INT J TUBERC LUNG DIS 12(3):S54–S62
TB-HIV SUPPLEMENT

Pulmonary tuberculosis among patients visiting a voluntary confidential counseling and testing center, Cambodia

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SUMMARY

SETTING: Voluntary counseling and confidential testing center (VCCT), Battambang District, Cambodia.

OBJECTIVES: To determine newly diagnosed pulmonary tuberculosis (PTB) prevalence and predicting factors, and assess the utility of TB-related symptoms and yield of sputum microscopy and culture.

DESIGN: Cross-sectional survey using interview, sputum smears and cultures and human immunodeficiency virus (HIV) testing.

RESULTS: Of 496 participants, 29 (5.8%) had culture-confirmed PTB while 19 (65.5%) were acid-fast bacilli (AFB) smear-positive. PTB prevalence was higher ($P <$ 0.001) in HIV-positives (20/124, 16.1%) than in HIV-negatives (9/372, 2.4%). On multivariable analysis, being HIV-positive, underweight (body mass index <18.5 kg/m^2), rapid weight loss and age ⩾35 years were predictors of PTB. Fever (93%) and hemoptysis (86%) had the highest sensitivity and specificity, respectively. The symptom complex of rapid weight loss, fever and hemoptysis detected all PTB cases (sensitivity 100%). Examination of three sputum smears with culture of the first sample detected 95% (19/20) of the HIV-associated PTB cases and 90% (26/29) overall.

CONCLUSIONS: TB is common in the VCCT setting, regardless of HIV status. The high prevalence of HIV and PTB among the participants warrants consideration of TB screening for all HIV suspects. Such screening through VCCT is feasible. Adding a single culture test to the evaluation of an initial sputum sample set will substantially increase case detection.

KEY WORDS: pulmonary tuberculosis predictors; HIV; sputum yield; Cambodia

CAMBODIA is among the world's high-burden countries for tuberculosis (TB).[1] Cambodia's National TB Program (NTP) operates under the National Center for Tuberculosis and Leprosy Control (CENAT). With a policy of decentralization, the NTP is administered through a provincial TB program office, district TB units and health centers.

The estimated annual incidence and prevalence of sputum smear-positive TB are 225 and 426 per 100 000 population, and those of TB all forms are respectively 508 and 762/100 000.[1,2] Human immunodeficiency virus (HIV) prevalence among persons aged 15–49 years was 1.9% in 2003.[3] The prevalence of latent TB infection (LTBI) among the general population is estimated at 64%.[4] A national TB-HIV prevalence survey estimated HIV seroprevalence among TB patients at 10% in 2005.[5]

The present survey was undertaken with the main objective of assessing the prevalence of newly diagnosed pulmonary TB (PTB) among all individuals visiting the community voluntary confidential counseling and testing (VCCT) center of the Battambang Provincial AIDS Office (PAO), regardless of HIV status. Other objectives were to determine predicting factors for PTB, to assess the operating characteristics of symptoms related to PTB and to determine the yield of sequential sputum microscopy and culture in diagnosing PTB.

STUDY POPULATION AND METHODS

Battambang Province is one of 22 provinces in Cambodia, with a population of 923 791. This cross-sectional survey was conducted in the main VCCT center located in Battambang District (population 300 573) during the period March-September 2005. The study was approved by the University of Alabama at Birmingham Institutional Review Board, the CENAT and the National Center for HIV/AIDS, Dermatology and STDs (NCHADS), Cambodia.

Subject recruitment and data collection

All subjects aged ⩾19 years who received routine pre-test counseling and underwent HIV testing at the VCCT

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center were eligible. Of 1220 subjects tested and referred for TB screening, 583 (48%) approached the study team located in the same building. Of these, 79 (14%) refused to participate in screening. An additional eight were excluded from the analysis: six (TB patients) were already on treatment for multidrug-resistant tuberculosis and two did not submit any sputum sample (Figure 1). The final sample size for analysis was thus 496. None of the subjects were on antiretroviral treatment (ART).

After providing informed consent, subjects were interviewed by the trained staff of the provincial TB program office. The transportation cost of participants to visit the center for sputum collection was covered; no other incentives or enablers were offered. The interview elicited information on general demographics, current symptoms related to TB and HIV/acquired immune-deficiency syndrome (AIDS) and previous TB disease and treatment. Rapid weight loss was defined as weight loss over weeks to months as self-reported by the participants. After the interview, each subject was requested to submit three sputum samples (for smear microscopy and culture examination), regardless of the presence or absence of TB symptoms; no chest X-ray was performed due to non-availability. The first sample was a spot specimen (post-interview), the second was an early-morning (home) sample and the third sample was a spot specimen.

The labeled specimens were examined at the Pasteur Institute of Cambodia. All positive smears on initial fluorochrome staining were confirmed by the Ziehl-Neelsen method for acid-fast bacilli (AFB).[6] Upon liquefaction and decontamination, all samples were cultured on Löwenstein-Jensen media regardless of smear status. Subjects with positive smear results were informed within 1 week and referred to a government referral hospital for free anti-tuberculosis treatment. Culture results were obtained within 8–10 weeks, but patients could be started on empirical TB treatment based on clinical judgment.

