Advances in the Diagnosis of Pulmonary Tuberculosis in HIV-Infected and HIV-Uninfected Children

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The identification of improved diagnostic tests for tuberculosis has been identified as a global research priority. Over the past decade, there has been renewed interest in the development and validation of novel diagnostic tools for pulmonary tuberculosis that are applicable to resource-poor settings. These techniques are aimed primarily at improving detection of the organism or a specific host immune response. Although most studies have focused on determining the accuracy of novel tests in adults, it is likely they will also have the capacity to significantly improve the diagnosis of childhood tuberculosis. Improving the quality of clinical samples obtained from children with suspected tuberculosis remains an important research priority while awaiting validation of novel diagnostic tests. This review will focus on a number of recent developments for the diagnosis of tuberculosis, with a specific emphasis on the application of these new tests to children in settings where tuberculosis is endemic.

Because confirming the diagnosis of pulmonary tuberculosis in young children is challenging, it is likely that childhood tuberculosis remains underrecognized and underreported. Nevertheless, it is estimated that children make up 10%–15% of the total global tuberculosis caseload [1]. Because tuberculosis in children may be rapidly progressive [2] and may more frequently disseminate or involve extrapulmonary sites, diagnostic delay or uncertainty is likely to result in increased morbidity and mortality.

In children, microbiologic confirmation by culture of the organism or demonstration of acid-fast bacilli remains the gold standard, but in practice, this is seldom achieved. First, it is difficult to obtain representative samples because young children are usually unable to expectorate, and extrapulmonary sites may be less accessible for sample obtainment. Second, because cavitary disease is unusual in younger children, results of smear microscopy are often negative, and mycobacterial culture is required.

In routine practice, in high-burden settings, clinicians seldom wait for the results of culture to be available before starting tuberculosis therapy in a child for whom the diagnosis is suspected [3]. This is because of a reluctance to delay therapy in children who may have rapidly progressive illness and also because the sensitivity of culture for diagnosis of tuberculosis in children is thought to be poor; thus, a negative culture result cannot be used as a rule-out test.

In this context, there is an urgent need for improved diagnostic algorithms and rapid and sensitive laboratory tests for tuberculosis in children. This review will focus on a number of advances in diagnosis of pediatric pulmonary tuberculosis in recent years that have resulted in incremental progress and will also highlight recent advances in the diagnosis of adult

tuberculosis that are currently undergoing evaluation in children.

CLINICAL AND RADIOLOGICAL DIAGNOSIS OF CHILDHOOD TUBERCULOSIS IS FREQUENTLY UNRELIABLE

The clinical presentation of pulmonary tuberculosis in childhood is often nonspecific, and the history of illness may be acute [2]. There is considerable subjectivity in the interpretation of radiological findings, particularly hilar lymphadenopathy [4]. These challenges are particularly acute in children infected with human immunodeficiency virus (HIV) [2] in the context of which other opportunistic infections may present with overlapping clinical and radiological findings.

Because childhood tuberculosis typically occurs in resource-poor settings, where access to highly trained health professionals is restricted, scoring systems have been developed to improve diagnostic accuracy. Such systems usually combine clinical and radiological evidence of disease, with a history of tuberculosis exposure or a positive tuberculin skin test (TST) result. There is considerable literature describing the performance of such systems; however, many systems are poorly validated, may not be generalizable to different epidemiological settings, and are not adapted for use in HIV-infected children [5]. A recent evaluation of 9 structured scoring systems clearly reveals the problem [6]. The proportion of 1445 children with suspected tuberculosis who were assigned a tuberculosis diagnosis by the 9 different systems varied from 6.9% to 89.2%. Agreement between these systems was slight, with a median pairwise κ statistic of 0.18. Although such systems may be useful when designed for and validated in particular epidemiological settings [2], caution should be exercised when generalizing the validity of a particular system.

