Evaluation of Real-time Polymerase Chain Reaction for Detection of the 16S Ribosomal RNA Gene of *Mycobacterium tuberculosis* and the Diagnosis of Cervical Tuberculous Lymphadenitis in a Country With a High Tuberculosis Incidence

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Background. Tuberculous lymphadenitis (TBL) is the most common form of extrapulmonary tuberculosis. Currently, the standard diagnostic test for TBL is culture, which takes more than several weeks to yield results. We studied a real-time polymerase chain reaction (PCR) for rapid detection of *Mycobacterium tuberculosis* in cervical lymph node specimens obtained from patients in a country where the tuberculosis incidence is high.

Methods. Patients with cervical lymphadenopathy were prospectively enrolled between April 2009 and March 2010. Clinical specimens obtained through fine-needle aspiration (FNA) and excisional biopsy were tested for *M. tuberculosis* by the COBAS TaqMan MTB Test, a real-time PCR assay for detecting the 16S ribosomal RNA gene of *M. tuberculosis*. Mycobacterial culture and histopathological findings from tissue biopsy specimens were used as a reference standard for sensitivity and specificity calculations.

Results. Of 73 patients, 41 received a diagnosis of TBL. For biopsy specimens, the sensitivity of real-time PCR was 63.4%, and the specificity was 96.9%. For FNA specimens, the sensitivity was 17.1%, and the specificity was 100%. The sensitivity of real-time PCR of biopsy specimens was comparable to that of tissue culture but significant lower than that of histopathological examination ($P < .01$).

Conclusions. Real-time PCR did not increase the yield for rapid diagnosis of TBL.

Mycobacterium tuberculosis has been a major threat to human health, causing approximately 1.3–1.6 million deaths worldwide annually [1]. To control *M. tuberculosis* transmission, an early and accurate diagnosis of tuberculosis, as well as delivery of appropriate tuberculosis treatment, is crucial [2]. Recently, the use of nucleic acid amplification tests (NAATs), especially polymerase chain reaction (PCR), has gained acceptance for rapid diagnosis of pulmonary tuberculosis [3–5]. Nevertheless, in the setting of tuberculous lymphadenitis (TBL), the most frequent type of extrapulmonary involvement [6–9], the usefulness of direct NAAT is less clear, and the diagnosis remains difficult because of nonspecific clinical presentations that may overlap with other infectious etiologies [10, 11] or malignancy [12, 13], the lack of sensitivity of direct acid-fast bacilli (AFB) staining, and delayed results of mycobacterial culture [14].

Recently, fine-needle aspiration (FNA) has assumed an important role as a possible noninvasive alternative to excisional biopsy for the diagnosis of peripheral lymphadenopathy by histopathologic examination [15, 16]. However, little is known about its clinical usefulness for direct NAAT [3, 17]. Therefore, we compared findings of real-time PCR for detection of the 16S ribosomal RNA (rRNA) gene of *M. tuberculosis* in cervical lymph node specimens obtained by FNA and by excisional biopsy for the diagnosis of TBL in a country where the tuberculosis incidence is high [18].

MATERIALS AND METHODS

A prospective cross-sectional study was undertaken among patients of the Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand, from April 2009 to March 2010. Adult patients (age, >18 years) for whom there was clinical suspicion of TBL or who presented with a ≥2-week history of cervical lymphadenopathy were enrolled. Patients with a diagnosis of nasopharyngeal carcinoma and patients who declined to sign an informed consent form were excluded. Those with fluctuant lymph nodes were also not enrolled, as their lymph nodes were not suitable for biopsy. Demographic and clinical characteristics in the medical records of patients were reviewed. The study protocol was approved by Ramathibodi Hospital at Mahidol University's ethics committee on human rights related to the research of human subjects.

Definitions
Results of mycobacterial culture and histopathological analysis of the excisional biopsy specimen from the lymph node were used as a reference standard for defining TBL. "Definite TBL" was defined as an *M. tuberculosis*-positive culture of a biopsied tissue specimen. "Probable TBL" was defined as an *M. tuberculosis*-negative culture in the presence of histopathological findings compatible with TBL (ie, granuloma with caseous necrosis), with clinical improvement after receipt of antituberculosis treatment.

