Sensitivity and Specificity of Real Time Polymerase Chain Reaction (RT-PCR) in Bronchial Washing for Diagnostic Pulmonary Tuberculosis at Maharat Nakhorn Ratchasima Hospital

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Objective: *To study sensitivity and specificity of real time polymerase chain reaction (RT-PCR) in bronchial washing for diagnostic pulmonary tuberculosis at Maharat Nakhorn Ratchasima Hospital.*
Material and Method: *A retrospective study of performed bronchial washing (BW) specimens for RT-PCR TB, AFB stain, and culture TB by conventional technique from 430 patients who had undergone bronchoscopic examination due to symptomatic abnormal CXR or Chest-CT with sputum samples negative or no sputum for AFB by the authors between December 1, 2008 and September 31, 2011. TB culture was gold standard in category A. Final diagnoses was confirmed with microbiological, clinicopathological finding and response to anti-TB treatment in category B. They were analyzed to study sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive and negative likelihood ratios of BW RT-PCR TB on diagnostic tool for detecting pulmonary TB. Statistical analysis of the data was described as percentage.*
Results: *Four hundred thirty patients were included in the presented study. Age range, sex, hemoptysis, smoking, CXR, and bronchoscope finding were not significant different between pulmonary TB and not pulmonary TB. The sensitivity, specificity, PPV, NPV, positive and likelihood ratios of bronchial washing for PCR-TB when applied to category A for diagnosing smear-negative pulmonary TB were 65.7%, 90.4%, 37.7%, 96.7%, 6.8, and 0.37 respectively. Category B were 43.2%, 93.3%, 62.3%, 86.4%, 6.4, and 0.6 respectively. After combination with BW AFB stain (bAFB), sensitivity was higher but specificity was less in both categories.*
Conclusion: *The low sensitivity of RT-PCR method might be low prevalence of active pulmonary TB in cases of the presented, the type of transfer and duration time (all of them were sent to a laboratory outside the hospital), the DNA extraction procedure, primer, the concentration of bronchial washing for DNA amplified, DNA extraction, and reproducible technique. However, BW RT-PCR should be done in a highly suspicious case due to rapid detection. False positive should be concerned in case of treated or old lesion from pulmonary TB.*

Keywords: *Bronchial washing, PCR-TB, Sensitivity, Specificity, IS6110*

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Pulmonary *Mycobacterium tuberculosis* (TB) is a major cause of morbidity and mortality worldwide. Early diagnosis pulmonary TB is necessary for effective treatment and to decrease the spread of the contagious disease. Sputum smear for acid fast bacilli (AFB) was developed in the 1880s and is still the same. The test alone is particularly ineffective to diagnose active pulmonary TB because about 40 to 50% of patients have sputum smear negative for AFB[1] and the lowest concentration (10^4 bacilli/ml) is required to detect by microscopic examination. Abnormal findings from chest x-rays (CXR) were used to diagnose pulmonary TB. It is very sensitive but lack specificity. CXR is also not good to detect active pulmonary TB in the early stage. Old lesions from CXR are hardly classified as scar lesion or current active disease.

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Culture TB was more sensitive than sputum AFB. It is capable to identify as few as 10 bacilli/ml of digested, concentrated specimens. The process of growing physically is required for species identification. The culture technique is still the gold standard for active TB. It is very sensitive if live *Mycobacterium* can be obtained in the specimen. However, it does not have live *Mycobacterium* in every specimen and usually required six to eight weeks (two weeks in liquid media)[2] for report.

The polymerase chain reaction (PCR) is a helpful additional method, especially in combination with bronchoscopy for rapid detection TB. Nowadays, it is developed to real time PCR (RT-PCR) technique that provided sensitivity and specificity equivalent to that of conventional PCR combined with southern blot analysis. RT- PCR has low contamination and is more rapid, vigorous, and reproducible than conventional PCR[3].

Bronchoscope with bronchial washing for AFB stain is a very helpful diagnostic tool for diagnostic pulmonary TB with negative sputum AFB. The PCR and direct culture were applied for detection of *Mycobacterium tuberculosis* complex in samples obtained from patients with suspicion of pulmonary and extra-pulmonary tuberculosis[4]. IS6100 is an insertion sequence of the IS3 family and it is present in multiple copies in the chromosome of *Mycobacterium tuberculosis*. PCR was detected in varied specimens for example, PCR in blood samples proved to be a rapid and specific technique, although one with low sensitivity[5], PCR-TB in urine were used for rapid diagnosis of extrapulmonary TB, good enough in quality[6]. PCR of pleural fluid had 19% sensitivity and 96% specificity compared to AFB staining (0% sensitivity and 100% specificity) and culture (4% sensitivity and 100% specificity)[7].