All subjects who were initially HIV-positive by the Determine HIV test (Abbott Laboratory, Weisbaden, Germany) were confirmed by the Uni-Gold™ test (Trinity Biotech Plc, Wicklow, Ireland). The CD4 count was measured using a Fast Count machine (Becton-Dickinson, Sparks, MD, USA). HIV-positive individuals were referred for enrollment and further evaluation at a government referral hospital or other facility. Those without TB disease were considered for isoniazid preventive treatment (IPT).

A case of PTB was defined as any single sputum culture-positive for *Mycobacterium tuberculosis* or at least two sputum smears positive for AFB (AFB-positive); an AFB-positive subject culture-positive for mycobacteria other than tuberculosis (MOTT) was not considered a PTB case. In this study, none of the MOTT cases ($n = 4$) were AFB-positive.

Statistical analysis

The outcome of interest was categorized as presence/absence of PTB. Continuous variables were reported as mean (with standard deviation) and median (with interquartile range). Continuous variables were compared using Student's unpaired *t*-test or Mann-Whitney-

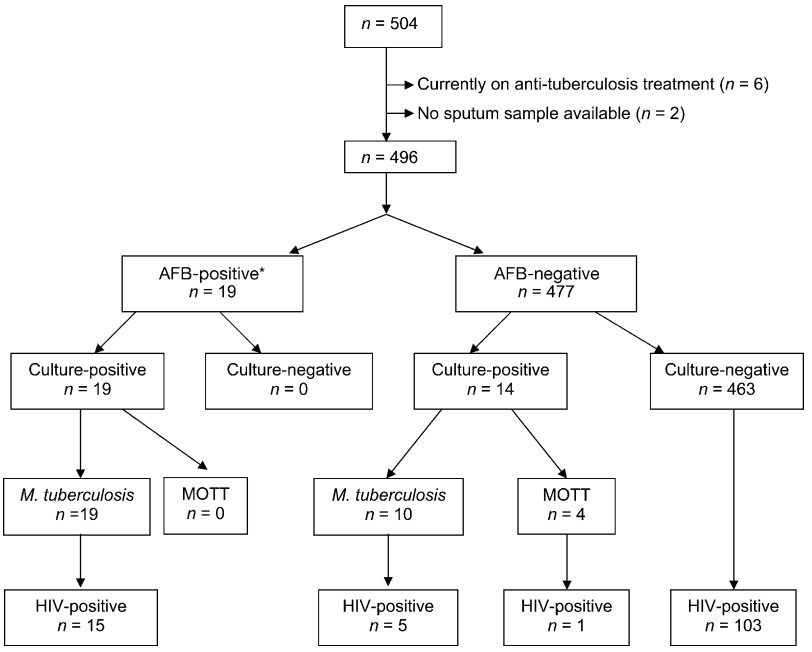

Figure 1 Flowchart of diagnostic outcomes and HIV status of the study participants. * AFB positive: at least two sputum samples smear-positive for AFB. AFB = acid-fast bacilli; MOTT = mycobacteria other than tuberculosis; HIV = human immunodeficiency virus.

Wilcoxon test (non-parametric test) for unpaired data. Categorical variables were reported as frequencies (with percentages) and the proportions were compared by Fisher's exact test.

Operating characteristics such as sensitivity, specificity, accuracy [(true-positives + true-negatives)/(true-positives + true-negatives + false-positives + false-negatives)], predictive values, likelihood ratios (LR) [(LR-positive = sensitivity/(1 − specificity); LR-negative = (1 − sensitivity) / specificity], and kappa (Cohen's)[7] were calculated. Multivariable regression analysis was done by logistic regression modeling. Odds ratios (ORs) were calculated with their 95% confidence intervals (CIs). Using 80% power and alpha = 0.05, the a priori sample size required to detect a statistically significant difference of PTB between HIV-positive and HIV-negative subjects was 423. Statistical significance was set at 0.05 (two-tailed). Analyses were conducted using SAS statistical software, version 9.1 (SAS Institute, Cary, NC, USA).