Procedures
After a patient provided informed consent, an initial FNA specimen was obtained for cytological examination. All patients subsequently underwent excisional biopsy. On the day of biopsy, FNA was repeated just before the biopsy specimen was excised, using the same technique as for the specimen obtained for cytological diagnosis. The aspirated material in the syringe and needle was flushed with 1 mL of normal saline and collected in a sterile bottle for PCR analysis. For excisional biopsy, the excised specimen was divided into 3 parts: the first underwent direct AFB staining and mycobacterial culture, using an automated BACTEC MGIT 960 System (BD

Biosciences, Sparks, MD); the second underwent histopathological examination; and the third underwent PCR analysis.

Real-Time PCR
DNA extraction of the clinical specimens obtained from the flushed FNA and biopsy specimens was performed using the QIAamp DNA Mini Kit (QIAGEN, Hamburg, Germany), according to the manufacturer's instructions [19]. The DNA samples were stored at −30°C for further testing. For detection of *M. tuberculosis*, the COBAS TaqMan MTB Test (Roche Molecular Systems, Branchburg, NJ) was used according to the manufacturer's instructions [20]. The test consisted of *Mycobacterium* genus–specific primers and *M. tuberculosis*–specific probes to detect a DNA sequence within the highly conserved 16S rRNA gene, with an internal control to identify any PCR inhibitor. One mycobacterium-negative control and 1 *M. tuberculosis*–positive control were included in each test run. Amplification and detection of DNA are performed automatically by the COBAS TaqMan 48 Analyzer, which determines the cycle threshold (C_T) for the target DNA along with a positive result. A C_T that is higher than the limit of the test system indicates no detection of target DNA and yields a negative test result [20]. Although determination of the C_T could be used for quantitative purpose [21], this test was validated only for use as a qualitative assay [20].

Statistical Analysis
Because of the lack of prior studies that used the COBAS TaqMan MTB test for diagnosing TBL, the estimated values for sample size calculation were based on a systematic review of conventional PCR, which showed sensitivities and specificities of 2%–100% and 28%–100%, respectively [22], and on an earlier study from our institution, which showed a sensitivity and specificity of 84% and 75%, respectively [23]. On the basis of an estimated sensitivity of 71%–99% (ie, 85% ± 14%) and specificity of 72%–100% (ie, 86% ± 14%), with a 95% confidence interval and a 2-sided α of 0.05, sample sizes of 25 patients with TBL and 24 patients without TBL were estimated. With an expected pretest probability of 50%, 50 patients with cervical lymphadenopathy would provide enough cases. However, to compare the sensitivities between FNA PCR and biopsy PCR, an expected difference of 30 percentage points between the 2 sensitivities was estimated. Therefore, a sample size of 35 patients was estimated to provide 80% power to detect an absolute difference in sensitivity of 30 percentage points (85% vs 55%) at a 2-sided α of 0.05.

Clinical characteristics between groups of patients were compared by the Fisher's exact test (for categorical variables) and the Mann-Whitney *U* test (for continuous variables). Parameters that may influence the biopsy PCR result were analyzed by univariate analysis with the Fisher's exact test.

Multivariate analysis by the binary logistic regression model was performed to determine the independent predictor of a positive biopsy PCR result, using the Enter method for all parameters with a P value of <.25 in the univariate model. The analyses were performed using SPSS, version 19 (IBM, Armonk, NY).

The statistical significance of the difference between each sensitivity and specificity was determined by the Z-test, using Stata/SE, version 10.0 (StataCorp, TX). The 95% CIs around sensitivity, specificity, and likelihood ratio were also determined with Stata/SE.

