Isolation and deisolation of patients admitted with presumptive pulmonary tuberculosis

Can it be shortened?

Fatehi E. Elzein, *FRCP, MScCTM,* ***Nisreen Alsherbeeni,*** *FRCP,* ***Mohammed Mursi,*** *MBBS,* ***Shoug F. Algoblan,*** *MBBS,* ***Abuzaid A. Abuzaid,*** *MBBS, Msc,* ***Ali M. Albarrak,*** *MD, FRCPC.*

ABSTRACT

الأهداف: تحديد العدد المثالي للطاخات عصيات البلغم الحمضية السريعة (AFB) وتفاعل سلسلة البوليميريز (PCR) اللازم لإيقاف عزل داء السل للمرضى الذين يشتبه بإصابتهم بالسل الرئوي.

المنهجية: كانت هذه دراسة مركزية بأثر رجعي قائمة على جميع المرضى الذين تم تشخيصهم مع مرض السل الرئوي المثبت خلال الفترة 2010م و 2018م. وقد أجريت هذه الدراسة في مدينة الأمير سلطان الطبية العسكرية (PSMMC) في الرياض، المملكة العربية السعودية، وهو مركز رعاية طبية من الدرجة الثالثة يتكون من 1،200 سرير. تم الحصول على البيانات من سجلات إشعار السل لدينا. فحصنا المرضى الذين لديهم لطاخات السل الإيجابية، أدرجت فقط 3 مسحات البلغم ل AFB في التحليل. أدرجت أيضا نتائج PCRللسل المتفطرة (MTB). في الدراسة، تم تقييم العائد التدريجي للمسحات الثانية والثالثة.

النتائج: بشكل عام، كانت مزرعة MTB إيجابية لعدد 240 مريضا. وكانت اللطخة والمزرعة إيجابية في 126 (52.5%) من المرضى، في حين أن 114 مريض كانت اللطخة سلبية والمزرعة إيجابية. من بين 126 مريضًا كانوا مصابين بالالتهاب الأذينيAFB ، تم اكتشاف 98 (77.8%) في العينة الأولى، و13(10.3%) في العينة الثانية، و 9 فقط (7.1%) في العينة الثالثة. كان تفاعل البلمرة المتسلسل لـ MTBإيجابيا في 122(96.8%) من المرضى. أربعة مرضى لم يخضعوا لاختبار PCR.

الخلاصة: اكتشف اختبار Xpert MTB/ المقاوم لريفامبيسين جميع المسحات الإيجابية للمرضى، بينما لم تسهم المسحة الثالثة بشكل كبير في عزل MTB.

Objectives: To determine the ideal number of sputum acid-fast bacilli (AFB) smears and polymerase chain reaction (PCR) required for discontinuing tuberculosis (TB) isolation among patients with suspected pulmonary TB.

Methods: This was a single-center, record-based retrospective study of all admitted patients diagnosed with culture-proven pulmonary TB between 2010 and 2018. The study was conducted at Prince Sultan Military Medical City (PSMMC) in Riyadh, Saudi Arabia, a large tertiary care center consisting of 1,200 beds. Data were obtained from our TB notification records. Patients with smear-positive TB were investigated. Only the first 3 sputum smears for AFB were included in the analysis. The PCR results for *Mycobacterium tuberculosis* (MTB) were also included in the study. The incremental yield of the second and third smears was assessed.

Results: Overall, 240 patients were MTB-culture positive. A total of 126 (52.5%) patients were smear and culture positive, whereas 114 were culture positive but smear negative. Of 126 patients who were AFB smear positive, 98 (77.8%) were detected in the first specimen, 13 (10.3%) in the second specimen, and only 9 (7.1%) in the third specimen. Polymerase chain reaction for MTB was positive in 122 (96.8%) smear-positive patients. Four patients did not undergo a PCR test.

Conclusion: A single Xpert MTB/resistance to rifampicin test detected all smear-positive patients, whereas the third smear did not significantly contribute to MTB isolation.

Saudi Med J 2019; Vol. 40 (10): 1008-1012
doi: 10.15537/smj.2019.10.24564

From the Infectious Diseases Unit (Elzein, Alsherbeeni, Mursi, Algoblan, Albarrak), Prince Sultan Military Medical City and from the Ministry of Health (Abuzaid), Riyadh, Kingdom of Saudi Arabia.