Table 1 Characteristics of study participants according to PTB status

Variable	PTB* (*n* = 29) *n* (%)	No PTB* (*n* = 467) *n* (%)	*P* value	Total (*N* = 496) *n* (%)
Age, years				
Mean (SD)	40.4 (12.9)	32.3 (9.4)		32.8 (9.8)
Median (IQR)	41 (31–48)	30 (25–38)	0.001†‡	31 (25–39)
Sex				
Men	18 (62.1)	289 (61.9)	1.00§	307 (61.9)
Women	11 (37.9)	178 (38.1)		189 (38.1)
Body mass index (kg/m^2)	(*n* = 29)	(*n* = 466)		(*n* = 495)
Mean (SD)	17.3 (2.7)	20.0 (2.8)	<0.001†¶	19.9 (2.8)
Median (IQR)	16.9 (15.8–18.5)	19.8 (18.3–21.5)		19.6 (18.1–21.4)
<18.5 (underweight)	21 (72.4)	126 (27.0)	<0.001†§#	147 (29.7)
18.5–24.9 (normal)#	8 (27.6)	313 (67.2)		321 (64.9)
⩾25–29.9 (overweight)#	—	27 (5.8)		27 (5.4)
HIV status				
Positive	20 (69.0)	104 (22.3)	<0.001†§	124 (25.0)
Negative	9 (31.0)	363 (77.7)		372 (75.0)
CD4 count (per mm^3)**	(*n* = 18)	(*n* = 90)		(*n* = 108)
Mean (SD)	58.3 (75.3)	230.4 (253.5)		200.3 (239.6)
Median (IQR)	30.5 (16–60)	136 (23–363)	0.01†‡	79 (23–325)
⩾200	1 (5.6)	38 (42.2)	0.003†§**	39 (36.1)
<200 and ⩾100	2 (11.1)	11 (12.2)		13 (12.0)
<100	15 (83.3)	41 (45.6)		56 (51.9)
TB symptom, any	29 (100)	345 (74.2)	<0.001§	374 (75.4)
Cough >3 weeks	17 (58.6)	177 (37.9)	0.03‡	194 (39.1)
Hemoptysis	6 (20.7)	63 (13.5)	0.27‡	69 (13.9)
Fever	27 (93.1)	268 (57.4)	<0.001‡	295 (59.5)
Loss of appetite	18 (62.1)	125 (26.8)	<0.001‡	143 (28.8)
Rapid weight loss††	24 (82.8)	156 (33.4)	<0.001‡	180 (36.3)
Night sweats	21 (72.4)	225 (48.2)	0.01‡	246 (49.6)
First symptom appeared, months	(*n* = 29)	(*n* = 345)		(*n* = 374)
<1	9 (31.0)	134 (38.8)	0.55§‡‡	143 (38.2)
⩾1 and <6	15 (51.7)	140 (40.6)		155 (41.4)
⩾6 to ⩽12	3 (10.3)	45 (13.0)		48 (12.8)
>12	1 (3.5)	20 (5.8)		21 (5.6)
Unknown	1 (3.5)	6 (1.7)		7 (1.9)
Any other symptoms/signs (HIV/AIDS)§§	19 (67.9)	197 (42.2)	0.01§	216 (43.6)
Diarrhea (>1 month)¶¶	3 (15.8)	35 (17.8)		38 (17.6)
Dysphasia¶¶	12 (63.2)	115 (58.4)		127 (58.8)
Skin rash (>1 month)¶¶	9 (47.4)	83 (42.1)		92 (42.6)
Lymph nodes (extra-inguinal)¶¶	11 (57.9)	51 (25.9)		62 (28.7)

* Any sputum culture-positive for *M. tuberculosis* or at least two sputum smears positive for AFB (AFB-positive). An AFB-positive subject with culture positive for MOTT was not considered as a PTB case. Four participants with culture positive for MOTT were included in the No PTB group; none of them were AFB-positive.
† Statistically significant.
‡ Non-parametric test (two-tailed).
§ Fisher's exact test (two-tailed).
¶ Student's unpaired *t*-test (two-tailed).
# 'Normal' and 'overweight' categories combined to calculate *P* value.
** Category ⩾200 compared with other categories to calculate *P* value; comparison of <100 vs. other; *P* = 0.004.
†† Rapid weight loss over weeks to months as reported by participant.
‡‡ Compared as <1 vs. ⩾1 and other; 'unknown' excluded to calculate *P* value.
§§ One of the PTB subjects for whom other symptoms/signs related to HIV/AIDS were not known is excluded.
¶¶ Percentage calculated out of *n* for the other symptoms/signs related to HIV/AIDS.
PTB = pulmonary tuberculosis; SD = standard deviation; IQR = interquartile range; HIV = human immunodeficiency virus; AFB = acid-fast bacilli; MOTT = mycobacteria other than tuberculosis.

RESULTS

General characteristics

The data of 496 subjects were analyzed (Figure 1). The median age was 31 years (Table 1); 61% of the subjects were aged <35 years, and 62% of the subjects were men. The sex ratio (women:men) among HIV-associated PTB subjects was 1:1.9, and for the overall study group it was 1:1.6 (Table 1). PTB subjects were significantly underweight compared with the non-PTB group (OR 7.1, 95%CI 3.1–16.4). Thirty one (6.2%) subjects had a prior history of TB treatment, and more of those in the PTB group had a history than in the non-PTB group (10% vs. 6%; $P = 0.46$).

PTB disease burden and HIV status

Of the 496 subjects, 29 (5.8%) had culture-confirmed PTB; four subjects positive for MOTT were not considered as PTB (Figure 1). PTB prevalence was higher in HIV-positives than HIV-negatives (16.1% vs. 2.4%; $P < 0.001$) (Table 1). Of the 29 PTB cases, 19 (65.5%) were AFB-positive (Figure 1). Of the 20 PTB cases among the HIV-positives, 15 (75%) were AFB-positive, compared with four of nine PTB cases (44%) among the HIV-negatives.

