Comparison of Polymerase Chain Reaction With Histopathologic Features for Diagnosis of Tuberculosis in Formalin-Fixed, Paraffin-Embedded Histologic Specimens

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Objective.—To investigate the relationship between various histopathologic features and the results of the tuberculosis (TB)—polymerase chain reaction (PCR) method in routinely submitted histologic specimens for the histopathologic diagnosis of TB.

Design.—We used 95 formalin-fixed, paraffin-embedded tissue blocks from 81 patients who were clinically suspected of having TB. We assessed the presence of histopathologic features including well-formed granuloma, poorly formed granuloma, caseous necrosis, and Langhans-type giant cells. We performed nested PCR for IS6110 and Ziehl-Neelsen staining for acid-fast bacilli (AFB).

Results.—Of the 81 patients studied, 53 patients had chronic granulomatous inflammation, whereas 28 patients had only chronic inflammation without definite granulomatous inflammation. Of the 53 cases with chronic granulomatous inflammation, 17 (32%) were AFB positive and 36 (68%) were TB-PCR positive. Among cases with chronic granulomatous inflammation, the percentage that were positive and negative by TB-PCR differed significantly with the presence of various histopathologic features. All of the 13 cases with well-formed granuloma, caseous necrosis, and Langhans-type giant cells were TB-PCR positive; however, 10 (36%) of the 28 cases with chronic inflammation without granulomatous lesions were also TB-PCR positive.

Conclusions.—TB-PCR is a rapid, sensitive method for the diagnosis of TB in routinely processed formalin-fixed, paraffin-embedded histologic specimens and is readily available in histopathology laboratories. We recommend use of TB-PCR when TB is suspected clinically, especially in cases of chronic inflammation without definite evidence of granulomatous inflammation.


The diagnosis of tuberculosis (TB) depends largely on the microscopic demonstration of acid-fast bacilli (AFB) and on mycobacterial culture. Methods for the diagnosis of TB have improved in recent years, and several molecular techniques have been introduced for clinical use. Of these, polymerase chain reaction (PCR) amplification for the detection of TB in formalin-fixed, paraffin-embedded archival tissues has been widely demonstrated in various tissue specimens, including lung, lymph nodes, and other tissues. Early diagnosis of TB allows prompt and specific antimycobacterial treatment. In terms of the speed of diagnosis, the TB-PCR method is advantageous because sensitivity of the Ziehl-Neelsen stain for AFB is low and bacterial culture for TB is protracted.

In terms of histopathologic diagnosis, TB can be diagnosed only as “a chronic granulomatous inflammation, suggestive of tuberculosis” on a routine surgical pathology report. However, histopathologic features of chronic granulomatous inflammation can be found in various conditions and diseases other than TB, such as foreign body reaction, fungal infection, sarcoidosis, cat scratch disease, leprosy, and brucellosis. Therefore, AFB stain, TB tissue culture, and TB-PCR should be performed to enable a definitive diagnosis of TB. The majority of previous studies have focused on the sensitivities and specificities of the TB-PCR method in formalin-fixed, paraffin-embedded tissues, and few reports have focused on the relationship between the various histopathologic features of TB and the results of TB-PCR. To address this issue, we investigated the relationship between TB-PCR results and histopathologic features, which included the presence of granuloma, Langhans-type giant cells, and caseous necrosis, in histologic specimens routinely submitted for the histopathologic diagnosis of TB.

MATERIALS AND METHODS

Histologic Specimens

We obtained tissue for this study from the archives of the Surgical Pathology Laboratory, Pusan National University Hospital for the years 2000 and 2001. We retrieved 95 formalin-fixed, paraffin-embedded tissue blocks from 81 patients (40 male patients and 41 female patients) who were clinically suspected to have TB. The affected anatomic sites from which tissues were obtained varied for the 81 patients and included lung (13 patients); pleura (13); soft tissues (11); lymph nodes (10); gastrointestinal tract (9), including the stomach, duodenum, ileum, and colon; larynx (7); middle ear (4); chest wall (3); and other (11), namely, the liver, skin, meninx, ureter, peritoneum, kidney, epididymis, eye, breast,

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and gallbladder. We reviewed the clinical records of these patients. For all 81 patients, the clinician in charge made a clinical diagnosis for the presence or absence of TB without knowledge of the PCR results, using clinical, laboratory, and epidemiologic data together with the observed clinical response to antituberculosis or other therapy. Two pathologists assessed the histopathologic features independently. The presence of well-formed granuloma, poorly formed granuloma, caseous necrosis, and Langhans-type giant cells was separately assessed and noted (Figure, A and B). We diagnosed cases having well-formed granuloma or poorly formed granuloma as having chronic granulomatous inflammation, regardless of the presence of caseous necrosis or Langhans-type giant cells. We diagnosed other cases showing only chronic inflammation without the features of granulomatous inflammation as having chronic inflammation. We also performed Ziehl-Neelsen staining for AFB for each tissue block (Figure, C). None of these tissues used in this study were sent for TB culture.