RESULTS

A total of 73 patients were studied. The patients' median age was 40 years (interquartile range [IQR], 27–53 years), and 21 (28.8%) were male. The majority (60.3%) of the patients were previously healthy (Table 1). At the time of enrollment, 7

Table 1. Characteristics of Patients With and Patients Without Tuberculous Lymphadenitis

Characteristic	Total Cases (n = 73)	TBL[a] (n = 41)	Non-TBL[b] (n = 32)	P
Age, years, median (IQR)	40 (27–53)	37 (25.5–52)	47.5 (27.5–55)	.15
Male sex	21 (28.8)	10 (24.4)	11 (34.4)	.44
No coexisting medical condition	44 (60.3)	28 (68.3)	16 (50.0)	.15
HIV infection	10 (13.7)	4 (9.8)	6 (18.8)	.32
History of TB	8 (11)	4 (9.8)	4 (12.5)	.72
Current anti-TB treatment	7 (9.6)	6[c] (14.6)	1 (3.1)	.13
Unilateral involvement	62 (84.9)	36 (87.8)	26 (81.2)	.52
LNs, no., median (IQR)	1 (1–3)	1 (1–3)	1 (1–2)	.51
Maximum LN size, cm, median (IQR)	2 (1.5–3)	2 (1.5–3)	2 (1–2.9)	.10
Tenderness	14 (19.2)	12 (29.3)	2 (6.2)	.02

Data are no. (%) of patients, unless otherwise indicated.

Abbreviations: HIV, human immunodeficiency virus; IQR, interquartile range; LN, lymph node; TB, tuberculosis; TBL, tuberculous lymphadenitis.

[a] Twenty-nine patients (70.7%) and 12 patients (29.3%) received a diagnosis of definite TBL and probable TBL, respectively.

[b] Eighteen patients had a definite diagnosis, including 7 with lymphoma, 4 with metastatic carcinoma, 3 with nontuberculous mycobacterium infections, and 1 each with sarcoidosis, Kaposi sarcoma, adenoma, and Kikuchi disease, whereas 14 patients had an unestablished diagnosis.

[c] One of 6 patients (16.7%) with TBL who were currently exposed to anti-TB therapy and 28 of 35 (80%) with TBL who were not exposed to anti-TB therapy had a culture positive for *Mycobacterium tuberculosis*.

patients (9.6%) had received antituberculosis treatment. The majority of patients (84.9%) had unilateral lymph node involvement, with a median of 1 palpable lymph node (IQR, 1–3 palpable lymph nodes) and a median maximum size of 2 cm (IQR, 1.5–3 cm). On the basis of biopsy findings, 41 patients (56.2%) received a final diagnosis of TBL, of whom 29 (70.7%) and 12 (29.3%) were considered to have definite and probable TBL, respectively. Compared with patients without TBL, a significantly higher proportion of patients with TBL had tenderness of the lymph node at presentation (29.3% vs 6.2%; $P = .02$). Among patients with TBL, exposure to antituberculosis therapy prior to biopsy was significantly associated with a lower chance of having an *M. tuberculosis*–positive tissue culture (16.7% vs 80%; $P < .01$). Eighteen patients (24.7%) received other final diagnoses, whereas 14 (19.1%) had unestablished diagnoses.