Received 21st May 2019. Accepted 15th September 2019.

Address correspondence and reprint request to: Dr. Fatehi E. Elzein, Infectious Diseases Unit, Prince Sultan Military Medical City, Riyadh, Kingdom of Saudi Arabia. E-mail: fatehielzein@gmail.com
ORCID ID: http://orcid.org/0000-0002-0465-3004

Tuberculosis (TB) infection remains a leading cause of morbidity and mortality worldwide, with an estimated 10.0 million people affected with TB disease in 2017, whereas an estimated 1.3 (range, 1.2-1.4) million deaths occurred among the human immunodeficiency virus (HIV)-negative population. An annual TB incidence rate of 10 (8.6-12)/100,000 population has been reported by the World Health Organization (WHO) in Saudi Arabia.[1] The "WHO End TB" strategy stresses on the need to prevent TB infection in settings with high risk of transmission including healthcare facilities.[2] Early suspicion and placing individuals with presumptive TB in an airborne infection isolation (AII), ideally using single-patient and negative-pressure ventilation rooms, are essential components of a hospital TB program. Furthermore, the current Center for Disease Control and Prevention (CDC) guidelines for the prevention of *Mycobacterium tuberculosis* (MTB) transmission in the healthcare setting recommended administrative controls that aim to reduce exposure to individuals with presumptive pulmonary TB and respiratory protection controls using disposable N95 respirators by healthcare workers.[3] Three respiratory specimens collected 8 to 24 hours apart, with at least 1 early morning specimen, should be sent to the laboratory for acid-fast bacilli (AFB) microscopy and culture. Isolation can be discounted if 3 smears are negative for AFB. However, AII is costly, scarce, or unavailable in many hospitals. The Middle East respiratory coronavirus (MERS-CoV) outbreak in the Arabian Peninsula has increased the demand for such AII rooms in countries like Saudi Arabia. Moreover, a systematic review identified a negative impact of isolation on the patients' mental health, whereas patients' safety and satisfaction were also unfavorably affected. In addition, few studies also found that the contact time between healthcare workers and isolated patients decreased.[4] Thus, measures for decreasing isolation time are needed. On the contrary, nosocomial outbreaks with sensitive, and more alarmingly, MDR TB have has been previously reported.[5,6] Consequently, a balance should be achieved to prevent disease transmission in health facilities.

Disclosure. Authors have no conflict of interests, and the work was not supported or funded by any drug company. Dr. Ali M. Albarrak is a member of the Editorial Team, and was therefore excluded from any final editorial decisions regarding this paper.

This study was conducted to determine the ideal number of sputum AFB smears and polymerase chain reaction (PCR) required for discontinuing TB isolation among patients with suspected pulmonary TB.

Methods. This is a single-center records based retrospective study of all patients diagnosed with culture-proven pulmonary TB between 2010 and 2018. The TB notification records were utilized to identify patients admitted with PTB. The patients' charts were reviewed by the authors for demographic data, sputum AFB smears, and polymerase chain reaction (PCR) and culture results. Only the first 3 sputum smears were included in the analysis. Polymerase chain reaction results for MTB were also included in the study.

The study was conducted at Prince Sultan Military Medical City (PSMMC) in Riyadh, Saudi Arabia, a large tertiary care center consisting of 1,200 beds. All patients attending the emergency department with suspected clinical and/or radiological features of pulmonary TB are immediately triaged to a negative pressure isolation room. Three sputum specimens are collected every 8 hours with one specimen collected early morning. Among patients who fail to expectorate, sputum induction is performed with 3% hypertonic saline nebulized in a negative pressure room. Occasionally, patients may require bronchoscopy to obtain appropriate specimen. In addition to routine sputum microscopy and culture, GeneXpert can be requested on all first specimens. Patients with smear-positive TB are kept in isolation, whereas individuals with alternative diagnosis or 3 negative smears are discharged.

We included all adults with at least one sputum culture positive for MTB. Patients with negative culture or non-tuberculous mycobacteria (NTM) were excluded. All patients were assessed according to the first positive smears and PCR results.