Among the HIV-positives, the CD4 count was significantly lower in the PTB group than in the non-PTB group (median 30.5 vs. 136.0, $P = 0.01$). The difference remained significant even after adjusting for age and sex. World Health Organization (WHO) clinical staging was available for 55 HIV-positives, nine of whom were PTB cases. Most subjects (46/55; 83.6%) were in Stage 3 (8/9 PTB cases were Stage 3 and 1/9 Stage 4). The remaining nine subjects were in Stages 1, 2 and 4 (three each). All three subjects in Stage 1 had a CD4 count >300/mm^3. The six subjects in Stages 2 and 4 had CD4 counts <125/mm^3. Thirty (68.1%) subjects in Stage 3 had a CD4 count <200/mm^3. All but one PTB subject had a CD4 count ⩽60/mm^3.

Multivariable regression analysis

In bivariate analysis, age ⩾35 years, body mass index (BMI) <18.5, HIV status, cough >3 weeks, fever, loss of appetite, rapid weight loss, night sweats and other symptoms/signs related to HIV/AIDS were significantly associated with PTB (Table 2). For multivariable logistic regression, we included only those factors for which the P value in bivariate analysis was <0.25. Age ⩾35 years, BMI <18.5, HIV positive status and (reported) rapid weight loss (over weeks to months) remained significant predictors of PTB. Addition of the interaction term 'HIV*age' to the model did not show statistical significance ($P = 0.41$).

Utility of symptoms

Two thirds of the subjects had one or more symptoms suggestive of PTB (Table 1). All the subjects in the PTB group had one or more symptom of PTB as compared to 74% in the non-PTB group ($P < 0.001$) (Table 1). Similarly, HIV-positives were more likely to have symptoms than HIV-negatives (84% vs. 73%, $P = 0.01$) (data not shown). Duration from onset of first symptom to day of interview was less than a month in 38% of the subjects (PTB 31% vs. non-PTB 39%, $P = 0.55$). Duration of symptoms ⩾1 month was longer in HIV-positives than in HIV-negatives (OR 1.8, 95% CI 1.1–3.0, $P = 0.02$) (data not shown). Also, PTB subjects were more likely to have HIV/AIDS-related symptoms than non-PTB subjects (OR 2.9, 95%CI 1.3–6.5, $P = 0.01$) (Table 1). A similar difference was

Table 2 Crude and adjusted odds ratios (with 95% confidence intervals and *P* values) for various factors predicting PTB

Predicting factor	Crude OR (95%CI)*	*P* value*	Adjusted OR† (95%CI)*	*P* value*
Age, years: ⩾35 vs. <35‡	3.5 (1.5–7.9)	0.003§	2.7 (1.1–6.5)	0.03§
Sex: men vs. women‡	0.9 (0.4–2.1)	0.90	—	—
BMI:¶ <18.5 vs. ⩾18.5‡	6.7 (2.9–15.7)	<0.001§	3.2 (1.3–8.1)	0.01§
HIV: positive vs. negative‡	7.8 (3.4–17.5)	<0.001§	4.0 (1.5–10.4)	0.004§
Cough >3 weeks: yes vs. no‡	2.3 (1.1–5.0)	0.03§	1.2 (0.5–2.9)	0.74
Hemoptysis: yes vs. no‡	1.7 (0.7–4.3)	0.28	—	—
Fever: yes vs. no‡	10.0 (2.4–42.6)	0.002§	2.9 (0.6–14.9)	0.21
Loss of appetite: yes vs. no‡	4.5 (2.1–9.7)	<0.001§	1.5 (0.5–3.9)	0.45
Rapid weight loss:# yes vs. no‡	9.6 (3.6–25.6)	<0.001§	3.7 (1.1–12.6)	0.03§
Night sweats: yes vs. no‡	2.8 (1.2–6.5)	0.01§	0.8 (0.3–2.4)	0.73
Other symptoms/signs:** yes vs. no‡	2.9 (1.3–6.5)	0.01§	1.0 (0.4–2.6)	0.94
Prior TB treatment: yes vs. no‡	1.8 (0.5–6.3)	0.36	—	—

* Wald confidence interval and χ^2 *P* value.
† Adjusted ORs calculated using multivariable logistic regression analysis.
‡ Reference category.
§ Statistically significant.
¶ BMI <18.5 = underweight, ⩾18.5 = normal/overweight. None of the participants were obese.
# Rapid weight loss over weeks to months as reported by participant.
** Any of the other symptoms/signs related to HIV/AIDS (chronic diarrhea, dysphasia, skin rash or extra-inguinal lymph nodes).
PTB = pulmonary tuberculosis; OR = odds ratio; CI = confidence interval; BMI = body mass index; HIV = human immunodeficiency virus; TB = tuberculosis.

observed for HIV-positives vs. HIV-negatives (66% vs. 36%, $P < 0.001$) (data not shown).