DNA Extraction and Nested PCR for IS6110

We extracted DNA from each block by standard proteinase K digestion followed by phenol-chloroform extraction, followed by isopropanol and ammonium acetate precipitation. To prevent carry-over tissue from contaminating subsequent samples, we cleaned the microtome blade with octane and 100% ethanol after sectioning each sample.

We performed the nested PCR as follows. We amplified a fragment from IS6110 using outer primers corresponding to nucleotides 1907 through 1926 and 2132 through 2151. After the first round of the reaction, we subjected 1 µL of the amplified product to a second round of PCR using the inner primers corresponding to nucleotides 1940 through 1959 and 2108 through 2127, which amplify a 188-base pair fragment (Figure, D). The total reaction volume used in each round was 25 µL. We performed PCR with 1.25 mM dNTP, 0.25 U of Taq polymerase (Perkin Elmer, Branchburg, NJ), 10 pM primer pairs, and template DNA (300 µg/mL) using a thermal cycler 9600 (Perkin Elmer). The PCR cycling conditions were 35 cycles of denaturation at 94°C for 30 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 60 seconds, with a final extension at 72°C for 10 minutes. We analyzed PCR products in a 2% agarose gel stained with ethidium bromide.

We handled all samples under sterile conditions and prepared them in a safety cabinet under a UV lamp. The control samples included known-positive and known-negative formalin-fixed, paraffin-embedded tissues processed in an identical manner and under the same conditions as the test samples. The positive control for PCR was formalin-fixed, paraffin-embedded tissue from lymph nodes that was both AFB and TB culture positive and that produced a 188-base pair PCR product. The negative control sample was formalin-fixed, paraffin-embedded tissue that showed no features of granulomatous inflammation and was AFB negative and TB culture negative. Additional negative controls included substitution of distilled water for the test template and a previous test sample that had yielded negative results.

Statistical Analysis

We evaluated the sensitivity and specificity of TB-PCR by using the clinical diagnosis as the criterion standard. We compared the TB-PCR results with the clinical diagnosis of TB, the results of AFB staining, and the various histopathologic features of the clinical specimens of the individual patients. We determined statistical significance using the chi-square test and the Fisher probability exact test, and significance was accepted at P < .05.

RESULTS

Comparison of Results for Tissue Samples Subjected to Histopathologic Diagnosis, AFB Staining, and TB-PCR

The results obtained from all of the tissue studies are shown in Table 1. In all patients studied, the corresponding tissues were not sent for TB culture; therefore, we could not compare the TB-PCR result with a TB culture result. Of the 81 patients studied, 53 had chronic granulomatous inflammation, and 28 had only chronic inflammation. Of the 53 cases having chronic granulomatous inflammation, 17 cases (32%) were AFB positive, and 36 cases

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es (68%) were TB-PCR positive. Of the 28 cases of chronic inflammation, none were AFB positive, and 10 (36%) were TB-PCR positive. The results of AFB staining and TB-PCR differed significantly between the cases with chronic granulomatous inflammation and the cases with chronic inflammation. That is, positive AFB and TB-PCR results were significantly more common in the cases with chronic granulomatous inflammation than in the cases with chronic inflammation.

A comparison of the results of TB-PCR and the results of AFB staining is shown in Table 2. Of the 46 cases that were TB-PCR positive, only 14 cases (30%) were AFB positive, and of the 17 AFB-positive cases, 14 cases (82%) were TB-PCR positive. Three cases that were TB-PCR negative and AFB positive were attributed to Mycobacterium species other than Mycobacterium tuberculosis (MTB).

### Correlation of TB-PCR Results With Clinical Diagnosis

Of the 81 patients, 55 were clinically diagnosed with TB. Of those 55, 78% (43/55) had chronic granulomatous inflammation and 22% (12/55) had chronic inflammation without definite granulomatous lesions. Compared with the clinical diagnosis, TB-PCR had a sensitivity of 78% (43/55) and a specificity of 88% (23/26). The positive predictive value for TB-PCR was 93% (43/46), and its negative predictive value 66% (23/35). We found that the TB-PCR result correlated significantly with the clinical diagnosis.