Although RT-PCR is commonly used to diagnose pulmonary TB when fiberoptic bronchoscopy was performed. The efficacy of this method was not established at Maharat Nakhorn Ratchasima Hospital. The presented was discovered sensitivity and specificity of BW RT-PCR for diagnostic pulmonary TB.

Material and Method

The retrospective study, approved by the institutional ethics committee, was conducted in the Division of pulmonary and critical, Department of medicine, Maharat Nakhorn Ratchasima Hospital, Thailand between October 1, 2008 and September 31, 2011.

Data selection

The electronic medical record data base at Maharat Nakhorn Ratchasima Hospital, a tertiary referral center, was reviewed for 430 patients who had abnormal CXR or CT-chest finding and sputum AFB negative status/no sputum between October 1, 2008 and September 31, 2011, who underwent fiberoptic bronchoscopy with biopsy and bronchial washing (BW) for both RT-PCR TB and BW culture for TB. Pulmonary TB was suspected depending on respiratory symptoms, abnormal chest x-ray, or chest CT. All of them were Human immune-deficiency virus (HIV) negative.

Fiber-optic bronchoscopy and bronchial washing

Fiberoptic bronchoscope was performed after conscious sedation with intravenous pethidine 25 mg injection about 30 to 45 minutes before procedure, 10% lidocain spray and solution were used as local anesthesia. The trachea and bronchus were examined and bronchial washing (BW) from affected segments, localized by CXR or Chest-CT and endoscopic assistance was collected. BW was performed by instilling 0.9% isotonic saline (NSS) at room temperature through the internal channel of the flexible bronchoscope and aspirated to the suction tubing connector. Usually 10 ml of NSS was instilled with each washing and estimated one-fourth to half of the fluid was retrieved in the suction tubing connector. Bronchial washing specimen was obtained by injecting each 10 ml of isotonic saline through the bronchoscope channel and followed by immediate suction with connector system. This was repeated several times until 35 to 40 ml was collected. Endobronchial biopsies (EB) (when endobronchial disease was encountered) were done. All BW samples were processed for Gram stain, AFB stain, RT-PCR and TB culture.

Real-time PCR for detection and identification of Mycobacterium tuberculosis complex

Commercially available PCR kits (Amplicor) were used in this study. It is a qualitative real-time detection TB/non-tuberculous mycobacteria (NTM). TB primers were designed at conserved hoxypssequence in IS6110 gene and MPB64 gene. The 8-methoxypsoralen (8-MOP) system is used to extinguish the template activity of contaminate DNAs. 8-MOP is known to intercalate into double-stranded nucleic acids and form a covalent interstrand crosslink after photo activation with incident light of wavelength of 320 to 400 nm. Open the PCR tube after UV irradiation (365 nm) for

20 minutes on amplified PCR products to prevent carry-over contamination; at least 5 ml of BW were tested. For the negative control, use 5 ml of the distilled water instead of nucleic acid. For the positive control, use 5 ml of the TB/NTM PC instead of nucleic acid.

Culture Mycobacterium tuberculosis complex

The culture TB was done with conventional method. Drugs sensitivity was tested. Culture TB was performed on the specimens after decontaminated process and concentration by the addition of 4% sodium hydroxide followed by centrifugation at 3,500 rpm for 15 minutes. The deposit from each specimen was inoculated into two Lowenstein-Jensen slopes and into paranitrobenzoic acid medium (PNB) for identified TB and non-tuberculous mycobacteria (NTM). The cultures were incubated for eight weeks and read weekly for growth of TB. All cultures were reported as positive contained a significant number of colonies. Bronchoscopic biopsy specimens were processed for histopathology. In the present study, the authors used 10% lidocain solution less than 10 ml with every procedure.

Diagnostic confirmation[8]

TB culture was gold standard in category A. Final diagnoses was confirmed with microbiological, clinicopathological finding and response to anti-TB treatment in category B.

Statistical analysis

The percentage of sensitivity, specificity, PPV, NPV, and likelihood ratio were calculated with two by two table by using two confirmation categories, category A and category B. Data was interpreted by descriptive statistics and expressed as number and percentages (%). All analyses were performed using SPSS 13.0 statistical software.