Table 2 shows findings of biopsy PCR and FNA PCR. There were 27 positive results of biopsy PCR, of which 1 was considered a false-positive result. The overall sensitivity and specificity of biopsy PCR for TBL diagnosis were 63.4% (95% CI, 46.9%–77.9%) and 96.9% (95% CI, 83.8%–99.9%), respectively. The likelihood ratio positive was 20.29 (95% CI, 2.9–141.6), and the likelihood ratio negative was 0.38 (95% CI, .25–.57). The group with definite TBL had a significantly higher PCR sensitivity, compared with the group with probable TBL (79.3% vs 25%; $P < .01$). Use of culture as the reference standard revealed that biopsy PCR had a sensitivity and specificity of 79.3% and 90.9%, respectively, whereas the likelihood ratio positive and likelihood ratio negative were 8.72 and 0.23, respectively. There were 7 patients with a positive result of FNA PCR, all of whom received a diagnosis of TBL and tested positive by biopsy PCR. The sensitivity and specificity of FNA PCR were 17.1% (95% CI, 7.2%–32.1%) and 100% (95% CI, 89.1%–100%), respectively. The likelihood ratio positive was infinity, and the likelihood ratio negative was 0.83 (95% CI, .72–.95). The sensitivity of biopsy PCR was significantly higher than that of FNA PCR (63.4% vs 17.1%; $P < .01$), whereas the specificities of these 2 approaches were comparable (96.9% vs 100%; $P = .31$). Among patients with definite TBL, the sensitivities of biopsy PCR and FNA PCR were 79.3% (95% CI, 60.3%–92.0%) and 20.7% (95% CI, 8.0%–39.7%), respectively. For the group with probable TBL, the sensitivities of biopsy PCR and FNA PCR were 25% (95% CI, 5.5%–57.2%) and 8.3% (95% CI, .2%–38.5%), respectively. By use of culture as the reference standard, the sensitivity and specificity of FNA PCR were 20.7% and 97.7%, respectively, and the likelihood ratio positive and likelihood ratio negative were 9.10 and 0.81, respectively.

Comparison of biopsy PCR with other conventional tests revealed that the sensitivity of biopsy PCR was significantly higher than that of the AFB stain (63.4% vs 26.8%; $P < .01$)

Table 2. Sensitivity and Specificity of Real-time Polymerase Chain Reaction of Excisional Biopsy and Fine-Needle Aspiration Specimens for the Diagnosis of Tuberculous Lymphadenitis

Patient Category	Total TBL			Definite TBL			Probable TBL			Non-TBL		
	Patients, Total No.	Patients, No. PCR+	Sensitivity, % (95% CI)	Patients, Total No.	Patients, No. PCR+	Sensitivity, % (95% CI)	Patients, Total No.	Patients, No. PCR+	Sensitivity, % (95% CI)	Patients, Total No.	Patients, No. PCR+	Specificity, % (95% CI)
Biopsy PCR												
All patients (n = 73)	41	26	63.4 (46.9–77.9)	29	23	79.3 (60.3–92.0)	12	3	25.0 (5.5–57.2)	32	1	96.9 (83.8–99.9)
Exclusion of HIV+ patients (n = 63)	37	22	59.5 (42.1–75.2)	25	19	76.0 (54.9–90.6)	12	3	25.0 (5.5–57.2)	26	1	96.2 (80.4–99.9)
Exclusion of anti-TB recipients[a] (n = 66)	35	24	68.6 (50.7–83.1)	28	22	78.6 (59.0–91.7)	7	2	28.6 (3.7–70.9)	31	1	96.8 (83.3–99.9)
Exclusion of HIV+ patients and anti-TB recipients (n = 57)[b]	32	21	65.6 (46.8–81.4)	25	19	76.0 (54.9–90.6)	7	2	28.6 (3.7–70.9)	25	1	96.0 (79.6–99.9)
FNA PCR												
All patients (n = 73)	41	7	17.1 (7.2–32.1)	29	6	20.7 (8.0–39.7)	12	1	8.3 (0.2–38.5)	32	0	100 (89.1–100)
Exclusion of HIV+ patients (n = 63)	37	5	13.5 (4.5–28.8)	25	4	16.0 (4.5–36.1)	12	1	8.3 (0.2–38.5)	26	0	100 (86.8–100)
Exclusion of anti-TB recipients[a] (n = 66)	35	6	17.1 (6.6–33.6)	28	5	17.9 (6.1–36.9)	7	1	14.3 (0.4–57.9)	31	0	100 (88.8–100)
Exclusion of HIV+ patients and anti-TB recipients (n = 57)[b]	32	5	15.6 (5.3–32.8)	25	4	16.0 (4.5–36.1)	7	1	14.3 (0.4–57.9)	25	0	100 (86.3–100)

Total tuberculous lymphadenitis (TBL) was diagnosed if results of histopathological analysis or tissue culture for *Mycobacterium tuberculosis* were positive. Definite TBL was a subset of total TBL in which culture was positive for *M. tuberculosis*. Probable TBL was a subset of total TBL in which culture was negative for *M. tuberculosis* but histopathological findings were compatible with TBL. Non-TBL was diagnosed if results of both tests were negative.