In the microbiology laboratory, all samples are cultured on solid media (Lowenstein-Jensen) and liquid media Mycobacterium growth indicator tubes (MGITs) and a smear was prepared for AFB detection. Cultures were considered negative if no growth was observed after 8 weeks of incubation. Smears that were prepared from concentrated samples were heat fixed and stained with Auramine and read under the fluorescent microscope for the presence of AFB. Positive smears were confirmed by over staining with ZN stain. Rapid detection of MTB complex DNA in the sputum or concentrated sediments that were either AFB smear positive or negative was performed by PCR test using GeneXpert RT-PCR MTB/RI.

Statistical analysis. Statistical analyses were performed using SPSS 20.0 (SPSS Inc., Chicago, IL, USA). Continuous data were expressed as mean±standard deviation (minimum - maximum) while categorized data were expressed as number (%). Cross tabulation was made between first and second sample results and between third and combined first and second samples. Cross tabulation was also made between PCR and AFB smears results. Categorical variables were compared using likelihood ratio. All p-values were 2-tailed, and $p \leq 0.05$ was considered statistically significant. The study was conducted after approval from the Research Ethical Committee of the Prince Sultan Military Medical City, Riyadh, Saudi Arabia.

Results. A total of 240 patients were MTB culture positive. The mean±SD age of the patients was 51.2±22.5 years. A majority of the patients were men (n=155, 64.6%). Only 7 (2.9%) of patients were HIV positive; however, 42 (17.5%) patients were not tested for HIV (**Table 1**).

A confirmed positive culture of MTBC was used as a reference standard. A total of 126 patients were smear and culture positive, whereas 114 were culture positive but smear negative. Polymerase chain reaction was performed in 83% of the study group, which was positive in 152 of 199 (76.4%) tested patients (**Table 1**). The overall sensitivity of the AFB smear and PCR were 52.5% and 76.4% respectively (**Table 2**). **Tables 3 & 4** shows cross tabulations between smears that showed positivity in the 1st or 2nd smear or both of them and results of the 3rd smear. Of 126 patients who were culture and AFB smear-positive, 98 (77.8%) were detected in the first specimen, 13 (10.3%) patients who were negative in the first specimen were positive in the second specimen. Only 9 (7.1%) were positive in the third specimen while negative in both first and second sample. The likelihood of positivity of the third specimen when both first and second smear were negative was 2.91 (LHR 2.9, p=0.23) (**Tables 3 & 4**). Six samples were negative for AFB beyond the third specimen (5 in fourth sample, one in the sixth sample); however, all of these patients were PCR positive based on the first sample.

Polymerase chain reaction was positive in 122 (96.8%) smear-positive patients (**Table 5**). Four patients did not undergo PCR; their smears were detected in the

Table 1 - Demographic and clinical characteristics of the patients with pulmonary tuberculosis (N=240).

Characteristic	n (%)
Gender	
Male	155 (64.6)
Female	85 (35.4)
Age (mean±SD)	51.2±22.5 (13.0-93.0)*
First sample	
Positive	98 (77.8)
Negative	28 (22.2)
Second sample	
Positive	88 (69.8)
Negative	34 (27.0)
Not done	4 (3.2)
Third sample	
Positive	80 (63.5)
Negative	37 (29.4)
Not done	9 (7.1)
Polymerase chain reaction	
Positive	152 (63.3)
Negative	47 (19.6)
Not done	41 (17.1)
HIV status	
Positive	7 (2.9)
Negative	191 (79.6)
Not done	42 (17.5)

Data are expressed number (%) as appropriate.
*(minimum – maximum)

Table 2 - Sensitivities of acid-fast bacilli (AFB) smear and polymerase chain reaction (PCR) using culture as gold standard.

Test	Positive n	Negative n	Sensitivity %	95% confidence intervals
Culture	240	-	-	-
1st specimen	98	28	40.8	34.6% to 47.3%
Combined (1st & 2nd)	111	15	46.3	39.8% to 52.8 %
Smear (cumulative)	126	114	52.5	45.9% to 58.9%
PCR	152	47	76.4	69.7% to 81.8%

Table 3 - Cross tabulation between 1st and 2nd acid-fast bacilli (AFB) smears.

