Testing for Tuberculosis Infection and Disease: The Expanding Role of Blood-based Assays

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Program Description
Screening and testing guidelines are an important component in the fight against tuberculosis (TB). The U.S. Centers for Disease Control and Prevention (CDC) over the past 15 years has developed and disseminated guidelines regarding the screening and diagnosis of TB that are still not fully implemented in the practice of medicine. The following program will discuss important clinical issues regarding the diagnosis of TB, with particular emphasis on identifying at-risk populations and the role of blood-based assays.

Learning Objectives
After completion of this activity, participants will be able to:
• Describe TB disease trends in the United States
• Identify the racial and ethnic groups disproportionately affected by TB disease
• Evaluate CDC recommendations concerning use of blood-based diagnostics such as Interferon-Gamma Release Assays (IGRAs) in the detection of TB infection
• Define and discuss the advantages and disadvantages of the various TB testing methods

Target Audience
The target audience for this activity includes Family Physicians/ Internal Medicine Specialists, Pediatricians, Nurse Practitioners, and Physician Assistants.

Disclosure of Conflicts of Interest
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The following faculty has reported real or apparent conflicts of interest that have been resolved:
• Kevin Winthrop, MD, MPH has disclosed that he has received research funding from Qiagen, has performed scientific consultant work for Abbvie, Pfizer, UCB, and Genentech, and has participated in data safety monitoring boards for RCTs conducted by UCB, Roche, Abbvie, Janssen, Biogen, Galapagos, and GSK.

The following reviewers and planners have reported real or apparent conflicts of interest that have been resolved:
• Barry A. Fiedel, PhD has nothing to disclose.
• Jennifer S. Smith, PhD has nothing to disclose.
• Robert Schneider has nothing to disclose.
• Elizabeth Stephens has nothing to disclose.

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This activity is supported by an independent education grant from QIAGEN.
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The information in this CME-designated monograph is based upon a Reach MD Grand Rounds Nation program of the same name. The faculty for this activity was Dr. Kevin Winthrop, Associate Professor of Infectious Diseases, Public Health and Preventative Medicine, Oregon Health and Science University, in Portland, Oregon. This monograph has been developed to provide the latest information on the use of various testing technologies and settings of testing for TB in the US, as well as information regarding the management of latent TB infection treatment.

Tuberculosis is a disease caused by the M. tuberculosis complex — this complex of organisms consists of Mycobacterium tuberculosis, but also related pathogens such as Mycobacterium bovis, M. canetti, M. africanum, and M. microti. These other organisms are rarer and much less frequently found in humans. Usually, patients are infected via inhalation of these microorganisms; other unusual modes of transmission include organ transplantation or ingestion of raw milk.

TB is usually transmitted via inhalation — a patient with TB coughs, and those around that person breathe in the TB bacilli and become infected. Those bacilli are taken up by alveolar macrophages, and once inside the macrophage the bacilli will replicate, then, cause apoptosis, and spread throughout the bloodstream. At that point, the body's immune system seeks to contain the spreading infection — there are a number of cytokines and cellular activation steps that subsequently take place. These responses include the TNF response, the interferon gamma response, and the TH1 responses. As the immune system limits the spread of the bacilli, it forms granulomas around the bacilli — these are conglomerations of lymphocytes and macrophages and other cells. They ingest bacilli, and attempt to kill the bacilli -- or at least keep it contained. This granuloma formation is driven by TNF alpha which is an important cytokine. In 90% of individuals, the infection remains latent. Latent TB infection, or LTBI, is a bit of a misnomer, because this is really a dynamic process. Latent TB infection should probably be labeled persistent infection. The bacilli are metabolically active in the latent state; however, they aren't very physiologically active. During active TB, the bacilli are replicating much more frequently and causing inflammation, infection, invasiveness, etc.

For 10% of patients, TB infection is a problem — and that is roughly the percentage that does develop active TB sometime during their lifetime. The highest risk of developing TB after infection occurs in the first two years after exposure. Again, most people develop latent TB and then 10% over their entire lifetime will regress, and the latent TB will activate or progress to active TB. Approximately 50% of that occurs in the first two years after infection. Thus, an individual has an approximate 5% risk of progressing from latent infection to active infection in the first two years after becoming infected, and this risk remains so for the rest of that person's life.

