Tuberculosis in children—is PCR the diagnostic solution?

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Polymerase chain reaction (PCR) has been recently incorporated as a diagnostic tool for the diagnosis of tuberculosis. The benefit of rapid results and greater sensitivity compared with traditional microbiological methods makes PCR a suitable technique in childhood tuberculosis, especially when diagnosis is difficult or when urgent diagnosis is needed. However, the possibility of false-positive results must be considered, especially if the clinical and epidemiologic context of the child make the diagnosis of tuberculosis improbable. The commercial ‘Amplicor PCR test’ lacks good sensitivity and specificity and it would be necessary to develop other commercial easy-to-use PCR kits that provides better yield.

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Tuberculosis continues to be a major health problem in the world. Annually, in developing countries, there are 1.3 million cases of tuberculosis among children under the age of 15 and 450 000 deaths resulting from the disease [1]. Thus, rapid diagnosis is essential for the prompt initiation of appropriate therapy and avoidance of progression of the disease [2].

The diagnosis of tuberculosis in children differs considerably from that in adults, because of the difficulties in obtaining microbiological confirmation. Pre-adolescent children usually develop paucibacilar forms of the disease and do not expectorate sputum easily. Consequently, conventional microbiological tests are frequently negative. Early-morning gastric aspirates from hospitalized children are the best clinical samples for acid-fast stain and mycobacterial culture [3]. Gastric aspirates from outpatients [4], induced sputum [5] or nasopharyngeal aspirates [6] would reduce the necessity of hospitalization, but the yield of these samples needs to be evaluated with more studies.

Acid-fast stain of gastric aspirates is usually negative in children with tuberculosis, and recovering several samples from each child who is positive is possible in less than 15% [7,8] of the cases. Although smears are usually specific for active tuberculosis [9], false-positive results have been reported [8,10] which have been attributed to saprophytic mycobacteria. These problems reduce the clinical utility of smears as a rapid diagnostic method in childhood tuberculosis.

The isolation of Mycobacterium tuberculosis from gastric aspirates from children with tuberculosis is also difficult. Mycobacterial cultures require several weeks and are positive in only 25–50% of the cases [8,11,12]; this rate increases considerably in infants less than 1 year of age [13]. Furthermore, the culture yield from other clinical samples such as bronchoalveolar lavage or cerebrospinal fluid (CSF) is even lower [14,15]. New liquid systems have not increased the sensitivity but have reduced the time to results to 3 weeks, which is still excessive when evaluating a child with suspected tuberculosis.

The limits of conventional bacteriology have stimulated the application of new diagnostic techniques. Great improvements in the direct detection of M. tuberculosis have resulted from methods using nucleic acid amplification procedures. In recent years, PCR has been used as a diagnostic tool in adult tuberculosis, with sensitivities of 95% in smear-positive samples and 48% in smear-negative samples [16]. Nevertheless, few studies have
evaluated the clinical utility of PCR in childhood tuberculosis, due to the relatively low number of children with the disease and the complexity of obtaining clinical samples.

According to the various studies published, the sensitivity of in-house PCR in assessing samples of gastric aspirates from children with pulmonary tuberculosis varies between 40% and 83% [17–21]. The reasons for this variability are the lack of uniformity in the methodology of sample processing, the amplified target of *M. tuberculosis* and the way detection of amplified DNA is performed. A study of 68 children obtained a sensitivity of 83.3% when using Chelex particles for DNA extraction and amplification of fragment of the IS6110 insertion element [17]. Consequently, the yield of PCR is much higher than that of the smear, and provides a rapid test in a child with suspected tuberculosis. Moreover, and contrary to what happens with adults, the reported sensitivity of PCR has usually been better than that of culture. This can be explained by the fact that only a small number of organisms is present in the samples from children and that a high proportion of mycobacteria may not be viable in vitro, as a result of the microbactericidal action of immune and inflammatory cells or the reduced viability attendant on processing and decontaminating specimens before culture.

