Instructions to the Author from Clinical Infectious Diseases

Instructions to Authors for publication in Clinical Infectious Diseases are available at:
https://academic.oup.com/cid/pages/General_Instructions