Among the symptoms, fever had the highest sensitivity (93%), while hemoptysis had the highest specificity (86%) (Table 3). Sensitivities for fever, loss of appetite, rapid weight loss and night sweats were higher in HIV-positives than HIV-negatives. In Table 4, selected combinations of significant factors from the multivariable model (Table 2) and most significant symptom complex are presented. The sensitivity of the symptom complex of either fever or hemoptysis or rapid weight loss was 100%, which was independent of HIV status (Table 4, #7). Thus, the probability of an individual with PTB not having a history of fever, hemoptysis or rapid weight loss was nil (LR-negative = zero). Similar conclusions can be made wherever sensitivity is equal to 100% and therefore LR-negative = zero (e.g., Table 4, complex #2). The specificity of all TB-related symptoms in HIV-positives was lower than that in HIV-negatives, except for hemoptysis. Overall, the accuracy of symptoms was lower in HIV-positives than HIV-negatives. As predictive values are affected by disease prevalence, LRs are reported. Using either a symptom or variable complex, it was easier to 'rule out' (LR-negative) PTB rather than 'ruling in' (LR-positive) PTB.

Yield of sputum microscopy and culture

Of the 496 subjects, 469 (95%) submitted at least two sputum samples, and 430 (87%) submitted three samples for examination. Including only the 430 subjects

Table 3 Operating characteristics of individual symptoms and predicting factors of PTB stratified by HIV status

	Operating characteristics							
Variable	Sensitivity %*	Specificity %†	PPV %‡	NPV %§	Accuracy %¶	LR-positive#	LR-negative**	Kappa %
Symptoms								
Cough >3 weeks	59	62	9	96	62	1.6	0.7	6
HIV-positive	55	59	20	87	58	1.3	1.0	8
HIV-negative	67	63	4	99	63	1.8	0.5	4
Hemoptysis	21	86	9	95	83	1.5	0.9	4
HIV-positive	15	86	18	84	75	1.1	1.0	2
HIV-negative	33	86	6	98	85	2.5	0.8	6
Fever	93	43	9	99	46	1.6	0.2	7
HIV-positive	100	30	21	100	41	1.4	0.0	12
HIV-negative	78	46	3	99	47	1.4	0.5	2
Loss of appetite	62	73	13	97	73	2.3	0.5	12
HIV-positive	65	57	22	89	58	1.5	0.6	12
HIV-negative	56	78	6	99	77	2.5	0.6	6
Rapid weight loss	83	67	13	98	67	2.5	0.3	14
HIV-positive	90	42	23	96	50	1.6	0.2	15
HIV-negative	67	74	6	99	73	2.5	1.0	7
Night sweats	72	52	8	97	53	1.5	0.5	5
HIV-positive	85	35	20	92	43	1.3	0.4	8
HIV-negative	44	57	2	98	56	1.0	1.0	0.1
Any of the above	100	26	8	100	30	1.3	0.0	4
HIV-positive	100	19	19	100	32	1.2	0.0	7
HIV-negative	100	28	3	100	30	1.4	0.0	2
'Significant' factors††								
Age ⩾35 years	65	62	10	97	62	1.7	0.5	8
HIV-positive	65	47	19	87	50	1.2	0.7	6
HIV-negative	67	67	5	99	67	2.0	0.5	4
BMI <18.5‡‡	72	73	14	98	73	2.7	0.4	16
HIV-positive	70	61	26	91	63	1.8	0.5	19
HIV-negative	78	76	7	99	76	3.3	0.3	10
HIV-positive	69	78	16	98	77	3.1	0.4	18

Note: The values in the first row of the variable are of the overall group.
'Gold standard' = PTB case (any single culture positive for *Mycobacterium tuberculosis* or at least two sputum smears positive for acid-fast bacilli).
* Sensitivity = true positives/(true positives + false negatives).
† Specificity = true negatives/(true negatives + false positives).
‡ PPV = true positives/(true positives + false positives).
§ NPV = true negatives/(true negatives + false negatives).
¶ Accuracy = (true positives + true negatives)/(true positives + true negatives + false positives + false negatives).
# Positive LR = sensitivity/(1 − specificity).
** Negative LR = (1 − sensitivity)/specificity.
†† From the multivariable logistic regression modeling (Table 2). One of the four significant factors, 'rapid weight loss', is presented along with the 'symptoms'.
‡‡ BMI = kg/m^2. Rapid weight loss over weeks to months as reported by participant.
PTB = pulmonary tuberculosis; HIV = human immunodeficiency virus; PPV = positive predictive value; NPV = negative predictive value; LR = likelihood ratio; BMI = body mass index.