Results

Baseline characteristics

Four hundred thirty patients underwent bronchoscopic exam for RT-PCR TB and culture TB, final diagnosis pulmonary TB were made with category A (culture TB-positive) and category B (depended on microbiological, clinicopathological finding, and response to treatment) (Fig. 1).

In category A, the number of patients with BW culture TB and BW RT-PCR positive were 35 (8.1%) and 61 (14.2%) in 430 specimens respectively.

The baseline characteristics of patients in category B are presented in Table 1. The number of patients in pulmonary TB (group A), not pulmonary TB [group B was lung cancer and group C was other (non-tuberculosis pneumonia and interstitial lung disease)] were 88 and 342 (232 and 110) respectively. Age range, sex, hemoptysis, smoking, CXR, and broncho-scope finding were not significantly different between pulmonary TB and not pulmonary TB, but there were older and more active smoking in not pulmonary TB (group B). Infiltration in CXR were common in group A (43.2%) and C (58.2%). Mass like lesions were the highest in group B (66%) and in group A (36.4%). Group B had the most endobronchial lesions (59.5%).

Three hundred forty two patients in category B were not pulmonary tuberculosis. Lung cancer was diagnosed in 232, other (non-tuberculosis pneumonia and interstitial lung disease) was diagnosed in 110 (pneumonia was diagnosed in 42, unknown diagnosed in 43, connective tissue disease in 10, cystic bronchiectasis in 9, and aspergilloma in 6). In case of pneumonia, non-tuberculous mycobacteria (NTM) were detected in four cases.

Correlation between diagnostic tools and final diagnosis

The diagnostic confirmation of pulmonary TB were shown in the flow chart, it was divided to category A and B. BW culture of TB was gold standard in category A. Correlation between method for

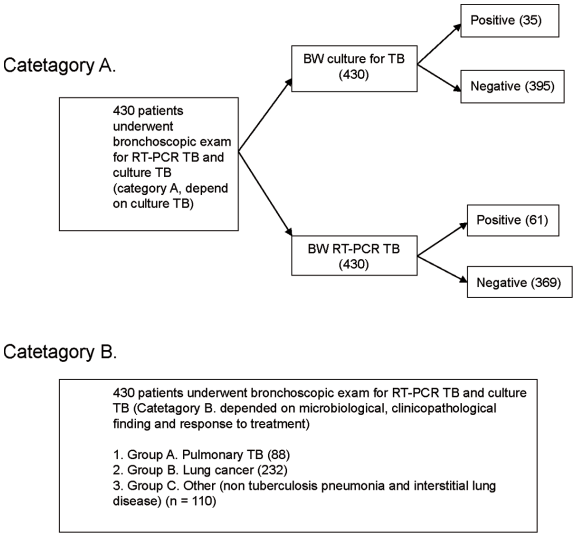

Fig. 1 Flow chart showing numbers of patients selected

Table 1. Baseline characteristics (n = 430)

	Group A, pulmonary TB (n = 88)	Non pulmonary tuberculosis		p-value
		Group B, lung cancer (n = 232)	Group C, other (non tuberculosis pneumonia and interstitial lung disease) (n = 110)	
Sex (male/female)	64/24	163/69	62/48	ns
Age (mean ± SD), years	58.1 ± 14.6	62.6 ± 11.0	55.5 ± 13.3	ns
Hemoptysis (yes/no)	17/71 (19.3%)	41/191 (17.7%)	16/94 (14.5%)	ns
Smoking				ns
Active	19 (21.6%)	62 (26.7%)	13 (11.8%)	
Ex-smoke	26 (29.5%)	74 (31.9%)	28 (25.5%)	
Never	43 (48.9%)	96 (41.4%)	69 (62.7%)	
Chest x-ray				ns
Mass like lesion	32 (36.4%)	153 (66.0%)	25 (22.7%)	
Infiltration	38 (43.2%)	59 (25.4%)	64 (58.2%)	
Military pattern	7 (7.9%)	1 (0.4%)	2 (1.8%)	
Nodule/nodules	4 (4.5%)	11 (4.7%)	6 (5.5%)	
Atelectasis	1 (1.1%)	1 (0.4%)	3 (2.7%)	
Cavity	5 (5.7%)	5 (2.2%)	5 (4.5%)	
Others	1 (1.1%)	2 (0.8%)	5 (4.5%)	
Bronchoscopic finding				ns
Endobronchial lesion	31 (35.2%)	138 (59.5%)	10 (9.0%)	
No endobronchial lesion	57 (64.8%)	94 (40.5%)	100 (91.0%)	