Second sample	First sample	
	Negative (n=28, 22.2%)	Positive (n=98, 77.8%)
Negative	15 (44.1)	19 (55.9)
Positive	13 (14.8)	75 (85.2)
Not done	-	4 (100.0)

Data are expressed as number (%)

Table 4 - Cross tabulation between 3rd acid-fast bacilli (AFB) smear and both 1st and 2nd smears.

First & second samples	Third sample		
	Negative (n=37, 29.4%)	Positive (n=80, 63.5%)	Not done (n=9, 7.9%)
Negative	6 (40.0)	9 (60.0)	-
Positive	31 (27.9)	71 (64.0)	9 (8.1)

Data are expressed as number (%).

Table 5 - Cross tabulation between sputum AFB smear and PCR.

PCR/smear	Negative (n=47, 19.6%)	Positive (n=152, 63.3%)	Not done (n=41, 17.1%)
Negative	47 (41.2)	30 (26.3)	37 (32.5)
Positive	-	122 (96.8)	4 (3.2)

Data are expressed as number (%).

first sample, one from the third sample, and 2 from the fourth sample. The PCR was 100% sensitive in detecting smear positive patients when the test is performed. None of the smear positive group was PCR negative. In smear-negative patients, the PCR was positive in 30 (26.3%) of cases with significant difference between the groups ($p \leq 0.001$).

Discussion. The CDC guidelines recommend that all patients with suspected pulmonary TB have 3 negative sputum AFB smears before the discontinuation of isolation.[3] Similarly, the European Centre for Disease Prevention and Control (ECDC) suggests a minimum of 2 sputum specimens subjected for AFB examination.[7] This study was conducted to determine the number of smears and/or PCR to determine deisolation of suspected TB patients. Overall, the sensitivity of AFB smear in identification of TB in our study was 52.5% (95% confidence interval [CI] 39.8%-52.8%). The first 2 smears together detected 88% (111 out of 126) of patients, whereas only 7.1% of patients were detected using the third serial smear alone. The contribution of the third sample was not significant with a likelihood ratio for positivity of 2.9 (p=0.23) These findings are in agreement with previous reports. Percentages of patients identified as AFB smear positive in at least the third specimen alone widely varied (0%, 5.3%, 8.6%, 3.2%, and 11.1%).[8] Studies performed in Colorado, USA, showed an incremental yield of the third specimen (2%), whereas in Tanzania, the increment was 2.5% in a cohort of 49,930 patients with suspected TB.[9] Similarly, 42 laboratories from 4 high-income countries showed that an additional yield from a third serial smear was 0.7% to 7.2%. However, a large number of smears, 122.7 to 796.3, were needed to detect one extra case with the third serial smear, whereas 164.8 to 2133.4 slides had to be examined to detect an incremental failure with the second serial smear.[10] In our cohort, 2 sputum smears would have missed 9 smear-positive patients over 9 years. However, the PCR would have prevented all these patients released to the general ward. Moreover, patients strongly suspected of PTB should be kept in isolation even with negative screening. Utilizing clinical decisions and/or PCR testing can help decrease immature deisolations.

Evidences that using the PCR can shorten the isolation time in PTB suspects have been growing.[11-14] Nucleic acid amplification test (NAAT) can rapidly diagnose TB and detect resistance. Xpert®MTB/RIF assay endorsed by the WHO is a rapid, cartridge-based NAAT. The result can be delivered within 2 hour.[15] In the case of respiratory specimens, the sensitivity and specificity of the Xpert® MTB/RIF assay were 100% (95% CI: 80-100%) and 100% (95% CI: 95%-100%).[16] In our study the PCR detected all smear positive patients. Similar to previous studies a single PCR in our study was equivalent to 3 serial smears finding.[14] The sensitivity and specificity of the PCR in the tested patients in this study was 100.0%. Interestingly when the PCR was performed, 30 patients (26.3%) of our smear negative patients turned to be sputum PCR positive. These patients could have transmitted the infection if released to the general ward. Of note, 10-20% of TB patients acquires their infection from smear and probably PCR negative patients; hence, deisolation should be guided by the clinical probability of the disease.[17-19]