### Correlation of the TB-PCR Results With Various Histopathologic Features

A comparison between the various histopathologic features of the cases and their TB-PCR results is summarized in Table 3. We found a statistically significant difference between the TB-PCR-negative and –positive cases with respect to the various histopathologic features. That is, all of the 13 cases with the typical histopathologic features of TB (a well-formed granuloma with caseous necrosis and Langhans-type giant cells) were TB-PCR positive. In addition, TB-PCR results were positive for 89% (24/27) of the cases with poorly formed granuloma with caseous necrosis and for 88% (14/16) of the cases with well-formed granuloma with caseous necrosis. The percentage of cases that were TB-PCR positive was significantly greater than the percentage that were TB-PCR negative for cases with caseous necrosis (86% positive, 30/35), Langhans-type giant cells (83% positive, 20/24), poorly formed granuloma (69% positive, 33/48), and well-formed granuloma (73% positive, 22/30) when these features were assessed individually. However, TB-PCR results were also positive in 10 (36%) of the 28 cases with chronic inflammation without definite granulomatous lesions (Table 1).

### COMMENT

In our study, we used PCR to analyze 95 routinely submitted formalin-fixed, paraffin-embedded tissue blocks from 81 patients with suspected TB. Many reports have confirmed that PCR amplification in formalin-fixed tissues detects TB DNA when only a few genomes are present.\textsuperscript{5,12,13} We used nested PCR for the MTB IS6110 sequence. This sequence is a repetitive mobile genetic element and is a good target for diagnosis because of its specificity and its presence in high copy numbers in most strains of the MTB complex (MTB, Mycobacterium bovis, Mycobacterium tuberculosis, and Mycobacterium bovis BCG).

### Table 1. Comparison of Cases by Histopathologic Diagnosis, AFB Stain Results and TB-PCR Results*

<table>
<thead>
<tr>
<th>Histopathologic Diagnosis</th>
<th>AFB Stain, No. of Cases</th>
<th>TB-PCR, No. of Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>Chronic granulomatous inflammation</td>
<td>36</td>
<td>17</td>
</tr>
<tr>
<td>Chronic inflammation without definite evidence of granulomatous inflammation</td>
<td>28</td>
<td>0</td>
</tr>
</tbody>
</table>

* AFB indicates acid-fast bacilli; TB, tuberculosis; and PCR, polymerase chain reaction.

### Table 2. Comparison Between the Results of TB-PCR and the Results of AFB Staining*

<table>
<thead>
<tr>
<th>TB-PCR</th>
<th>AFB Stain, No. of Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td>Positive</td>
<td>14</td>
</tr>
<tr>
<td>Negative</td>
<td>3</td>
</tr>
</tbody>
</table>

* AFB indicates acid-fast bacilli; TB, tuberculosis; and PCR, polymerase chain reaction.

### Table 3. Correlation Between TB-PCR and the Various Histopathologic Features of Cases*

<table>
<thead>
<tr>
<th>Histopathologic Features</th>
<th>Result of TB-PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td>WFG + CN + LGC</td>
<td>13</td>
</tr>
<tr>
<td>Positive</td>
<td>33</td>
</tr>
<tr>
<td>Negative</td>
<td>22</td>
</tr>
<tr>
<td>PFG + CN</td>
<td>24</td>
</tr>
<tr>
<td>Positive</td>
<td>22</td>
</tr>
<tr>
<td>Negative</td>
<td>26</td>
</tr>
<tr>
<td>CN</td>
<td>30</td>
</tr>
<tr>
<td>Positive</td>
<td>16</td>
</tr>
<tr>
<td>Negative</td>
<td>20</td>
</tr>
<tr>
<td>LGC</td>
<td>26</td>
</tr>
<tr>
<td>Positive</td>
<td>13</td>
</tr>
<tr>
<td>Negative</td>
<td>33</td>
</tr>
<tr>
<td>PFG</td>
<td>15</td>
</tr>
<tr>
<td>Positive</td>
<td>22</td>
</tr>
<tr>
<td>Negative</td>
<td>24</td>
</tr>
</tbody>
</table>