Pulmonary TB = group A; not pulmonary TB = group B and C; ns = not significant; BW = bronchial washing
Pathology for granulomatous disease 18 in group A, positive for malignancy 113 patients in group B

Table 2. Correlation between BW culture TB and BW RT-PCR TB in category A (n = 430)

	BW culture TB		Totals
	Absent	Present	
RT-PCR positive	38	23	61
RT-PCR negative	357	12	369
Totals	395	35	430

BW = bronachial washing; AFB = acid fast bacilli stain; RT-PCR = real time polymerase chain reaction

Table 3. Correlation between BW culture TB and bAFB in category A (n = 430)

	BW culture TB		Totals
	Negative	Positive	
bAFB positive	4	14	18
bAFB negative	391	21	412
Totals	395	35	430

bAFB = bronchial washing for acid fast bacilli (AFB) stain; BW = bronchial washing

diagnosis pulmonary TB in category A were presented in Table 2-4.

In Table 2, the number of BW culture positive for TB were 35 (8.1%). This means that the prevalence was 8.1%. Of the 395 (91.9%) that had BW culture negative, 61 (14.2%) were RT-PCR-positive, 369 (85.8%) were RT-PCR negative and 357 (83%) were both negative BW culture and RT-PCR.

Table 3. The number of bAFB positive were 18 (4.2%), 14 of them were proved for *Mycobacterium tuberculosis*. Four hundred twelve patients were

Table 4. Correlation between any methods and BW culture for TB in category A (n = 430)

	BW culture TB		Totals
	Negative	Positive	
bAFB positive and/or RT-PCR positive	41	28	69
RT-PCR and bAFB negative	354	7	361
Totals	395	35	430

bAFB = bronchial washing for AFB stain; RT-PCR = real time polymerase chain reaction

bAFB negative but 21 (5.1%) of them were positive for culture TB.

Correlation between BW RT-PCR and bAFB in category A were presented (Table 4). Either bAFB or BW RT-PCR were positive in 69 (16%) of 430 patients, and 28 (40.6%) in 69 were culture positive. Both bAFB and RT-PCR were negative in 361, of which seven (1.9%) of them were culture positive.

Correlation between BW RT-PCR and bAFB in patients were classified with category B (Table 5). BW RT-PCR TB were positive in 38 (43.2%) the 88 TB patients who were diagnosed pulmonary TB

Table 5. Correlation between RT-PCR and bAFB for pulmonary TB in category B

	Pulmonary TB (n = 88)		Not pulmonary TB (n = 342)	
	RT-PCR positive n = 38 (%)	RT-PCR negative n = 50 (%)	RT-PCR positive n = 23 (%)	RT-PCR negative n = 319 (%)
bAFB positive	10 (11.4)	4 (4.5)	0 (0)	4 (1.2)
bAFB negative	28 (31.8)	46 (52.3)	23 (6.7)	315 (92.1)

bAFB = bronchial washing for AFB stain; RT-PCR = real time polymerase chain reaction
all for bAFB-positive in non pulmonary TB group were non *Mycobacterium tuberculosis* (5), proved by BW culture TB

Table 6. Diagnostic validity of AFB staining, RT-PCR in BW of category A and B (n = 430)