Table 4 Operating characteristics of various combinations of symptoms and predicting factors of PTB

Symptoms/variables complex	Operating characteristics							
	Sensitivity* %	Specificity† %	PPV‡ %	NPV§ %	Accuracy¶ %	Positive LR#	Negative LR**	Kappa %
'Significant' factors††								
1 Age‡‡/BMI§§/weight loss/HIV	100	32	8	100	36	1.5	0.0	5
2 Age‡‡/BMI§§/weight loss	100	35	9	100	38	1.5	0.0	6
HIV-positive	100	12	18	100	26	1.1	0.0	4
HIV-negative	100	41	4	100	43	1.7	0.0	3
3 Age‡‡/BMI§§/HIV	100	40	9	100	44	1.7	0.0	7
4 Age‡‡/weight loss	97	43	10	99	46	1.7	0.1	8
HIV-positive	100	17	19	100	31	1.2	0.0	6
HIV-negative	89	50	4	99	51	1.8	0.2	4
5 BMI§§/HIV	93	59	12	99	61	2.3	0.1	13
6 Weight loss/HIV	90	57	12	99	59	2.1	0.2	11
Symptom complex								
7 Fever/hemoptysis/weight loss (overall)	100	35	9	100	39	1.5	0.0	6
HIV-positive	100	20	19	100	33	1.2	0.0	7
HIV-negative	100	40	4	100	41	1.7	0.0	3

'Gold standard' = PTB case (any single culture positive for *M. tuberculosis* or at least two sputum samples smear-positive for AFB).
* Sensitivity = true positives/(true positives + false negatives).
† Specificity = true negatives/(true negatives + false positives).
‡ PPV = true positives/(true positives + false positives).
§ NPV = true negatives/(true negatives + false negatives).
¶ Accuracy = (true positives + true negatives)/(true positives + true negatives + false positives + false negatives).
# Positive LR = sensitivity/(1 − specificity).
** Negative LR = (1 − sensitivity)/specificity.
†† From multivariable logistic regression modeling (Table 2).
‡‡ Age ⩾35 years.
§§ BMI < 18.5 = underweight.
PTB = pulmonary tuberculosis; PPV = positive predictive value; NPV = negative predictive value; LR = likelihood ratio; BMI = body mass index; HIV = human immunodeficiency virus; AFB = acid-fast bacilli.

would have excluded four PTB cases (culture-positive *M. tuberculosis*) from analysis. The yield of sputum and culture was greater in the HIV-associated PTB cases ($n = 20$) than in the overall PTB group (Figure 2). Nineteen (65.5%) of the 29 PTB cases were AFB-positive; all were culture-positive for *M. tuberculosis* (Figures 1 and 2). Therefore, 34.5% of the cases would not have been diagnosed without culture. The sensitivity of either of the first two sputum samples being smear-positive was equal to any of the three sputum-smears being positive (76%, 22/29) (Figure 2). The sensitivity of either of first two positive smears or first positive culture was 90% (26/29); the addition of culture of the second sample added one more case, increasing the sensitivity to 93% (27/29). Thus, smear microscopy and culture of the first two samples detected 93% of the cases. The two cases that could not be detected on the first two samples (smear and/or culture) had a third sputum sample culture-positive for *M. tuberculosis*; the third smear in these two cases was also negative for AFB.

DISCUSSION

Cambodia remains a high TB burden country with a large reservoir of latently infected individuals, estimated at 64%.[4] Despite recent declines, its HIV rate remains one of the highest reported in Asia.[8] In Battambang Province, the estimated TB-HIV co-infection rate is 23%, twice the national figure.[5] The decentralized NTP infrastructure is therefore challenged at the operational level to devise a TB screening and detection strategy. Evidence is needed to assist the Ministry of Health and key stakeholders in mapping future directions for the continuum of care strategy for HIV-infected persons.

The potential contribution of VCCT centers toward TB control has been considered,[9,10] and the utility of screening for TB in such centers has been previously documented outside of Asia.[10–12] No study, however, has prospectively evaluated serial cultures for diagnosis along with the sensitivity, specificity and predictive values of signs/symptoms of TB and HIV/AIDS and compared HIV-infected with non-HIV-infected subjects. The government-run VCCT center chosen as the study site was the first established in the region and is located away from the referral hospital. It is accessed by organizations referring patients from several districts and the military. Despite the number of subjects who declined to be studied, the sample size was sufficient to assess issues key to understanding PTB in the region, simultaneously among HIV-infected and non-infected subjects.

The proportion of subjects accessing VCCT with TB symptoms is high and the duration of symptoms was longer in the HIV-infected cases. All PTB patients reported symptoms; 74% of those without PTB did so also. As extra-pulmonary TB was not assessed, it is probable that such cases were among the non-PTB group and likely had symptoms. However, focusing