MICROBIOLOGIC CONFIRMATION OF DISEASE IS CHALLENGING

Microbiologic confirmation of tuberculosis in children by culture has not been part of routine care in high-burden settings because of the unavailability of facilities, the difficulty in obtaining samples, the poor performance of smear microscopy, and the perception that microbiologic yield is low. However, several studies have now confirmed that microbiologic confirmation is feasible and useful to exclude drug-resistant tuberculosis [3]. In areas with high HIV and tuberculosis coinfection, confirmation is particularly valuable, because treatment of both infections is associated with pill burden and complex drug interactions. For example, drug interactions may occur between the rifamycins, especially rifampicin, and some of the nonnucleoside reverse-transcriptase inhibitors or protease inhibitors [7]. In HIV-infected children, culture confirmation may also be

important to distinguish nontuberculosis mycobacterial infection and disseminated bacille Calmette–Guérin from *Mycobacterium tuberculosis* disease.

Methods Used to Enhance Specimen Recovery

Gastric Aspiration. For many years, 3 consecutive early morning gastric lavage (GL) or aspirate (GA) samples have been the standard of care. However, the culture yield from GL has been disappointing; specimens must be taken on 3 sequential days for optimal yield, and the procedure is unpleasant, relatively invasive, and usually requires hospitalization. Thus, GL is difficult in ambulatory settings, where most children seek care.

Sputum Induction. Sputum induction has been successfully used in a number of studies as an alternative to GL to obtain a lower respiratory tract specimen for culture diagnosis. After pretreatment with an inhaled bronchodilator, nebulization with hypertonic (3%–5%) saline is performed, and secretions are obtained by suctioning or by expectoration in older children [8].

A number of studies have now shown that collection of induced sputum is feasible, safe, and effective even in infants. In 2 large studies involving hospitalized infants in a tertiary care facility in South Africa (median ages, 9 [9] and 13 [8] months), samples were successfully obtained from 95% of children. In the first study, one induced sputum sample yielded more positive culture results (10% of samples) than 3 did sequential GL samples (6% of samples), although in the second study, the cumulative yield from 3 induced sputum samples (87%) was greater than that of 3 GL samples (65%; $P = .018$ [8, 9]). One induced sputum sample was equivalent to 3 GL samples [8]. The yield was similar in HIV-infected and HIV-uninfected children.

Two studies evaluated the yield from sputum induction in less ill children presenting for care at a peripheral level. Among children with mild illness admitted to a case-verification ward as part of an infant tuberculosis vaccine trial, the yields of single induced sputum and GL samples were equivalent; however, positive culture results (from 2 GL to 2 induced sputum samples) were obtained for only 10% of admitted children [10]. More recently, a study performed in a primary care clinic in South Africa, where 270 children (median age, 38 months) with suspected tuberculosis had 2 induced sputum specimens obtained on sequential days, reported microbiologic confirmation in 11% of cases. Of importance, microbiologic confirmation improved the diagnostic yield, identifying an additional 18 children who would not have received treatment based on clinical judgment, an increase in diagnostic yield of 22% [11].

Induced sputum samples have been successfully used for confirmation of childhood pulmonary tuberculosis in other high-burden countries [12].

Precautions must be taken to prevent nosocomial transmission during sputum induction. The procedure should be

performed in a well-ventilated room equipped with UV lighting or in the open air, and sufficient time should be allowed between procedures. Appropriate particulate respirators (N95 or FFP2) should be used by staff.

Sputum induction has a number of advantages over GL. It can be performed as an outpatient procedure, it is relatively easy to perform, and the yield is higher. Combined data from a number of studies involving infants and young children that included thousands of sputum induction procedures show that sputum induction is safe, without any documented serious adverse events [8–10, 13]. The remaining challenge is to change health care workers' perception that microbiologic diagnosis is not possible in children and to achieve more widespread implementation of sputum induction in children in health care facilities.

Nasopharyngeal Aspiration. Nasopharyngeal aspiration (NPA), achieved by passing a cannula through a nostril into the nasopharynx, is an attractive diagnostic procedure because it is minimally invasive and easy to perform. Early studies involving persons with suspected tuberculosis from Peru [14] and Uganda [15] suggested that the culture yield from NPA was similar to that from GA (30% vs 38%; median age, 5 years [14]) or from sputum induction (24% vs 22%; median age, 48 months [15]). In contrast, in Yemen ($n = 213$; median age, 5 years) [16], solid mycobacterial culture results were positive for 7% of NPA samples, 9% of GA samples, 8% of expectorated sputum samples, and 14% of induced sputum samples. Similarly, in 2 Peruvian studies involving 165 [17] and 218 [18] children, the yield from NPA samples was lower than that from GA samples (3.8% vs 6.8% and 10% vs 5.5%, respectively).