	bAFB stain alone		BW RT-PCR alone		bAFB and/or BW RT-PCR	
	n/N (%)	95% CI	n/N	95% CI	n/N	95% CI
Sensitivity						
Category A	14/35 (40.0%)	0.24-0.58	23/35 (65.7%)	0.47-0.80	28/35 (80.0%)	0.62-0.90
Category B	14/88 (15.9%)	0.09-0.25	38/88 (43.2%)	0.32-0.54	42/88 (47.7%)	0.37-0.58
Specificity						
Category A	391/395 (98.9%)	0.97-0.99	357/395 (90.4%)	0.86-0.93	354/395 (89.6%)	0.86-0.92
Category B	338/342 (98.8%)	0.97-0.99	319/342 (93.3%)	0.89-0.95	315/342 (92.1%)	0.88-0.95
PPV						
Category A	14/18 (77.8%)	0.52-0.93	23/61 (37.7%)	0.25-0.51	28/69 (40.6%)	0.29-0.53
Category B	14/18 (77.8%)	0.52-0.93	38/61 (62.3%)	0.48-0.74	42/69 (60.8%)	0.48-0.72
NPV						
Category A	391/412 (94.9%)	0.92-0.97	357/369 (96.7%)	0.94-0.98	354/361 (98.1%)	0.95-0.99
Category B	338/412 (82.0%)	0.78-0.85	319/369 (86.4%)	0.82-0.89	315/361 (87.3%)	0.83-0.90
Positive LR						
Category A	39.50	13.73-113.56	6.80	4.64-10.04	7.70	5.52-10.76
Category B	13.60	4.59-40.30	6.42	4.04-10.19	6.04	3.96-9.22
Positive LR (w)						
Category A	3.50	1.42-8.60	0.60	0.41-0.88	0.68	0.48-0.96
Category B	3.50	1.42-8.60	1.65	1.13-2.41	1.55	1.09-2.20
Negative LR						
Category A	0.60	0.46-0.79	0.37	0.23-0.60	0.22	0.11-0.43
Category B	0.85	0.78-0.93	0.61	0.51-0.73	0.57	0.46-0.69
Negative LR (w)						
Category A	0.05	0.03-0.08	0.03	0.02-0.06	0.02	0.01-0.04
Category B	0.22	0.18-0.27	0.16	0.12-0.20	0.15	0.11-0.19

bAFB = bronchial washing for acid fast bacilli stain; BW RT-PCR = bronchial washing real time polymerase chain reaction; CI = confidence interval; Category A = culture TB proven; Category B = microbiological, clinicopathological finding and response to anti-TB treatment; PPV = positive predictive value; NPV = negative predictive value; LR = likelihood ratio (w) = weight by prevalence (prevalence = 0.08 in category A, prevalence = 0.20 in category B)

(category B). There are 14 patients had the positive results of both bAFB and cultured of TB. BW RT-PCR TB had false positive 23 (6.7%) in 342 of not pulmonary TB patients

Diagnostic validity of bAFB, RT-PCR and any methods in BW of both category A and B

The validity of RT-PCR TB, bAFB and combination both of them were evaluated. Pulmonary TB was diagnosis with two categories that were described in the flow charts. Detection rates were compared between bAFB, BW RT-PCR and bAFB and/or BW RT-PCR. The sensitivity, specificity, PPV, NPV, positive LR and negative LR in category A were 40%, 98.9%, 77.8%, 94.9%, 39.5, and 0.6 respectively. In BW RT-PCR of category A were 65.7%, 90.4%, 37.7%, 96.7%, 6.8, and 0.37 respectively. Combination of both methods (in category A), these results were 80%, 89.6%, 40.6%, 98.1%, 7.7, and 0.22 respectively. The sensitivity of bAFB and/or BW RT-PCR was higher than bAFB and BW RT-PCR alone. Specificity was lower after combination of methods but sensitivity was higher. Bronchial washing for bAFB alone had lower sensitivity but very high specificity. All of bAFB, BW RT-PCR and any methods were lower sensitivity in category B; on the contrary specificity of them were not inferior. After weight by prevalence positive LR were diminished of both categories and all techniques.

Discussion

The presented was established the sensitivity and specificity of BW RT-PCR TB for diagnostic pulmonary TB at Maharat Nakhorn Ratchasima Hospital.

All patients in the present study had abnormal CXR or Chest CT, negative sputum AFB/no sputum, and clinical was suspicious pulmonary TB. Although smear negative pulmonary TB was commonly diagnosis in routine practice and most of them were received empirically anti-TB treatment but radiographic findings were lack of specificity to confirm diagnosis pulmonary TB. Fiber-optic bronchoscope was performed to get the samples for the definite diagnosis of pulmonary TB in sputum smear negative patients. Futhermore, the sensitivity and specificity of bAFB and BW RT-PCR in the presented can be established.

Yoon et al (1992)[9] studied PCR-TB from sputum compared with sputum culture of TB of patients with pulmonary tuberculosis or other pulmonary disease using primers targeting the IS6110, conventional technique, sensitivity of patients with tuberculosis was 93.7%, specificity 63%, similar in Schluger et al (1994)[10] assayed the presence of the IS6110 by PCR-TB from 65 patients to diagnose active pulmonary TB (confirmed diagnosis by microbiology, pathology and clinical history), the sensitivity and specificity were 100 and 70% respectively, for the diagnosis of any TB infection (active, treated, asymptomatic) sensitivity and specificity of PCR were 87.5% and 90% respectively, that meant PCR-TB in those studies were lower sensitivity but higher specificity in cases were previously infected or treated.