* TB indicates tuberculosis; PCR, polymerase chain reaction; WFG, well-formed granuloma; PFG, poorly formed granuloma; CN, caseous necrosis; and LGC, Langhans-type giant cell.
Mycobacterium bovis bacillus Calmette-Guérin, Mycobacterium africanum, Mycobacterium microti) and its apparent absence in other species of mycobacteria. However, a recent report showed that some MTB strains do not contain the IS6110 insertion sequence in their genomes, which may explain any reduced sensitivity of PCR.14 The nested PCR used in the present study used primers that amplify a short fragment of IS6110, a repetitive insertion sequence of MTB, and has a detection limit of 10 fg (equivalent to 2 MTB cells) when determined on culture-sourced DNA.15-17 In addition, nested PCR is more sensitive than 1-step PCR, especially in some circumstances, such as amplification from formalin-fixed, paraffin-embedded tissue specimens. In view of the higher sensitivity of nested PCR using IS6110, special attention must be paid to the capacity of MTB DNA to remain in the tissue specimen after drug therapy. Hernandez-Pando et al18 showed the persistence of DNA from MTB in superficially normal lung tissue during latent infection, and Salian et al5 suggested that healed tuberculous granulomas, which were culture and AFB stain negative for MTB, would sometimes be positive for MTB DNA. Thus, when MTB DNA is found in tissue specimens, a further evaluation of other laboratory and clinical information is needed, especially before a final diagnosis of TB is made.

In this study, we did not use mycobacterial culture of tissue specimens as a reference for the TB-PCR because corresponding specimens were not submitted for TB tissue culture. Instead, we used the clinical diagnosis as a comparative reference for TB-PCR results. The 78% sensitivity of TB-PCR is similar to or lower than that of other reports.5,8-11 Initially, we believed that TB-PCR was a suitable method for the diagnosis of TB in routinely processed, formalin-fixed and paraffin-embedded histologic specimens. However, application of the PCR method to formalin-fixed, paraffin-embedded tissue has shown several limitations, including DNA structural changes due to prolonged formalin fixation.19,20 Perhaps the most intriguing finding of this study was that a substantial proportion of cases with chronic inflammation but without definite granulomatous lesions were also TB-PCR positive (36%, 10 of 28 cases). In view of the histopathologic diagnosis, TB was diagnosed only as "chronic granulomatous inflammation with caseous necrosis, consistent with tuberculosis." For confirmation, AFB culture and AFB staining results are required. Because of the low sensitivity of AFB staining and the nonavailability AFB culture specimens, TB-PCR would be mandatory for rapid diagnosis. However, the cost of TB-PCR is higher than that of routine morphologic examination and AFB staining, so we examined morphologic parameters of chronic granulomatous inflammation, which might enable prediction of the results of TB-PCR. In our study, all of the cases with the typical histopathologic features of TB (well-formed granula with caseous necrosis and Langhans-type giant cells) were TB-PCR positive. In addition, 69% (33/48) of the cases with only poorly formed granula were positive by TB-PCR. These results suggest that the classic histopathologic features of chronic granulomatous inflammation are compatible with the TB-PCR results. It is noteworthy that more than one-third of cases with chronic inflammation but without definite granulomatous lesions (36%, 10/28) were also positive by TB-PCR. Immunosuppression or the small size of biopsy specimens might explain these results. In immunosuppressed patients, such as patients infected with the human immunodeficiency virus (HIV), the tissue reaction to TB includes a spectrum of changes that may not involve the presence of granuloma, despite the presence of TB pleuritis.21 In addition, because the size of submitted histologic specimens is usually small, fully developed histopathologic features of TB, such as a well-formed granuloma with caseous necrosis, are infrequently seen. In our study, of the 10 TB-PCR–positive cases having only chronic inflammation without granulomatous inflammation, 7 cases had only small biopsy specimens, and 1 case was that of an HIV-infected person. Therefore, if TB is suspected clinically, we recommend use of the TB-PCR method, especially in cases with small biopsy specimens or cases from immunosuppressed patients, even when cases show only chronic inflammation without definite evidence of granulomatous inflammation.

In conclusion, TB-PCR is a rapid, sensitive method for diagnosing TB in routinely processed formalin-fixed, paraffin-embedded histologic specimens, and it is readily available in histopathology laboratories. However, laboratories should implement strict controls to avoid contamination and false-positive results. We recommend using a combination of AFB staining, histopathologic features of specimens, and TB-PCR to definitively confirm the diagnosis of TB in routinely submitted formalin-fixed, paraffin-embedded specimens.

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References


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