Alternative Methods to Augment Specimen Recovery. Alternative methods include bronchoalveolar lavage (BAL), obtainment of stomach contents with use of a string test [19], ear swabs, and fine needle aspiration of an enlarged lymph node [20]. BAL is a resource-intensive and invasive procedure that has a lower yield for mycobacterial culture, compared with GL; therefore, BAL is not indicated for microbiologic confirmation of tuberculosis [21]. The string test is not suitable for young children (<4 years of age) and has not been well studied in a pediatric population, although it has been shown to be well tolerated by older children (median age, 8 years) [19]. An ear swab provides a useful sample that can be easily obtained but is limited to situations in which there is a discharging ear. Fine needle aspiration and culture is a very useful adjunct to culture of respiratory specimens when an enlarged lymph node is present and when staff have been appropriately trained in the procedure [20].

Noninvasive Specimens
Because young children swallow sputum, stool may contain viable *M. tuberculosis*; however, stool has proved to be a poor specimen for culture in 2 Peruvian studies, with culture yields from stool samples and GA samples, of 1.4% and 6.8% [17] and 1.8% and 10% [18], respectively, possibly because of the difficulty in adequately decontaminating the sample without destroying mycobacteria.

Urine samples are simple to collect and, although unlikely to be culture positive except in cases of urinary tract tuberculosis, may be useful for molecular diagnostics or antigen detection.

Rapid Liquid Culture Methods
Automated liquid culture systems, such as the mycobacterial growth indicator tube (MGIT) (Becton Dickinson) offer significantly more rapid diagnosis than does conventional solid culture (13.2 vs 25.8 days in a meta-analysis) [22]; however, availability is limited because of cost and resource requirements. The microscopic observation drug susceptibility (MODS) technique, which involves visual inspection of growth in a liquid culture system, has been proposed as a low-cost alternative. MODS is substantially more sensitive than solid culture for pediatric samples [18] and offers the advantage of simultaneous detection of drug resistance. Implementation is somewhat complex at present because of the need for reagents from multiple suppliers, and the method requires highly trained operators.

Simple, rapid, and accurate identification of *M. tuberculosis* complex in cultured samples is now possible with use of an inexpensive immunochromatographic assay (Capilia TB) [23].

ALTERNATIVES TO CULTURE FOR DETECTION OF *M. tuberculosis*

Until very recently, the performance of nucleic acid amplification assays (NAAs) for the detection of *M. tuberculosis* DNA or RNA in patient samples has been disappointing. In adults, commercial assays display high specificity (pooled specificity, 97% [24]) but poor sensitivity for smear-negative samples (pooled estimate, 66% [25]). Although there have been no large studies evaluating the performance of these commercial assays in children, in one study, the sensitivity of the Roche Amplicor MTB test was disappointing (44%) [26]. The performance of in-house assays has been highly variable [27], probably because of variability in patient populations, DNA extraction and amplification protocols, and operator experience. As a result, in-house assays have not gained widespread acceptance.

Because NAA results can potentially be delivered in hours or days rather than weeks, a positive NAA result from a highly specific assay (such as the commercially available tests) may be useful to rapidly confirm a diagnosis, even if only for a subset of patients. However, the widespread adoption of NAA has been restricted by the need for specialized molecular laboratories and experienced staff, which are seldom available in high-burden settings.

Polymerase chain reaction (PCR)–based line probe assays, such as the Genotype MTBDRplus (Hain Lifescience), are now

increasingly used for the rapid detection of drug resistance [28]. Such tests offer sensitive and specific detection of isoniazid and rifampicin resistance directly from smear-positive samples or from positive culture samples. These assays have not specifically been assessed in pediatric populations; however, because the majority of pediatric samples are smear negative, their role would be primarily for detection of resistance in positive culture samples. Such assays require dedicated molecular facilities and highly trained operators.

The development of real-time PCR detection of *M. tuberculosis* represents an important advance. Real-time PCR allows simultaneous amplification and detection of PCR product, without the need for manipulation of PCR products and the associated risk of amplicon contamination. Furthermore, real-time PCR permits the detection of several different PCR products in the same reaction, allowing simultaneous detection of tuberculosis and drug resistance mutations.