Wong et al (1998)[11] studied 190 patients with chest radiographic lesions and negative sputum smears for acid-fast bacilli were performed fiberoptic bronchoscopy (pulmonary TB 67.18%, lung cancer 26.56% and other 6.25%). The sensitivity, specificity, PPV and NPV of conventional PCR-TB when applied to bronchial aspirate specimens for diagnosing smear-negative pulmonary TB were 97.2%, 73.2%, 82.7% and 95.2% respectively. PCR-TB might have an added place to transbronchial biopsies in the rapid diagnosis active pulmonary tuberculosis with smear negative.

Hidaka et al (2000)[12] studied in 98 patients (24 active pulmonary TB, 28 sequalae of pulmonary TB and 46 non-tuberculosis pulmonary disease). The sensitivity of the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) in both of bronchial washing and bronchial brushing and both of them mixed together were 76, 70, and 91% respectively.

The presented had sensitivity of RT-PCR nearly Hidaka's PCR-RFLP was explained with similar low numbers of active pulmonary TB patients. In case of high prevalence of active pulmonary they showed high sensitivity and lack of specificity of PCR-TB. Combination of any technique was proved to enhance sensitivity.

RT-PCR TB in the presented showed lower sensitivity to previous conventional PCR[13]. The ineffectiveness of RT-PCR in the presented was explained with multiple factors, including number of active pulmonary TB in the presented had about one-fifth of all (category B), the type of fixative and fixation time (all of them were sent to a laboratory outside the hospital), the DNA extraction procedure, primer, the concentration of bronchial washing for DNA amplified, DNA extraction and reproducible technique. False positive of BW RT-PCR in the presented was explained with old lesion from previous pulmonary tuberculosis or scar tumor that was not mentioned in data characteristic. Four cases

of non-*Mycobacterium tuberculosis* were detected in the presented, species types were not identified due to limitation of technique.

The limitations of the presented were retrospective study, included non-pulmonary TB patients too much, not mentioned about prior treated or scar from pulmonary TB that effected the results of culture and RT-PCR. Sputum RT-PCR TB should be done for enhances sensitivity or avoids invasive procedure for diagnostic pulmonary TB. Specificity of RT-PCR in the presented was effective to exclude active pulmonary TB in case was not highly suspicious TB and negative RT-PCR. The bAFB in the presented was rapid method to detection pulmonary TB but less sensitive and cannot exclude non-tuberculous mycobacteria (NTM), more helpful when combined with BW RT-PCR.

Conclusion

BW RT-PCR for diagnosis pulmonary TB should be done in highly suggestive case of active pulmonary TB because it was useful for rapid detection. The sensitivity was higher when combination with bAFB. History of pulmonary TB or old lesion can result in lower sensitivity. The BW culture of TB should be routinely performed for confirmation of pulmonary TB, NTM, and MDRTB due to cost-effective (600 baht/specimen) even though it needs the longer time.

Potential conflicts of interest

None.

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ความไวและความจำเพาะของการตรวจพันธุกรรมของวัณโรคในน้ำล้างหลอดลมเพื่อวินิจฉัยวัณโรคปอดที่โรงพยาบาลมหาราชนครราชสีมา

นภัทร เขียวอ่อน, สุธัญญา ช่วงงาม, สุภารัตน์ มิตรสูงเนิน, จนิสสยา เปี่ยมเอม, จารุกรณ์ วิศาลสวัสดิ์

วัตถุประสงค์: *เพื่อศึกษาความไวและความจำเพาะของการตรวจสารพันธุกรรมของวัณโรคในน้ำล้างปอดเพื่อการวินิจฉัยวัณโรคปอดของผู้ป่วยในโรงพยาบาลมหาราชนครราชสีมา*