Abbreviations: anti-TB, antituberculosis drugs; CI, confidence interval; FNA, fine-needle aspiration; HIV, human immunodeficiency virus; PCR, polymerase chain reaction; PCR+, positive results of real-time PCR; TBL, tuberculous lymphadenitis.

[a] Exclusion of patients who had recently been receiving anti-TB prior to biopsy.

[b] One HIV-infected patient had recently been receiving anti-TB prior to biopsy.

Table 3. Sensitivity and Specificity of Conventional Tests and Real-time Polymerase Chain Reaction of Excisional Lymph Node Biopsy Specimens for the Diagnosis of Tuberculous Lymphadenitis

Diagnostic Test	Total TBL (n = 41)		Definite TBL (n = 29)		Non-TBL (n = 32)	
	Specimens, No.	Sensitivity, % (95% CI)	Specimens, No.	Sensitivity, % (95% CI)	Specimens, No.	Specificity, % (95% CI)
Histopathological analysis[a]	38	92.7 (80.1–98.5)	26	89.7 (72.6–97.8)	0	100 (89.1–100)
Culture	29	70.7 (54.5–83.9)	29	100 (88.1–100)	0	100 (89.1–100)
AFB stain	11	26.8 (14.2–42.9)	9	31.0 (15.3–50.8)	2	93.8 (79.2–99.2)
Real-time PCR	26	63.4 (46.9–77.9)	23	79.3 (60.3–92.0)	1	96.9 (83.8–99.9)
Addition of real-time PCR to one of the following tests[b]						
Histopathological analysis	41	100 (91.4–100)	29	100 (88.1–100)	1	96.9 (83.8–99.9)
Culture	32	78.0 (62.4–89.4)	29	100 (88.1–100)	1	96.9 (83.8–99.9)
AFB stain	30	73.2 (57.1–85.8)	25	86.2 (68.3–96.1)	3	90.6 (75.0–98.0)
Histopathological analysis or culture[b]	41	100 (91.4–100)	29	100 (88.1–100)	0	100 (89.1–100)

Total TBL was diagnosed if results of histopathological analysis or tissue culture for *Mycobacterium tuberculosis* were positive. Definite TBL was a subset of total TBL in which culture was positive for *M. tuberculosis*. Non-TBL was diagnosed if results of both tests were negative.

Abbreviations: AFB, acid-fast bacilli; CI, confidence interval; PCR, polymerase chain reaction; TBL, tuberculous lymphadenitis.

[a] Granuloma with caseous necrosis.

[b] Results were interpreted as positive if results of at least one of the 2 tests was positive.

but significantly lower than that of histopathological analysis (63.4% vs 92.7%; $P < .01$) (Table 3). The sensitivity of biopsy PCR was also lower than that of culture (63.4% vs 70.7%), but the difference was not statistically significant ($P = .48$). The specificities of all tests were high and comparable with each other (100% [95% CI, 89.1%–100%] for histopathological analysis, 96.9% [95% CI, 83.8%–99.9%] for PCR, and 93.8% [95% CI, 79.2%–99.2%] for AFB staining). The addition of biopsy PCR to conventional confirmatory tests resulted in a higher sensitivity for diagnosing TBL, compared with conventional tests alone, but these differences did not reach statistical significance (100% for biopsy PCR plus histopathological analysis, compared with 92.7% for histopathological analysis alone [$P = .08$], and 78% for biopsy PCR plus culture, compared with 70.7% for culture alone [$P = .45$]). The addition of biopsy PCR to AFB staining of biopsied tissue specimens raised the sensitivity for diagnosing TBL to 73.2%, but this finding was not significantly greater than the sensitivity of biopsy PCR alone (73.2% vs 63.4%; $P = .34$).