However, the need for manual sample processing and DNA extraction, both highly operator-dependent steps, remains an obstacle to widespread implementation. There has therefore been considerable interest in the recently published large evaluation study of the GeneXpert MTB/RIF assay in adult patients [29]. This technology uses a cartridge-based automated DNA extraction / real-time PCR amplification and detection system that is virtually operator independent. The test is able to simultaneously detect the presence of *M. tuberculosis* DNA and rifampicin resistance. The performance of the test for adults with suspected tuberculosis was excellent, with a single test having a sensitivity of 98.2% for smear-positive and 72.5% for smear-negative tuberculosis. The specificity of the test among patients without tuberculosis was 99.2%. This technology represents a major advance in delivering highly sensitive NAA technology in a format that is suitable for use at the microscopy center level in developing countries. Results from the first evaluation studies of this technology in pediatric patient populations are expected soon.

Because small fragments of *M. tuberculosis* DNA from disintegrating bacilli in the lungs and elsewhere may be filtered through the glomeruli, the detection of transrenal DNA in urine has been studied. Results from adult studies are, however, extremely variable (sensitivity range, 7%–79% [30]), probably related to differences in patient populations and sample collection and DNA extraction and amplification protocols. The detection of transrenal DNA has not yet been evaluated in a pediatric population.

The detection of mycobacterial antigens, such as lipoarabinomannan (LAM) is an alternative to nucleic acid detection in urine. LAM detection may be achieved in an enzyme-linked immunosorbent assay (ELISA) format or in a lateral flow test, potentially useful at the point-of-care. The sensitivity of a LAM ELISA in adult studies has varied widely [31]; however, the test is most sensitive among patients with tuberculosis and advanced HIV infection. There is a need to evaluate the performance of this test in children with suspected disseminated tuberculosis and those with HIV coinfection.

INTERFERON–γ RELEASE ASSAYS AND TUBERCULOSIS DISEASE

Interferon (IFN)–γ assays (IGRAs) (QuantiFERON-TB Gold assay [QFT-G], QuantiFERON-TB Gold In-Tube assay [QFT-GIT; Cellestis], and T-SPOT.TB assay [Oxford Immunotec]) are increasingly being used as replacement tests for the TST in adults [32]. However, data supporting their use specifically in children are less clear and several questions concerning the use of IGRAs in children remain unresolved [33].

Although a positive IGRA or TST result may indicate recent *M. tuberculosis* exposure to support a diagnosis of tuberculosis disease in a child with suspected tuberculosis, the true sensitivity of IGRAs and TST for the diagnosis of pulmonary tuberculosis can be determined only in children with culture-confirmed tuberculosis disease. Of all the children for whom results of an IGRA are available from published studies, ~2% had culture-confirmed tuberculosis disease. Results from studies that included ≥10 children with culture-confirmed tuberculosis disease suggest a comparable sensitivity of 70%–90% for IGRAs and TST among children in high-income countries (Table 1). Studies that have compared the results of both assays (T.SPOT.TB and QFT-G or QFT-GIT) in children with culture-confirmed tuberculosis disease in these settings appear to indicate a comparable sensitivity between them. By contrast, in low-income settings, IGRAs appear to be potentially less sensitive for the detection of tuberculosis disease in children. The underlying explanation for this discrepancy remains unknown.

In HIV-infected children, the sensitivity of an IFN-γ enzyme-linked immunospot assay (ELISPOT) was significantly higher than TST in young malnourished HIV-infected children in two studies in South Africa [34, 35]. However, a similar sensitivity was reported for QFT-G and TST in 36 HIV-infected children in a study from Romania [36].

Because the gold standard of culture confirmation in children often remains elusive, a diagnosis of highly probable tuberculosis disease is often made on the basis of compatible clinical symptoms and signs, a tuberculosis contact history, and chest radiograph findings consistent with tuberculosis [37]. In many studies, the sensitivity of IGRAs in children with culture-confirmed and highly probable tuberculosis disease is reported to reflect routine pediatric clinical practice. The sensitivity of IGRAs, compared with TST, in studies that have included ≥10 children with culture-confirmed or probable tuberculosis disease is shown in Table 2.