All of our smear positive patients were detected with a single PCR examination. It is not yet clear whether a single expert or 2 examinations should guide discontinuation of AII. Single NAA test can decrease the demand for negative pressure rooms by approximately 75% when compared to serial sputum AFB smears.[12] The sensitivity of 1 Xpert, 2 Xperts, 2 smears, or 3 smears compared to culture was 0.85 (95% CI: 0.61-0.96), 0.95 (95% CI: 0.73-1.0), 0.70 (95% CI: 0.46-0.88), and 0.80 (95% CI: 0.56-0.93), respectively. The NPV of the 4 strategies was also similar (0.99 for 3 smears, 0.98 for 2 smears, 0.99 for 1 Xpert, 1.00 for 2 Xperts. The FDA has recently approved testing of 1 or 2 sputum PCRs as an alternative to serial AFB smears for discontinuation of airborne precautions.[20]

Despite current CDC recommendations that nucleic acid amplification tests (NAATs) be performed on at least one respiratory specimen from all patients evaluated for TB, molecular testing for TB in the United States is underutilized, due to lack of awareness or due to costs and logistical considerations.[3] Remarkably and following increasing awareness, our current PCR request rate in suspected PTB improved to 83%.

We believe our findings support studies endorsing a single PCR to shorten the isolation period in suspect pulmonary TB. The third serial smear specimen had a low incremental yield and would lead to delay in removing patients from AII rooms. This is associated with economic burden and can contribute to patients' stigmatization and adversely affect their outcome. Polymerase chain reaction findings and critical clinical

decisions will guard against immature deisolation in these patients. Our data did not allow for calculation of the days saved and the economic impact of NAA. However, a recent review showed molecular testing to be associated with shorter median isolation period (2.9 versus 2.5 days), time to hospital discharge (6.0 versus 4.9 days) and lower costing with savings of $13347 per isolated TB negative patients.[21]

Study limitations. It is a retrospective, single center study. In the cohort analyzed, the number of patients with HIV co-infection was very low that the outcome may not be generalized to areas where the prevalence of HIV/TB co-infection is high. The exact timing of the sample collection and release of patients from isolation could not be identified in all patients prohibiting calculation of time and economic saving. One strength of our study is the high PCR request rate (83%). Furthermore, NAAT performed on our smear negative patients detected 30 (26.3%) of culture positive, smear negative patients. This can contribute to the diagnosis and help in the isolation decision.

In conclusion, a single PCR examination helps discontinue negative pressure room isolation while a third sputum smear did not contribute significantly to the deisolation decision in patients suspected of pulmonary TB.

Acknowledgment. *We extend our thanks to our TB co-coordinators, Moaidh Alzahrani and Saud Aldalbahi, for their help in the study and to Professor Enas Hamed for the statistical analysis. We would like to thank Editage (www.editage.com) for English language editing.*