For patients with definite TBL, the sensitivity of biopsy PCR was significantly higher than that of the AFB stain (79.3% vs 31.0%; $P < .01$) but was not significantly lower than that of histopathological analysis (79.3% vs 89.7%; $P = .28$). The addition of biopsy PCR to histopathological analysis did not significantly increase the sensitivity for diagnosing definite TBL (100% for both tests vs 89.7% for histopathological analysis alone; $P = .08$). The addition of biopsy PCR to AFB staining

raised the sensitivity for diagnosing definite TBL to 86.2%, but the increased value was not significantly greater than the sensitivity of biopsy PCR alone (86.2% vs 79.3%; $P = .49$).

The median C_T of all specimens with a positive biopsy PCR result was 40.3 (IQR, 36.5–42.1). A comparison of patients with definite and those with probable TBL showed a lower median C_T in the group with definite TBL (38.7 vs 42.7; $P = .02$). The C_T of one positive biopsy PCR result in the group without TBL was 42.1.

The results of FNA cytological analysis were available in 62 of 73 cases. Compared with FNA PCR, compatible FNA cytological analysis [24] had a significantly higher sensitivity (78.4% for cytological analysis vs 16.2% for FNA PCR; $P < .01$) but a lower specificity (80% vs 100%; $P = .02$) for diagnosing TBL (Figure 1). The addition of FNA PCR to FNA cytological analysis raised the sensitivity to 81.1% (95% CI, 64.8%–92.0%), but this value was not significantly higher than the sensitivity of cytological examination alone ($P = .77$).

Results of analysis of factors associated with a positive biopsy PCR result are shown in Table 4. There was a nonstatistically significant trend toward a negative result of biopsy PCR among patients recently exposed to antituberculosis therapy. According to results of binary logistic regression analysis, the only parameter that showed a significant association with a positive result of biopsy PCR was a tissue culture positive for *M. tuberculosis*.
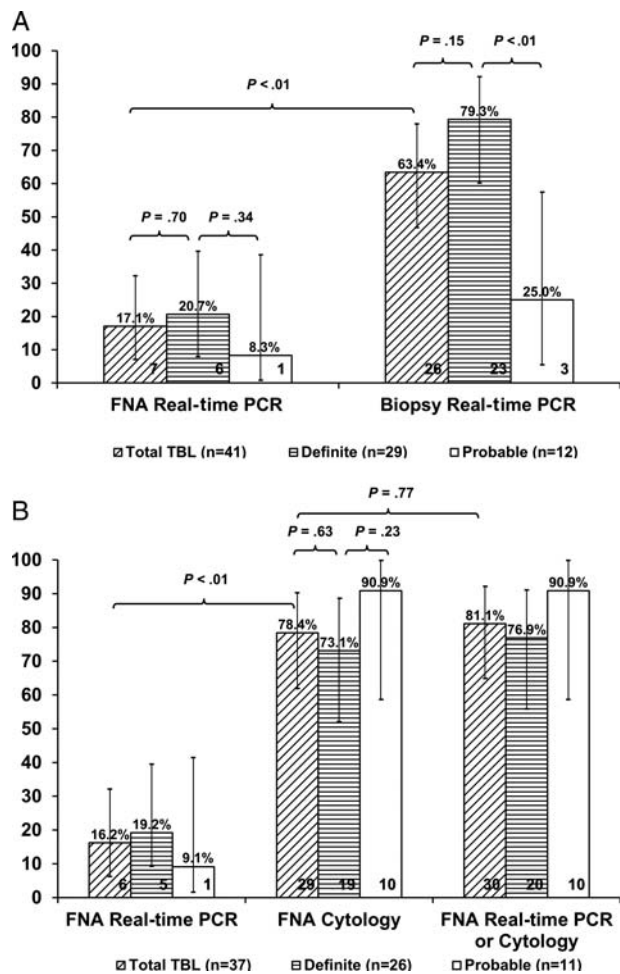
Figure 1. *A*, Comparison of the sensitivities of real-time polymerase chain reaction (PCR) of specimens obtained by fine-needle aspiration (FNA) or excisional biopsy (n = 73). *B*, Comparison of the sensitivities of real-time PCR, cytological analysis, and a combination of real-time PCR and cytological analysis of FNA specimens (n = 62). Eleven cases were excluded because of unavailability of results of FNA cytological analysis. Error bars show 95% confidence intervals. FNA cytological patterns that are compatible with tuberculous lymphadenitis include (1) epithelioid granuloma without necrosis, (2) epithelioid granuloma with necrosis, or (3) necrosis without epithelioid granuloma [24]. Abbreviations: FNA, fine-needle aspiration; PCR, polymerase chain reaction; TBL, tuberculous lymphadenitis.