People living in Western Europe and North America have very low rates of TB, primarily less than 20:100,000. Southeast Asia and Sub-Saharan Africa are the areas of highest TB incidence in the world. In the United States, TB incidence continues to decline. In fact, the TB incidence in the United States is at historic lows. In 2013, which was the last full year of data reporting, the incidence rate was 3:100,000.

In terms of the states with the greatest prevalence rates of TB in the US, they are those that generally have the most number of immigrants, or individuals who were born outside the US. California, Texas, Florida, New York — these
are the states with greater ethnic diversity, more immigration and correspondingly, more TB. Alaska and Hawaii also have higher TB rates. Again, this reflects both immigration as well as higher rates of TB in some of the native populations in Alaska.

In terms of the proportion of patients that are developing TB who are foreign-born, in 1993 this was about 30%. In 2013, it increased to approximately 65%. Although the overall percentage of individuals infected with TB has diminished the last four or five years, the proportion of foreign-born individuals with TB has increased. There are less case-to-case transmissions of TB currently than occurred 20 years ago. Most of these cases are individuals who were infected outside the United States, who immigrated to the US, and then at some point after arriving, they progressed from LTBI to active TB.

There are certain races or ethnicities that have higher TB rates. The highest number of cases occurs in the Asian population, 32%; Hispanic is 29%, and black or African American is 22%; In terms of race, rates are decreasing in all races/ethnicities.

As to the primary care provider’s role in diagnosing and managing TB, the United States Centers for Disease Control and Prevention (CDC) published a guidance in 2011. An updated guidance document was published in 2013. The CDC has recognized the importance the primary care providers play, not just internists and family practitioners, but physician assistants, nurse practitioners, and others in acute care settings. The CDC noted that “[Clinicians] play a vital role in TB control throughout communities in the United States. Hospital or clinic-based medical practitioners, including those working in emergency departments, are usually the first source of medical care for persons with TB disease. These providers may also provide ongoing management of TB patients.”

As to determining just when to initiate the diagnostic process for TB infection, one of the most important factors is clinical suspicion. Clinical suspicion is driven by awareness and understanding of the risk factors for TB infection. This will ensure that the right diagnostic tests are ordered, and that the individual receives appropriate clinical management.

Regarding risk factors for TB, the number one risk factor is foreign birth. This is followed by individuals who lived abroad in a country where TB is endemic. Another strong risk factor is having had prior contact with an individual infected with TB. This last risk factor is becoming rarer in the US. However, clinicians should still ask all patients suspected of having TB if they have had contact with a TB patient. Patients should also be asked if they themselves have ever...
been tested or treated for TB. This should be asked of patients known to have been in settings of higher TB transmission, including jails, prisons, or homeless shelters. IV drug use should also trigger a need to ask about, or test, for TB.

Progression to active TB disease has a strong basis in the individual’s immunocompetency status. HIV is one of the more common immunodeficiency states, and it is a strong risk factor for progression from LTBI or latent TB to active TB disease. Other common immunodeficiency/immunosuppressive states include diabetes, end-stage renal disease, and organ transplantation. The use of autoimmune or inflammatory therapies, such as the anti-TNF drugs and other biologic therapies, are also risk factors for activating TB. Silicosis, being underweight, smoking, and having prior bowel surgery also increase the likelihood of activating latent TB.

In regard to screening for latent TB, the two diagnostic tools available to do so are the tuberculin skin test (TST) or the interferon gamma release assays (IGRA). If either test is positive, it would necessitate the obtaining of a chest radiograph.

In regard to the epidemiology of latent TB infection in the United States, data from the federal NHANES database, based on the results of tuberculin skin tests, provides this information. The age group with the highest incidence of LTBI is individuals over 65 years of age — with a prevalence rate of 4.8%. In younger individuals — aged 15-24 years of age — the prevalence rate is 0.6%.