A combination of both TST and IGRA has been found to increase sensitivity in several settings, suggesting that this

Table 1. Comparison of Results From Studies Comparing the Sensitivity of Interferon–γ Assays and Tuberculin Skin Test (TST) in Children With Culture-Confirmed Tuberculosis Disease

Study	Sensitivity, % (95% CI)[a]		
	QFT-G/QFT-GIT	ELISPOT/T.SPOT.TB	TST
Low tuberculosis prevalence			
Kampmann 2009 (n = 25)	0.80 (.59–.93)	0.58 (.37–.78)	0.88 (.72–.96)
Detjen 2007 (n = 28)	0.93 (.77–.99)	0.93 (.77–.99)	1.0 (.88–1.00)
Bamford 2009 (n = 49)	0.78 (.64–.89)	0.67 (.46–.83)	0.82 (.68–.92)
Bakir 2008 (n = 15)	…	0.73 (.47–.89)	…
Haustein 2009 (n = 16)	0.75 (.47–.92)	…	0.67 (.38–.87)
Hermann 2009 (n = 15)	0.78 (.60–.90)	…	0.84 (.67–.94)
Latorre 2009 (n = 13)	0.75 (.46–.91)	0.92 (.7–1.00)	…
Tsolia 2010 (n = 12)	1.00 (.70–1.00)	…	10 (.91)
Cruz 2010 (n = 13)	…	0.92 (.65–.99)	0.77 (.49–.92)
High tuberculosis prevalence			
Nicol 2005 (n = 12)	…	0.83 (.54–.95)	…
Hansted 2009 (n = 23)	…	1.00 (.87–1.00)	1.00 (.87–1.00)
Nicol 2009 (n = 10)	…	0.50 (.23–.76)	0.80 (.49–.94)
Liebeschuetz 2004 (n = 57)	…	0.83 (.76–.93)	0.35 (.21–.51)
Warier 2009 (n = 15)	…	0.53 (.30–.75)	1.00 (.82–1.00)
HIV-infected			
Liebeschuetz 2004 (n = 30)	…	0.74 (.54–.88)	0.36 (.18–.58)
Davies 2009 (n = 22)	…	0.64 (.41–.83)	0.33 (.15–.58)
Stavri 2009 (n = 36)	…	0.47 (.30–.65)	0.40 (.27–.93)

Abbreviations: Includes studies in which ≥10 children had confirmed tuberculosis. CI, confidence interval; ELISPOT, enzyme-linked immunospot assay; HIV, human immunodeficiency virus; QFT-G, QuantiFERON-TB Gold assay; QFT-GIT, QuantiFERON-TB Gold In-Tube assay; TST, tuberculin skin test.

[a] Not every individual in each study had all tests done; stated sensitivity (95% CI) calculated using number who had test as denominator.

may the optimum approach to exclude tuberculosis in children with increased certainty [38–40]. When both tests are used, consideration should be given to the timing of each test with respect to the other. Because the TST reagent includes peptides that are incorporated into IGRA, the potential of a prior TST to boost a subsequent IGRA response remains a concern. Data that have investigated the potential of a prior TST to boost a subsequent IGRA response have reported inconsistent results, depending on the timing of the second IGRA [41, 42].

Reflecting the current target market for IGRAs, the majority of studies involving children have focused on the use of IGRA for the diagnosis of latent tuberculosis (LTBI). Because of the absence of a recognized gold standard, the sensitivity of IGRAs for the detection of LTBI in children is more difficult. Without a gold standard, analysis of the results of IGRAs and TST with respect to a tuberculosis contact history or gradient of exposure is one way to potentially determine the superiority of one test over another for the detection of LTBI. Although results are conflicting, results of IGRAs appear to correlate better with defined *M. tuberculosis* exposure, compared with TST, in most [39, 43] but not all studies [44, 45]. However, in view of the uncertainty and lack of a definitive explanation underlying discordant results between TST and IGRA (mostly TST positive and IGRA negative), coupled with the increased vulnerability of young children to

progress to tuberculosis disease after *M. tuberculosis* exposure, many pediatricians may feel uncomfortable withholding preventive treatment in children with TST-positive and/or IGRA-negative results [33, 46, 47]. The predictive value of a positive TST for the development of tuberculosis disease has been well described in children [48], whereas data on the positive and negative predictive value of IGRAs in children remain scarce [49].