DISCUSSION

Despite a growing number of studies showing promising results of NAAT for rapid detection of *M. tuberculosis* in respiratory specimens [17], there are relatively few data on the use of NAAT to evaluate nonrespiratory specimens, especially those obtained from lymph nodes [17, 22], and of these data, there have been conflicting results with regard to the diagnostic performance of NAAT [22]. In the present study, we used the COBAS TaqMan MTB Test, a real-time PCR–based system,

for direct *M. tuberculosis* detection in cervical lymph node specimens in a tuberculosis-endemic country [18]. To our knowledge, we are among the first few investigators [25, 26] who have studied real-time PCR for direct detection of mycobacteria in lymph node specimens.

Recently, excellent results were shown by the COBAS TaqMan MTB Test for direct detection of *M. tuberculosis* in respiratory specimens, with an overall sensitivity of 92% [5]. Compared with the COBAS AMPLICOR MTB Test [27], the newer TaqMan assay has shown a higher sensitivity for detecting *M. tuberculosis* in respiratory samples [28]. According to Yang et al [5], the TaqMan assay previously performed well in the analysis of respiratory specimens and was highly sensitive for smear-negative samples, suggesting a probable benefit for testing specimens that contain a smaller number of *M. tuberculosis* colony-forming units. Nevertheless, the present study revealed that the TaqMan test did not perform as well in the direct detection of *M. tuberculosis* in lymph node specimens.

Results of our PCR analysis of an excisional biopsy specimen were comparable to findings from 1 study that used the AMPLICOR test [29]. However, we observed that the TaqMan test had a significantly lower yield in the analysis of FNA specimens: the sensitivity was <20%, which was an unexpectedly lower than the sensitivities of 47%–75% reported by studies that used the AMPLICOR test [29, 30].

In previous studies, PCR inhibitors were detected in a higher proportion of extrapulmonary specimens, compared with respiratory specimens [31, 32]. However, the PCR system used in this study has the ability to identify PCR inhibition and to report specimens containing such inhibitors as invalid. In our study, only 3 specimens, all of which were obtained by biopsy, were reported as invalid. Therefore, other factors are likely responsible for the test's decreased performance.

TBL has several unique features. First, the infection is paucibacillary in nature [33]. Second, the organisms tend to clump together [34] and are mostly unequally distributed in the specimen [35]. Third, a walled-off epithelioid granuloma is a common pathological reaction [36], while an abscess formation is not present in most cases [37]. In our study, the lower sensitivity of FNA PCR might be explained by a smaller volume of specimen obtained by FNA, as compared to tissue biopsy, given that almost none of our patients' lymph nodes were fluctuated and that only a small amount of aspirated material could be obtained from these nodes. In a study involving pediatric patients with mycobacterial lymphadenitis, the sensitivity of real-time FNA PCR was higher than that of biopsy PCR [26]. However, the volume of aspirated material and the interval between FNA and biopsy were not mentioned, and the majority of infecting organisms were *Mycobacterium avium*. A higher specimen volume has been previously shown to increase the sensitivity of the test, especially for AFB

Table 4. Parameters That May Influence the Result of Real-time Polymerase Chain Reaction Performed on Lymph Node Biopsy Specimens From 73 Patients

Parameters	Total (n = 73)	Positive Real-time PCR (n = 27)	Negative Real-time PCR (n = 46)	P^a	Adjusted P^b
History of tuberculosis	8 (11)	2 (7.4)	6 (13)	.70	
Current anti-tuberculosis treatment	7 (9.6)	2 (7.4)	5 (10.9)	1	
Positive AFB stain	13 (17.8)	7 (25.9)	6 (13)	.21	.50
Compatible histopathologic findings[c]	38 (52.1)	24 (88.9)	14 (30.4)	<.01	.20
Positive culture for *M. tuberculosis*	29 (39.7)	23 (85.2)	6 (13)	<.01	<.01

Data are number (%) of patients.