For many years, the tuberculin skin test (TST) was the only available TB test. Purified protein derivative (PPD) is injected into the skin in the subcutaneous space and this causes cellular recruitment and a Th1-type response — interferon gamma release — and activation of T-cells at the site. This inflammatory response results in an induration. The larger the induration, the more likely someone is truly infected with TB. Typically, a 10 mm cut point of induration is used to denote a positive test result; however, if someone is immunocompromised, a lower cut point is often accepted to improve sensitivity in someone who may not mount as great a response. By improving sensitivity, there is a corresponding decrease in specificity. Those two concepts are important — the lower the cut point, the more likely the TB infection is going to be diagnosed; however, it is more likely the result will be a false-positive. In patients at high risk — patients with HIV, patients on biologics or steroids, someone who has been recently infected — there is often a willingness to lower the cut point to 5 mm. There are a number of problems associated with the TST. One, is they are difficult to properly place in the arm. Secondly, they are difficult to read in terms of getting an accurate measurement of the induration. Compared to 30 years ago, healthcare providers are not being properly trained in the administration and reading of the TST. And the proper reading of the size of the induration is critical to making the right decision as to whether an individual is infected with TB. The difference between a healthcare provider reading an induration as 10mm versus 9mm — a difference of 1mm — can be the difference between an accurate diagnosis, and an inaccurate one. False-positive results are often seen with the TST — particularly in individuals who have been vaccinated with BCG. The bovine form of TB that is used for BCG vaccination, can result in a TST being false positive. False-positive TSTs also occur in patients exposed to nontuberculous Mycobacterium. There is also poor positive predictive value in low-prevalence populations. In individuals with low inherent risk factors for TB, many of the positive TSTs that occur in these individuals will be false positives. Finally, allergy is another limitation of the TST. False-negative

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**Problems with TST**

- Poor inter-reader reliability
  - 9 mm (negative) vs. 10mm (positive)?

- False-positives/specificity
  - NTM infection
  - Prior Bacille-calmette Guerin (BCG) vaccination

- Poor positive-predictive value in low prevalence populations (like US)
skin tests, particularly in people who are immunosuppressed or quite elderly, may be seen with the use of TST.

Enter the era of the interferon gamma release assays – IGRAs. Two IGRAs are commercially available. The first is the T-SPOT manufactured by Oxford Immunotec. The second is the QuantiFERON Gold In-Tube test that is manufactured by QIAGEN. These two tests employ similar technology to test for TB. The IGRAs assess response to synthetic overlapping peptides that represent specific M. tuberculosis proteins, such as early secretory antigenic target-6 (ESAT-6) and culture filtrate protein 10 (CFP-10). These proteins are present in all M. tuberculosis and they stimulate measurable release of IFN-γ in most infected persons, but they are absent from BCG vaccine strains and from most nontuberculous mycobacteria.

Whole blood is taken from the patient and this blood is exposed to these antigens, and then the amount of lymphocyte reactivation or interferon gamma production from reactive lymphocytes are measured. The T-SPOT measures the number of cells being reactive, the QuantiFERON Gold In-Tube measures the amount of lymphocyte-produced interferon gamma. Each of these tests is measuring specific responses to TB, consequently, false-positive results due to BCG do not occur with the use of IGRAs. This is an important advantage that the IGRAs have over the TST. Another advantage is that the test requires only a single visit in order to obtain the blood — and there is no need for a follow-up visit. False-positive IGRAs can theoretically occur — these are usually the result of some environmental Mycobacterium or, nontuberculous Mycobacterium that do contain ESAT and CFP.

In 2010 the CDC addressed the role of IGRAs in diagnosing TB in the publication “Updated Guidelines for Using Interferon Gamma Release Assays to Detect Mycobacterium tuberculosis Infection — United States, 2010.” the Guidelines noted that the IGRAs were preferred for people who had been vaccinated with BCG and for those individuals unlikely to return for their skin test reading. For children 5 years of age and younger, the tuberculin skin test is preferred. It is noted that in young children sensitivity could be improved by using both the TST and an IGRA.

An important consideration underlying the diagnosis of TB is the positive predictive value of the test being utilized. Positive predictive value refers to the probability that a positive test is a “true positive.” In terms of the positive predictive value of these tests, these are driven by background prevalence of TB in that given locale. In a locale where there is a high prevalence of TB, the majority of positive tests will be true positives. In a setting that has a very low prevalence of TB, most positive TB tests will, in actuality, be false-positives.