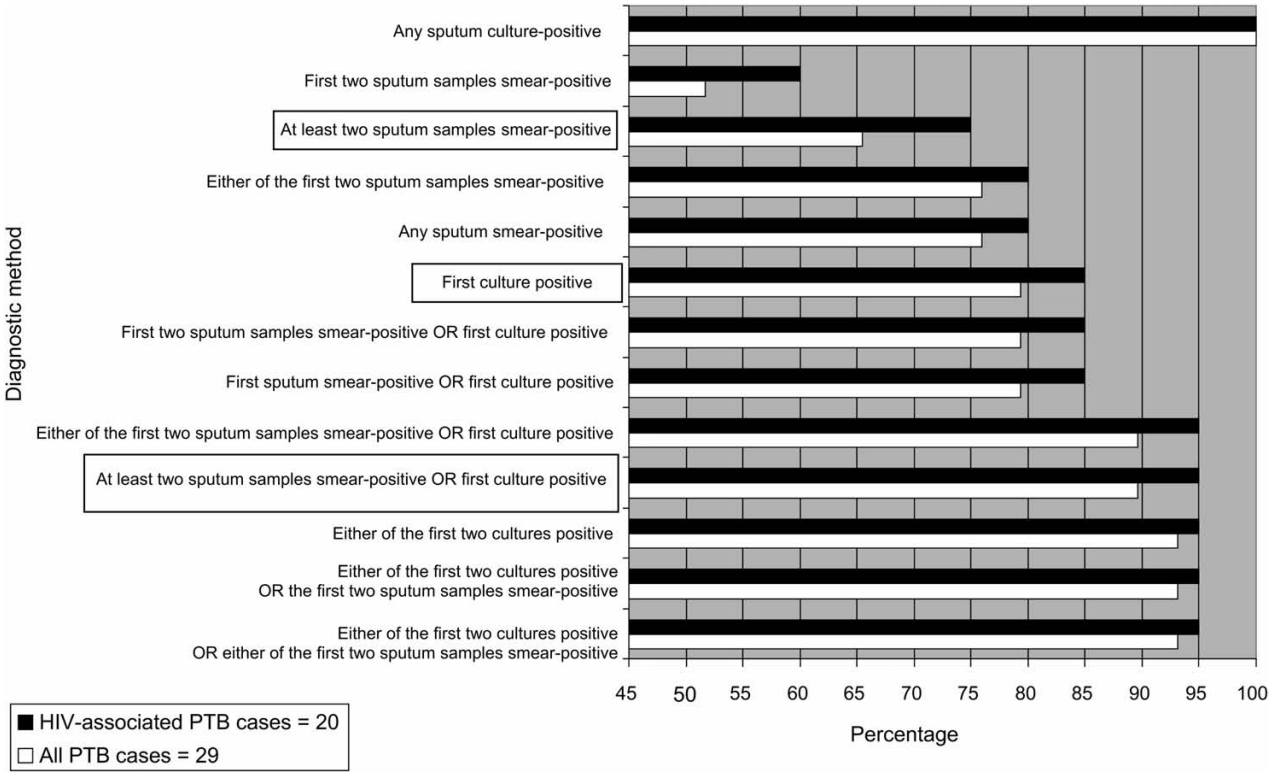

Figure 2 Yield of sputum smear microscopy and culture in all PTB cases (n = 29) and in HIV-associated PTB cases (n = 20). HIV = human immunodeficiency virus; PTB = pulmonary tuberculosis.

on the detection of infectious TB, regression modeling demonstrates that a history of rapid weight loss and a low BMI (underweight) predict PTB. Rapid weight loss was also found to be an important screening tool for PTB elsewhere among advanced HIV-infected patients.[13] An analysis of symptoms and significant factors (Tables 3 and 4) demonstrates varying predictive values according to HIV status. While pulmonary-related symptoms (cough, hemoptysis) are more sensitive indicators of disease in HIV-negative cases, systemic symptoms are more sensitive indicators in HIV-positive cases. Overall, sensitivity of cough in this setting may have been low due to the cut-off point of 3 weeks used for screening. Assessment of symptom accuracy, however, shows each one to be less accurate for HIV-infected cases. An analysis of various 'complexes' of symptoms and variables (Table 4) demonstrates their possible utility for health workers when comparing the sensitivity of these complexes to the significant variables identified in the multivariable model. The complex of 'fever, hemoptysis or weight loss' yields a sensitivity equally as high as the model for all patients. Knowledge of HIV status is not required for this determination. Our results differed from other studies,[14–17] which likely reflect different care settings, populations and screening methods.

Although the causes of smear-negative PTB using standard AFB staining methods in a high HIV setting have been investigated, serial sputum cultures were not used.[18] The results found in this prospective study, using three sputum smear and culture examinations, are informative. The high proportion of smear-positive cases for the entire cohort could reflect the advanced stage of TB disease and/or the high sensitivity of fluorescence microscopy in well-trained hands, even in resource-poor settings.[19,20] Similarly high yields using fluorochrome-based staining have been reported in retrospective analyses of hospital-based cohorts in developed settings.[21,22] The addition of a first (spot) sputum culture to three routine smear examinations increases the confirmed diagnostic yield in the current study by 37% among all cases and by 27% among HIV-positive cases. Single-spot sputum cultures have been used successfully elsewhere, including among HIV-infected patients.[21,23] In settings without access to fluorescence microscopy, the benefit of culture will likely be more substantial.

In conclusion, TB screening initiated through VCCT is feasible and important in detecting a large number of previously undiagnosed PTB cases. Such active case finding may avert death and worsening morbidity if access is given to prompt treatment. The high prevalence of PTB among the HIV-negative subjects indicates that some confusion may exist between the two diseases, with many subjects seeking HIV testing thinking that they are HIV-infected, unaware of a possible TB diagnosis. Such confusion, in the context of high TB disease rates, highlights the need for community education and implementation of infection control measures in VCCT centers which are potential

high-risk settings for TB transmission. Finally, with the new 2006–2015 WHO Global Plan[24] and its emphasis on intensified case finding and enhanced laboratory infrastructures, adding a single culture test to the evaluation of an initial sputum sample set will substantially increase case detection. The feasibility of performing culture, however, depends on expanding existing laboratory infrastructures, which is occurring in some settings.

