Do the eyes have it? Performance of molecular detection of tuberculosis on fresh and paraffin embedded tissues, including those with no visible tissue

Health
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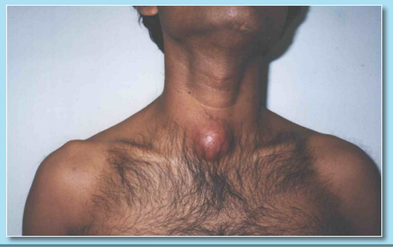
Background
Little work has been done on the performance of tuberculosis PCR with respect to the quality of tissue specimens. Laboratories often receive liquid samples with no visible tissue for testing. The sensitivity of TB PCR on these specimens is unknown.
Methods
Culture and PCR results from all tuberculosis-positive tissues from January 2011 to January 2013 were analysed, noting whether tissue was not visible in the specimen. Sensitivity of microbiological methods were compared, correlating with histopathology and clinical information to ensure specificity. Sensitivity of culture was calculated excluding patients already treated for tuberculosis.
Results
65 patients had 81 positive samples; 69 by PCR and 43 by culture. Using Excluding previously treated patients, 51 of 57 (89%) were positive by PCR, versus 43 of 61 (70%) by culture. There were no false positives. 44 samples with "no visible tissue" were tested. 4 were PCR-positive and one equivocal; one of the five was culture-positive. Two samples with no visible tissue were false negatives, as another sample from the same site was TB-positive.
Conclusion
Sensitivity of TB PCR is superior to culture on tissue specimens. Of the seven patients with no visible tissue and a final microbiological diagnosis of tuberculosis at the site, 4 of 7 had positive PCR tests, one equivocal and two negative. 4 (9% of all tests performed on samples without visible tissue) were not confirmed by another method, and would have been missed had the test not been performed, which indicates testing these samples has utility. The quality of the specimen does, however, deserve comment, as the two (5%) known false negatives are of concern.

Introduction

Extrapulmonary tuberculosis can be difficult to diagnose, as culture and histopathology have long turn-around times, and the sensitivity of microscopy is low[1]. Tuberculosis PCR (TB PCR) can be performed on fresh or formalin-fixed, paraffin-embedded tissues, and provides a rapid method of diagnosis. The sensitivity of PCR on tissue specimens varies widely and has been measured to be from 17 – 90%[1, 2].

Not infrequently, the molecular section of the laboratory has noted that there is minimal, or no, visible tissue present in the specimen container when fine needle aspirates or even core biopsies are received. No data could be found on the sensitivity of PCR when specimen quality was taken into account. We suspected that it could reduce sensitivity of the test. Therefore, we decided to issue a comment highlighting to the requesting doctor that no tissue could be seen in the specimen, and that the impact of this on the sensitivity of the test was unknown. In light of this, the performance of TB PCR on tissues was reviewed, with particular reference to those with no visible tissue.



Methods

TB PCR is performed on fluids and tissues, using different DNA extraction methods. Specimens that appear fluid in consistency are centrifuged, the pellet is sonicated to release DNA, and then extracted using the EasyMag automated platform. Tissues undergo Proteinase K treatment, followed by sonication and manual extraction using the Qiagen minikit. 10 – 20 micrometre sections of formalin-fixed, paraffin-embedded (FFPE) tissues are deparaffinised with xylene prior to a similar extraction. The in-house, real-time PCR targets the 65kDa heat shock protein and IS6110.

All tissues with positive tuberculosis cultures, microscopy or TB PCR examined in the laboratory from January 2011 to December 2012 were included in the analysis. The presence of a comment to indicate that the specimen contained no visible tissue was recorded. Sensitivity of microbiological methods were compared, correlating with histopathology and clinical information to ensure specificity. Sensitivity of culture was calculated excluding patients already treated for tuberculosis.

Results

Positive tissues since Jan 2011	TB PCR positive	Smear positive	Culture positive	Other mycobacterial species
Positive by any microbiological method (81)	69/76 (91%)	9/72 (13%)	43/72 (60%)	None
Gold standard: culture positive (43)	32/38 (92%)	7/43 (16%)		None
Positive by culture or PCR on fresh tissue (73)	63/70 (90%)		43/72 (60%)	None
Positive on FFPE tissue	6/6	N/A	N/A	N/A
Positive with no visible tissue	4/5 (1 equivocal)	1/5	1/5	None
Positive by any microbiological method, excluding patients already treated	51/57 (89%)		43/61 (70%)	

Table 1: *Sensitivity of TB PCR on tissues according to specimen type and previous TB treatment.*

There were 65 patients with 81 tissue samples positive for tuberculosis. All patients had correlating clinical and histopathological evidence of tuberculosis. A comment to indicate that the specimen contained novisible tissue was appended to 44 tissues during this period.