References

1. World Health Organization. WHO global tuberculosis report 2018. Pharmacological Reports. Geneva: World Health Organization; 2018.
2. World Health Organization. WHO Guidelines on tuberculosis infection prevention and control [Update 2019]. Geneva: World Health Organization; 2019. Available from: https://www.who.int/tb/publications/2019/guidelines-tuberculosis-infection-prevention-2019/en/
3. Jensen PA, Lambert LA, Iademarco MF, Ridzon R, CDC. Guidelines for preventing the transmission of Mycobacterium tuberculosis in health-care settings, 2005. ***MMWR Recomm Rep*** 2005; 54 (RR-17): 1-141.
4. Abad C, Fearday A, Safdar N. Adverse effects of isolation in hospitalised patients: A systematic review. ***J Hosp Infect*** 2010; 76: 97-102.
5. Sepkowitz KA, Friedman CR, Hafner A, Kwok D, Manoach S, Floris M, et al. Tuberculosis among urban health care workers: A study using restriction fragment length polymorphism typing. ***Clin Infect Dis*** 1995; 21: 1098-101.
6. Edlin BR, Tokars JI, Grieco MH, Crawford JT, Williams J, Sordillo EM, et al. An outbreak of multidrug-resistant tuberculosis among hospitalized patients with the acquired immunodeficiency syndrome. ***N Engl J Med*** 1992; 326: 1514-1521.
7. Migliori GB, Zellweger JP, Abubakar I, Ibraim E, Caminero JA, De Vries G, et al. ***Eur Respir J*** 2012; 39: 807-819.
8. Wilmer A, Bryce E, Mdcm JG, Wilmer A, Bryce E, The JG, et al. The role of the third acid-fast bacillus smear in tuberculosis screening for infection control purposes: A controversial topic revisited. ***Can J Infect Dis Med Microbiol*** 2011; 22: 5-7.
9. Ipuge YAI, Rieder HL, Enarson DA. The yield of acid-fast bacilli from serial smears in routine microscopy laboratories in rural Tanzania. ***Trans R Soc Trop Med Hyg*** 1996; 90: 258-261.
10. Rieder HL, Chiang CY, Rusen ID. A method to determine the utility of the third diagnostic and the second follow-up sputum smear examinations to diagnose tuberculosis cases and failures. ***Int J Tuberc Lung Dis*** 2005; 9: 384-391.
11. Chaisson LH, Roemer M, Cantu D, Haller B, Millman AJ, Cattamanchi A, et al. Impact of GeneXpert MTB/RIF assay on triage of respiratory isolation rooms for inpatients with presumed tuberculosis: A hypothetical trial. ***Clin Infect Dis*** 2014; 59: 1353-1360.
12. Campos M, Quartin A, Mendes E, Abreu A, Gurevich S, Echarte L, et al. Feasibility of shortening respiratory isolation with a single sputum nucleic acid amplification test. ***Am J Respir Crit Care Med*** 2008; 178: 300-3005.
13. Millman AJ, Dowdy DW, Miller CR, Brownell R, Metcalfe JZ, Cattamanchi A, et al. Rapid molecular testing for TB to guide respiratory isolation in the U.S.: A cost-benefit analysis. ***PLoS One*** 2013; 8: 1-8.
14. Lippincott CK, Miller MB, Popowitch EB, Hanrahan CF, Van Rie A. Xpert MTB/RIF assay shortens airborne isolation for hospitalized patients with presumptive tuberculosis in the United States. ***Clin Infect Dis*** 2014; 59: 186-192.
15. Boehme CC, Nabeta P, Hillemann D, Nicol MP, Shenai S, Krapp F, et al. Rapid molecular detection of tuberculosis and rifampin resistance. ***N Engl J Med*** 2010; 363: 1005-1015.
16. Malbruny B, Le Marrec G, Courageux K, Leclercq R, Cattoir V. Rapid and efficient detection of Mycobacterium tuberculosis in respiratory and non-respiratory samples. ***Int J Tuberc Lung Dis*** 2011; 15: 553-555.
17. Behr MA, Warren SA, Salamon H, Hopewell PC, Ponce De Leon A, Daley CL, et al. Transmission of Mycobacterium tuberculosis from patients smear-negative for acid-fast bacilli. ***Lancet*** 1999; 353: 444-449.
18. Hernández-Garduño E, Cook V, Kunimoto D, Elwood RK, Black WA, FitzGerald JM. Transmission of tuberculosis from smear negative patients: A molecular epidemiology study. ***Thorax*** 2004; 59: 286-290.
19. Xie YL, Cronin WA, Proschan M, Oatis R, Cohn S, Curry SR, et al. Transmission of Mycobacterium tuberculosis from Patients Who Are Nucleic Acid Amplification Test Negative. ***Clin Infect Dis*** 2018; 67: 1653-1659.
20. National Tuberculosis Controllers Association. Consensus statement on the use of Cepheid Xpert MTB / RIF ® assay in making decisions to discontinue airborne infection isolation in healthcare settings. [Updated 2016 April. 2019 June 29] Available from: http://www.tbcontrollers.org/docs/resources/NTCA_APHL_GeneXpert_Consensus_Statement_Final.pdf
21. Chaisson LH, Duong D, Cattamanchi A, Roemer M, Handley MA, Schillinger D, et al. Association of Rapid Molecular Testing with Duration of Respiratory Isolation for Patients with Possible Tuberculosis in a US Hospital. ***JAMA Intern Med*** 2018; 178: 1380-1388.