Acknowledgements

The authors thank the patients and the staff of the provincial AIDS and TB offices for their participation. They also thank Dr K Sok, Deputy Director of Battambang Provincial Health Department, Dr M T Eang, Director of CENAT, Dr M C Vun, Director of NCHADS, and Mr E Chanthol from Family Health International, Phnom Penh, Cambodia for their invaluable support to this study. They are grateful to Dr D-T Chen from the University of Alabama at Birmingham for his consultation regarding biostatistical issues.

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RÉSUMÉ

CONTEXTE : Centre d'accompagnement librement consenti et de test confidentiel (VCCT), district de Battambang, Cambodge.

OBJECTIFS : Déterminer la prévalence de la tuberculose pulmonaire (TBP) nouvellement diagnostiquée et ses facteurs prédictifs, et évaluer l'utilité des symptômes liés à la TB et le rendement de l'examen microscopique et de la culture des crachats.

SCHÉMA : Enquête transversale utilisant l'interview, les frottis et les cultures de crachats ainsi que le test pour le virus de l'immunodéficience humaine (VIH).

RÉSULTATS : Sur 496 participants, 29 (5,8%) souffrent d'une TBP confirmée par la culture ; 19 (65,5%) ont une bacilloscopie positive des frottis. La prévalence de la TBP est plus élevée ($P < 0,001$) chez les sujets séropositifs pour le VIH (20/124 ; 16,1%) que chez les séronégatifs (9/372 ; 2,4%). A l'analyse multivariée, le fait d'être séropositif pour le VIH, d'avoir un poids insuffisant (indice de masse corporelle $<18,5$ kg/m^2), une chute rapide de poids et un âge $\geqslant 35$ ans sont des facteurs prédictifs de la TBP. La sensibilité la plus élevée concerne la fièvre (93%) et la spécificité l'hémoptysie (86%). Le complexe de symptômes chute rapide de poids avec fièvre et hémoptysie a détecté l'ensemble des cas de TBP (sensibilité 100%).

L'examen de trois frottis de crachats avec culture du premier échantillon a détecté 95% des cas de TBP associés au VIH (19/20) et 90% de l'ensemble (26/29).
CONCLUSIONS : Dans le contexte d'un centre de VCCT, la TB est courante quel que soit le statut VIH. La prévalence élevée du VIH et de la TBP parmi les sujets justifie la prise en compte d'un dépistage de la TB chez tous les suspects de VIH. Un tel dépistage est réalisable au travers du VCCT. L'addition d'un simple test de culture à l'évaluation de l'ensemble des échantillons initiaux de crachats augmentera de manière substantielle la détection des cas.

RESUMEN

MARCO DE REFERENCIA : El centro de orientación y prueba diagnóstica voluntarias y confidenciales (VCCT) en el distrito de Battambang, Camboya.
OBJETIVOS : Determinar la prevalencia y los factores de predicción de los casos nuevos de tuberculosis pulmonar (TBP) y evaluar la utilidad diagnóstica de los síntomas asociados con la TB y el rendimiento de la baciloscopia y el cultivo del esputo.
MÉTODOS : Estudio transversal con entrevista, baciloscopia y cultivo del esputo y prueba diagnóstica del virus de inmunodeficiencia humana (VIH).
RESULTADOS : De los 496 participantes, en 29 (5,8%) el cultivo confirmó el diagnóstico de TBP y 19 (65,5%) tuvieron baciloscopia positiva. La prevalencia de TBP fue más alta en los pacientes con serología positiva para el VIH (20/124 ; 16,1%) que en los pacientes negativos (9/372 ; 2,4% ; $P < 0{,}001$). En el análisis multifactorial, se definieron como variables independientes de TBP el bajo peso (índice de masa corporal $<18{,}5$ kg/m^2), la rápida pérdida de peso y la edad de $\geqslant 35$ años. La fiebre demostró la más alta sensibilidad (93%) y la hemoptisis la mayor especificidad (86%). Con la asociación de rápida pérdida de peso, fiebre y hemoptisis se detectaron todos los casos (sensibilidad 100%). El examen de tres muestras de esputo con cultivo de la primera muestra detectó 95% de los casos de TBP asociada con la infección por el VIH (19/20) y 90% de todos los casos (26/29).
CONCLUSIÓN : La TB es frecuente en los VCCT del VIH, independientemente del estado de la serología. La alta prevalencia de infección por el VIH y TB en los usuarios de estos centros justifica considerar la detección sistemática de la TB en todos los individuos con presunción de infección por el VIH. Tal estrategia es factible en dichos centros. La realización de un cultivo en la valoración inicial de cada muestra de esputo aumentaría considerablemente la detección de casos de TB.