The main clinical benefit of PCR in the diagnosis of childhood tuberculosis has been found in a group of patients who usually have negative smear and culture results: only hilar adenopathy on chest radiograph, no clinical symptoms and unidentified source case, or not from within the household. In these cases, in contrast to traditional methods, PCR sensitivity does not seem to decrease significantly [18]. Another advantage of PCR can be seen in cases of severe tuberculosis, such as meningitis or miliar tuberculosis, where a delay in the diagnosis can have fatal consequences. The results of PCR in cerebrospinal fluid and other clinical samples from children with tuberculous meningitis can assist in the rapid diagnosis and prompt commencement of specific therapy [19].

PCR can detect nucleic acids from dead as well as live *M. tuberculosis* and therefore can remain positive for long periods in patients undergoing tuberculosis therapy. Thus, this method should be used only for the initial diagnosis and not for follow-up evaluations of patients who are receiving antimycobacterial drugs [22]. Nevertheless, this characteristic of the PCR can be used to corroborate the clinical diagnosis of children undergoing specific therapy when the radiologic evolution seems to be unsatisfactory. Children with a clinical diagnosis of tuberculosis usually start specific therapy without microbiological confirmation, due to the low yield of conventional microbiological techniques. However, in spite of appropriate therapy, some children present increases in the parenchymal lesion and the size of the lymph nodes, as well as atelectasis and air trapping. In these cases, if the diagnosis is uncertain, PCR can be a complementary tool that supports the diagnosis of tuberculosis in the child receiving antituberculous therapy [18].

The specificity of PCR in children with tuberculosis has been inconsistent. Some of the studies published have found that positive PCR results are specific to tuberculosis [17,18], while others have reported false-positive results in children with non-tuberculous diseases and have obtained a specificity of 80–90% [19,20]. These false-positive results have been attributed to contamination with exogenous DNA or amplicon, and force each laboratory to take extreme measures to avoid contamination. Consequently, positive PCR results should always be interpreted carefully, taking into consideration the clinical and epidemiologic context of the child with suspected tuberculosis.

A controversial aspect of PCR in the diagnosis of children with tuberculosis is that positive results have been reported not only in children with tuberculous disease, but also in those with tuberculosis infection without apparent disease [17–21]. This puts into question the ability of PCR to distinguish between infection and disease in children [19]. Nevertheless, a study concluded that children with tuberculosis infection and positive PCR results had mediastinal adenopathies on CT-scan that were not evident on chest radiographs [18]. This group of patients could present microbiological activity detected not only by PCR, but also by culture [8]. Consequently, these would not be false-positive results but rather a consequence of PCR’s higher sensitivity.

Recently, a commercial PCR kit (AmpliCone test, Roche Diagnostic Systems, Branchburg, NJ, USA) has been developed to avoid the cumbersome and time-consuming ‘in-house’ PCR techniques [23]. Later, a second-generation kit was marketed (Cobas–AmpliCone, Roche Diagnostic Systems) which automates the amplification and
detection, simplifies laboratory set-up, and decreases the amount of hands-on labor [24]. Published studies in children have usually performed ‘in-house’ PCR, and this limits the relevance of their conclusions for clinical practice. Our working group published a study comparing an ‘in-house’ PCR technique and an Amplicor test [25]. The sensitivity of the Amplicor test was lower than that of ‘in-house’ PCR techniques (44% versus 65%) and similar to that of culture (44%). Furthermore, the Amplicor test gave false-positive results in four children with non-tuberculous diseases (specificity: 93%). In another study of 21 children with tuberculosis, Amplicor was positive in only three children (14.3%), although these patients seemed to have a very low bacillary load, because only two children were culture positive (9.5%) and none of them were smear positive [26]. Therefore, the Amplicor test does not seem to be the ideal PCR test for children with tuberculosis.

In summary, the PCR technique has been incorporated as a diagnostic tool for the diagnosis of tuberculosis in recent years. The benefit of rapid results and greater sensitivity compared with traditional methods makes PCR a suitable technique in assessing childhood tuberculosis, especially when diagnosis is difficult or when urgent diagnosis is needed. However, until advances in PCR performance specificity are obtained, positive PCR results must be carefully considered, particularly if the clinical and epidemiologic context of the child makes the diagnosis of tuberculosis improbable. The commercial Amplicor PCR test lacks good sensitivity and specificity, and it would be necessary to develop other commercial easy-to-use PCR kits that provide better yields. In the meantime, PCR will be a supporting tool, together with radiograph and traditional microbiological methods used to diagnose a child with suspected tuberculosis.

REFERENCES