Abbreviations: AFB, acid-fast bacilli; *M. tuberculosis*, *Mycobacterium tuberculosis*; PCR, polymerase chain reaction.

[a] Univariate analysis by the Fisher's exact test.

[b] Multivariate analysis by the binary logistic regression model, using the Enter method for all parameters with a *P* value of <.25 in the univariate model.

[c] Granuloma with caseous necrosis.

smear–negative specimens [38]. Type of study design, the consistency of specimen collection, the selection of the target of DNA amplification, the type of NAAT, and the reference standards should also be accounted for in the difference in the reported test performance [22].

Unlike respiratory specimens, for which a higher yield of the NAAT was noted for AFB smear–positive samples [5, 17], we did not show that a positive AFB stain increased the diagnostic yield of the test, but the low proportion of AFB stains with positive results in the present study should be considered. Of note, we showed that the combination of real-time PCR with conventional confirmatory tests, including cytological analysis, histopathological analysis, and culture, did not significantly improve the sensitivity for diagnosing TBL and potentially decreased the specificity. However, a sample size that was calculated not to compare the finding between different test methods but to evaluate the real-time PCR might have to be taken into account as a possible explanation for the statistically nonsignificant results.

Although the sensitivity was only fair to good overall, the specificity of the TaqMan assay was high, probably because of the use of stringent histopathological criteria to minimize the chance of misclassifying culture-negative TBL as non-TBL, the use of primers and probes that were specifically designed to target DNA sequences within the highly conserved 16S rRNA gene of *M. tuberculosis* [20, 22], and the lower chance of DNA cross-contamination for the real-time PCR technique [21].

Despite a low sensitivity, the application of real-time PCR might still be of interest in cases of nondiagnostic histopathological analysis because of the much shorter time to results. When likelihood ratios were taken into account, the positive real-time PCR result would significantly raise the probability of TBL, while the negative result slightly lowered the probability of TBL [39]. In other words, TBL could not be ruled out by the negative real-time PCR test result.

From our finding, we illustrated that culture positivity was strongly associated with real-time PCR positivity. This piece of information underlines the possibility that direct real-time PCR testing for *M. tuberculosis* would be less beneficial among cases of refractory lymphadenopathy that had been exposed to antituberculosis treatment.

For quantitative real-time PCR, the C_T had an inverse relationship with DNA load [21]. However, the COBAS TaqMan MTB Test has not been validated for quantitative analysis [20]. Here, we showed that the C_T may be helpful in indicating true and false positivity of the real-time PCR test. The lower C_T might represent culture-positive specimens, and the high C_T might suggest false-positive results of biopsy PCR, particularly when used with other diagnostic histopathological analysis.

Our study was conducted in a country where the tuberculosis incidence is high [18], which allowed an opportunity to include a high number of cases with an index of suspicion for tuberculosis. Nevertheless, there were a few limitations in the present study. First, we excluded cases that were not suitable for excisional biopsy, as histopathological analysis was part of the reference standard. During the study period, 6 culture-positive TBL cases that presented with fluctuating lymph nodes were not enrolled. The sensitivity of FNA PCR might have been higher if all cases with fluctuating lymph nodes had been included, because fluctuant lesions are ideally approached with FNA [16]. Second, we did not evaluate the sensitivity of culture and direct staining of FNA specimens, given the small volume of samples obtained. Therefore, the sensitivity of culture, compared with that of FNA PCR, is unknown.

Our data raise uncertainty about the performance of NAAT for direct detection of TBL, given the differences in

NAAT performance between our study and other available studies [25, 29, 30].

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