วัสดุและวิธีการ: *เป็นการศึกษาย้อนหลังโดยการเก็บข้อมูลของผู้ป่วยจำนวน 430 ราย ที่มารับการตรวจปอดและหลอดลมโดยกล้องส่องหลอดลมชนิดโค้งงอโดยผู้นิพนธ์ เนื่องจากมีภาพรังสีปอดหรือเอกซเรย์คอมพิวเตอร์ปอดผิดปกติประกอบกับมีอาการทางระบบการหายใจ และไม่พบเชื้อวัณโรคจากการตรวจเสมหะหาเชื้อวัณโรค หรือ เก็บเสมหะไม่ได้ ตั้งแต่วันที่ 1 ตุลาคม พ.ศ. 2551 ถึง 31 กันยายน พ.ศ. 2554 ผู้ป่วยทั้งหมดถูกแบ่งเป็นผู้ป่วยวัณโรคปอดและไม่ใช่วัณโรคปอด โดยใช้เกณฑ์ดังนี้ ประเภท เอ ใช้ผลการเพาะเชื้อวัณโรคจากน้ำล้างปอดเป็นมาตรฐาน ประเภท บี ใช้ผลทางจุลชีววิทยา, พยาธิวิทยา และการตอบสนองต่อการรักษา ผู้ป่วยทั้งหมดถูกประมวลผลเพื่อศึกษาถึงความไว, ความจำเพาะ, โอกาสที่จะเป็นวัณโรคถ้าผลตรวจเป็นบวก, โอกาสที่จะไม่เป็นโรคถ้าผลตรวจเป็นลบ และความน่าจะเป็นไปได้ถ้าผลเป็นบวกหรือลบ ของการตรวจสารพันธุกรรมจากน้ำล้างปอดในการวินิจฉัยวัณโรคปอด การวิเคราะห์ทางสถิติบรรยายเป็นเปอร์เซ็นต์*

ผลการศึกษา: *ในจำนวนผู้ป่วย 430 ราย ที่เข้าเกณฑ์การศึกษา ช่วงอายุ, เพศ, ประวัติไอออกเลือด, ประวัติสูบบุหรี่, ภาพรังสีปอดและผลการตรวจหลอดลมไม่มีความแตกต่างกันอย่างมีนัยสำคัญทางสถิติ ในผู้ป่วยที่เป็นและไม่ได้เป็นวัณโรคปอด เมื่อนำมาประมวลผลในผู้ป่วยประเภท เอ สำหรับวินิจฉัยวัณโรคปอดที่ผลการตรวจเสมหะเป็นลบ ความไว, ความจำเพาะ, โอกาสที่จะเป็นโรคถ้าผลตรวจเป็นบวก, โอกาสที่จะไม่เป็นโรคถ้าผลการตรวจเป็นลบ และความน่าจะเป็นไปได้ถ้าผลเป็นบวกหรือลบ ของการตรวจสารพันธุกรรมของวัณโรคจากน้ำล้างปอดเป็น 65.7%, 90.4%, 37.7%,96.7%, 6.8 และ 0.37 ตามลำดับในผู้ป่วยประเภท เอ 43.2%, 93.3%, 62.3%, 86.8%, 6.4 และ 0.6 ตามลำดับในผู้ป่วยประเภท บี เมื่อประมวลผลร่วมกับการย้อมเชื้อวัณโรคจากน้ำล้างหลอดลม พบว่ามีความไวเพิ่มขึ้นแต่ความจำเพาะลดลงทั้งสองกลุ่ม*

สรุป: *ความไวของการตรวจสารพันธุกรรมของวัณโรคในน้ำล้างหลอดลม ค่าที่ได้ต่ำกว่าที่ควร อาจเป็นผลมาจากความชุกของผู้ป่วยที่เป็นวัณโรคปอดในการศึกษานี้ไม่มาก, วิธีและเวลาในการขนส่ง (ตัวอย่างทั้งหมดถูกส่งตรวจนอกโรงพยาบาล), กระบวนการในการสกัดดีเอ็นเอ, สารตั้งต้น (primer), ความเข้มข้นของน้ำล้างหลอดลมในกระบวนการเพิ่มสารพันธุกรรม, เทคนิคในการสกัดและเพิ่มปริมาณสารพันธุกรรม อย่างไรก็ตามการตรวจสารพันธุกรรมของวัณโรค (RT-PCR) ควรทำในกรณีที่สงสัยว่าน่าจะเป็นวัณโรคปอดอย่างมาก เนื่องจากทราบผลเร็ว ควรระวังผลบวกลวงในกรณีที่เคยได้รับการรักษาวัณโรคมาก่อนหรือเป็นแผลเป็นเดิมจากวัณโรคปอด*