In total 28 specimens from 24 patients were PCR positive, culture-negative. 10 of these 24 patients had previous or current TB; 6 were culture-positive at another site; another 3 had granulomatous inflammation on histology; 3 had other clinical evidence of TB and a response to treatment; one of the remaining was deceased with pneumonia and it was discovered post-mortem; and the other had recent BCG vaccination. Excluding those with prior TB treatment, the deceased patient and the BCG patient, 12 are true false negative cultures **(see Figure 1)**.

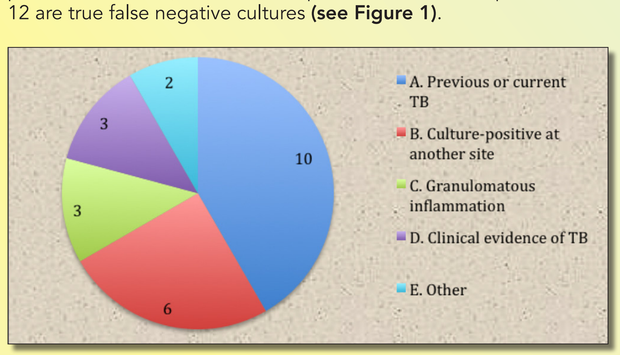


Figure 1: *TB PCR-positive, culture-negative samples. B, C and D represent true false-negative cultures.*

Discussion

Overall, the performance of TB PCR on tissues was excellent, with a sensitivity of 89% compared to 70% for culture in the group of patients who had not already received tuberculosis treatment.

Samples were only included in this analysis if they were positive by at least one method – either smear, culture or TB PCR. This means it is possible that specimens from some patients with tuberculosis have not been detected by any microbiological modality, and these are the ones that concern us. If no visible tissue is received for molecular analysis, it is likely that a small amount has been received for culture also. As evidence that this scenario may occur, four patients had completely negative results on one specimen, but were diagnosed with TB when another similar specimen tested positive. Three of these had no visible tissue seen, and the fourth had no comment issued to that effect. For one patient, this was a fine needle aspirate of a paravertebral collection, where a subsequent pus aspirate was PCR and culture positive. Another patient had a fine needle aspirate of lymph node which was culture and PCR negative; a subsequent biopsy of a lymph node from the same group, in paraffin, was PCR positive, and a bone marrow was culture positive. A third patient had knee tissue tested which was PCR and culture negative; somewhat surprisingly, a wound swab from the same area was PCR positive, but still culture negative. This patient's respiratory samples were culture positive.

As evidence that the testing may be useful, even if no tissue is seen, five patients had positive results. In one, the smear was strongly positive and culture was positive. In the other four, TB PCR was the only positive test. In one, the first was equivocal, prompting a repeat sampling procedure. Two were culture negative and in the other, culture was not performed.

Conversely, if no comment was made and some visible tissue can be assumed, there were 29 tuberculosis-positive specimens received after October 2011. 2 were PCR negative, culture-positive, giving a comparative sensitivity for PCR of 93%.

Regarding the paraffin-embedded specimens, there were 6 positives from 4 patients; none had been cultured. This is thus a very useful test, as the diagnosis would not have been microbiologically confirmed in these 4 patients otherwise. A single culture-positive specimen which also had PCR after formalin fixation was found to be negative. Of course, there may be other cases where PCR on paraffin specimens that have not been cultured is negative, and these cases would not be confirmed at all.

Conclusion

TB PCR has a superior sensitivity compared with mycobacterial culture for the diagnosis of TB on tissue specimens. Of the six patients with no visible tissue and a final microbiological diagnosis of tuberculosis at the same site, 5 of 7 specimens had positive tests, and two were negative. Overall, the numbers of tests are too low to provide any statistical inference of the usefulness of this test, and the lack of a gold standard for patients with negative cultures means that the true sensitivity of the test is unknown. However, 4 of the 5 were not culture positive, and would have been missed had the test not been performed. This makes it useful to perform TB PCR on specimens without visible tissue. In total, 44 specimens without visible tissue were tested, including at least 2 (5%) false negatives; an unknown number of these may have been from patients with tuberculosis. A comment alerting the clinician to the potential reduced sensitivity of testing on such a poor sample should be issued.

References

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