Several recent studies involving children have questioned the performance of the QFT-G and QFT-GIT in young children <5 years of age [50, 51]. In these studies, a great number of indeterminate assay results have been reported, primarily as a result of failure of the positive control response in the assay. Reduced IFN-γ production in response to mitogen stimulation in young children has been described [52], and several studies have shown an age-related change in the magnitude of T helper cell cytokine secretion [53, 54].

The true role of IGRAs for the detection of tuberculosis disease or LTBI in children remains to be determined. In high-income countries where the detection of individuals with LTBI remains the priority, many advocate a role for IGRAs in confirming a positive TST result. In contrast, the role of IGRAs, if any, for the detection of tuberculosis disease or to support a diagnosis in children in low-income countries remains questionable [55]. In these settings, currently available IGRAs

Table 2. Comparison of Results From Studies Comparing the Sensitivity of Interferon–γ Assays and Tuberculin Skin Test (TST) in Children With Definite or Probable Tuberculosis Disease

Study	Sensitivity, % (95% CI)[a]		
	QFT-G/QFT-GIT	ELISPOT/T.SPOT.TB	TST
Low tuberculosis prevalence			
Kampmann 2009 (n = 63)	0.63 (.53–.73)	0.50 (.40–.60)	0.71 (.61–.80)
Connell 2006 (n = 9)	1.00 (.73–1.00)	...	1.00 (.64–1.00)
Connell 2008 (n = 9)	0.89 (.60–.99)	1.00 (.73–1.00)	1.00 (.70–1.00)
Bamford 2009 (n = 195)	0.59 (.53–.65)	0.53 (.46–.59)	0.63 (.56–.69)
Bianchi 2009 (n = 16)	0.94 (.75–.99)	...	0.88 (.63–.97)
Hermann 2009 (n = 32)	0.78 (.64–.88)	...	0.87 (.74–.95)
Cruz 2010 (n = 30)	...	0.77 (.59–.89)	0.87 (.70–.95)
High tuberculosis prevalence			
Nicol 2005 (n = 47)	...	0.72 (.60–.81)	...
Dogra 2006 (n = 11)	0.64 (.40–.83)	...	0.82 (.56–.95)
Nicol 2009 (n = 58)	...	0.40 (.27–.53)	0.52 (.38–.65)
Liebeschuetz 2004 (n = 133)	...	0.84 (.74–.92)	0.81 (.72–.90)
Warier 2009 (n = 53)	...	0.42 (.32–.54)	1.00 (.94–1.00)
HIV-infected			
Liebeschuetz 2004 (n = 30)	...	0.74 (.54–.88)	0.36 (.18–.58)
Davies 2009 (n = 22)	...	0.64 (.51–.75)	0.29 (.17–.46)

Abbreviations: CI, confidence interval; ELISPOT, enzyme-linked immunospot assay; HIV, human immunodeficiency virus; QFT-G, QuantiFERON-TB Gold assay; QFT-GIT, QuantiFERON-TB Gold In-Tube assay; TST, tuberculin skin test.

[a] Not every individual in each study had all tests done; stated sensitivity (95% CI) calculated using number who had test as denominator.

do not appear to fulfill the criteria to impact significantly on the tuberculosis burden. First, commercial IGRAs are unable to distinguish LTBI from tuberculosis disease. Although the search for novel biomarkers with the ability to distinguish between LTBI and tuberculosis disease continues [56], the challenge of incorporating new diagnostic tests into existing tuberculosis programs remains problematic. Second, IGRAs remain operationally too complex and costly for use in such settings. Third, IGRAs provide only indirect evidence of *M. tuberculosis* infection and do not assist with identification of drug resistance.

Additional research is required to identify improved, more accurate, and simple immune-based blood tests for use in young children in high tuberculosis burden settings. In high-income countries, unraveling the underlying reasons behind discordant results between the TST and IGRAs would represent a major advance.

CONCLUSION

The identification of improved diagnostic tests for tuberculosis has been identified as a global research priority. Although test developers typically focus on adult populations, it is likely that many of the new generation of tests will have applicability for childhood tuberculosis. There is a need to continue to advocate for test development targeted at this vulnerable and neglected population and to rapidly assess the performance of novel tuberculosis diagnostic tests in pediatric populations.

Notes

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