With regard to the relative sensitivity of IGRAs, a case-controlled study was conducted in Peru — a country with a very high prevalence of TB. The study compared the sensitivity of the TST and the QF-IT, a QFT-GIT, and a QFT-IA. The results are shown in the table below:

<table>
<thead>
<tr>
<th>Prevalence</th>
<th>QFT-IT</th>
<th>QFT-IT</th>
<th>TST</th>
</tr>
</thead>
<tbody>
<tr>
<td>1%</td>
<td>4%</td>
<td>0.46</td>
<td>0.08</td>
</tr>
<tr>
<td>4%</td>
<td>85%</td>
<td>0.78</td>
<td>0.08</td>
</tr>
<tr>
<td>80%</td>
<td>99%</td>
<td>0.90</td>
<td>0.08</td>
</tr>
</tbody>
</table>

The positive predictive value for LTBI in low prevalence regions is shown in the table above. The positive predictive value (PPV) for the TST is 0.08, which is much lower than the PPV for the IGRAs.
high prevalence of TB (approximately 60% of the population are infected). This study population encompassed a number of immunocompromised patients with rheumatoid arthritis. These patients were tested with a TST and a QuantiFERON Gold In-Tube assay. In the control group, about 60% of people reacted positive to both tests. In the RA population, however, 45% reacted positive for the QuantiFERON Gold In-Tube assay, and only 27% reacted positive to the skin test — a two-fold difference. It should also be noted that there was a great deal of prednisone use in the RA group. This study demonstrated the greater sensitivity of IGRAs than the TST.

The high sensitivity of IGRAs is particularly important given the pipeline of biologic agents currently under development to manage the spectrum of immunosuppressive diseases. These agents target a number of pathogenic mechanisms — including JAK-inhibitors, anti-IL-6 inhibitors, IL-23 blockers, interferon gamma receptor antibodies, etc. All of these agents will likely increase the risk of TB. So, utilizing diagnostic tests that optimize the detection of TB infection in immunocompromised patients will take on even greater importance.

When screening immunosuppressed patients, there are several cardinal rules to live by. The first is that a priori probability reigns supreme. That is, if a patient’s risk factors, history, and living locale increase their risk for being infected with TB, then a positive screening test is probably a true positive. On the other hand, if a patient’s circumstances are such that they are most likely not infected with TB, then a negative TB test is probably accurate. Consequently, if they have risk factors and they’re immunosuppressed, in order to increase sensitivity the use of two screening tests — the TST and IGRA — is recommended. Finally, if a clinician is suspicious of the result of the screening test, then the test should be repeated. This is particularly true for patients with no risk factors with a positive test. Conversely, it is recommended that someone who’s immunosuppressed and comes from a highly endemic area, such as the Philippines, or if they are on 60 mg of prednisone, and the results of their TB screening test is negative, that they be further evaluated by using additional screening tests.

And lastly, what is the role of nucleic acid amplification testing (NAAT) in TB diagnosis? NAAT is PCR-based testing that looks for sequences specific to MTB. NAATs are very sensitive and very specific. If a patient is suspected of having pulmonary TB, sputum or respiratory samples for smear and culture will most likely be obtained. Dr. Winthrop’s institution recommends obtaining 3 samples. One of those samples is sent for a NAAT, and if the smear is negative that means there are fewer bacilli and it makes these tools less sensitive, probably on the order of 60% sensitive versus 98-99% sensitivity — or 95% sensitivity if the smear is positive. False positive NAAT results are very unlikely. Having screened an individual for latent TB infection, and the results have been confirmed as positive, the next step is determining the optimal management course. This can consist of 9 months of isoniazid therapy, 4 months of rifampin, or the new once-weekly rifapentine-INH combination for 12 weeks. A study published in the New England Journal of Medicine demonstrated that this 3-month, once-weekly therapy was as effective and as safe as 9 months of isoniazid.

In conclusion, blood-based interferon gamma release assays (IGRAs) can play an important role in helping to identify individuals with LTBI infection or active TB disease. Primary care clinicians — family physicians and internists, pediatricians, and NPs and PAs — are on the front line of providing health care services to these individuals and populations, and represent a key force in the efforts to eliminate TB.