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Abstract

Title of Dissertation: An Evaluation of the Latent Tuberculosis Control Program in the United States Military at Accession

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Although the Centers for Disease Control and Prevention (CDC) recommend targeted testing for latent tuberculosis infection (LTBI), the military has continued universal testing for all recruits. Furthermore, it has been suggested that the interferon-gamma release assays (IGRAs) may be more specific and cost-effective than the tuberculin skin test (TST). This dissertation examines the impact of proposed changes in screening policy at accession, including both targeted testing and the use of IGRAs for screening.

The epidemiology of tuberculosis TB in the US military is similar to the general population with an incidence that is low and declining. Risk factors for developing active TB are similar to the general US population. Therefore, effective screening and treatment at time of accession is a critical element of the military's TB control program.

Targeted testing was evaluated in US Army recruits at Fort Jackson using a questionnaire, the TST, both commercially-available IGRAs. Prediction models were

developed which demonstrated that targeted testing based on presence of four risk factors could eliminate 91% of testing with a sensitivity of 79% and specificity of 92%.

TST positive, IGRA negative discordance was strongly associated with increasing BST size, suggesting that cross-reactivity to NTM may be an importance source of this discordance and false positive TSTs. Nevertheless, the tests largely identified different people as positive, suggesting that the majority of positives in heterogeneous, low-prevalence populations are false positives using any of the three commercially-available LTBI diagnostic tests.

Finally, the cost-effectiveness analysis found that targeted testing provided a better value than universal testing, a finding which was robust in sensitivity analysis. The IGRAs had a similar value to the TST but were slightly more costly, although the incremental cost-effectiveness ratios were very sensitive to small changes in model assumptions.

As the US military is a heterogeneous, low-prevalence population, the use of targeted testing is recommended at accession in to military service. The TST and IGRAs were found to have similar performance characteristics and cost. Since the overall specificity of the TST and IGRAs are similar, targeted testing should also be performed when using the IGRAs, as with the TST.

An Evaluation of the Latent Tuberculosis Control Program in the United States Military at Accession

by

James Dominic Mancuso

Doctoral Dissertation submitted to

the Faculty of the Department of Preventive Medicine and Biometrics Graduate Program

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Dedication

I dedicate this work to my wife, Kathy, and our three sons, Alex, Luke, and Jake. They have supported me through all of my educational and military adventures even when it took me away from them for long periods of time. I love them very much and hope that someday the boys understand why their dad likes school so much. My own father and mother, James and Patricia Mancuso, have also been an inspiration and for my education and constant source of loving guidance and advice throughout my life. They are the best examples of the kind of person I hope to be, and all that I am and achieve I owe to them.

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Chapter 1—Introduction

The foundation of the current strategy to prevent tuberculosis (TB) in the US military is universal testing for latent tuberculosis infection (LTBI) using the tuberculin skin test (TST). However, the risk of active TB in the active duty US military is low, with only 10 cases in 2006 and a rate of 1.4 cases per 100,000 person-years from 1998 to 2005, compared to an age-adjusted US rate of 5.0 per 100,000 over the same time interval. Rates of TB in both populations continue to decline. Nevertheless, while testing for LTBI in the US has shifted to targeted testing of only persons at high risk, the military's policy of universal testing for LTBI has resulted in a large volume of testing. Over 250,000 TSTs are performed at accession each year. Many have questioned whether this predominantly low-risk population receives substantial preventive benefit from this therapy. This is mainly due to the low positive predictive value of the test in a low prevalence population, but also because of the many factors that lead to false-positive results, such as: variability in administration, product variability, and cross-reactions to non-tuberculous mycobacteria or Bacille-Calmette Guérin (BCG) vaccine. The military's universal TB testing program is costly, time-intensive, leads to pseudoepidemics of false positive skin tests, puts service members at risk for adverse drug events from therapy, and distracts from more effective TB control efforts such as targeted preventive therapy and aggressive case finding of active TB. Implementation of a targeted testing program is more cost-effective and would reduce unnecessary testing, treatment and adverse events from therapy.

The Global Burden and Epidemiology of Tuberculosis

Tuberculosis remains one of the leading causes of disability and death throughout the world. The World Health Organization (WHO) reported in 2006 that there were 9.1 million incident cases and 1.7 million deaths from TB.[14] Additionally, the challenge of TB control is exacerbated by the problem of multi-drug resistant tuberculosis (MDR-TB), of which there were 0.5 million cases, and by the 0.7 million co-infected with HIV.[14] Despite decreasing rates of TB seen in Western Europe and the US, the epidemics of infection with Human Immunodeficiency Virus (HIV) [15] and MDR-TB [16] are responsible for the increasing rates of TB seen in Africa [17] and Eastern Europe. Furthermore, outbreaks of drug-resistant tuberculosis with high case-fatality ratios have recently been demonstrated in what has been called a "perfect storm" of these risk factors, including HIV co-infection, nosocomial transmission, and extensively drug-resistant tuberculosis (XDR-TB).[18, 19] Finally, one-third of the world's population is considered to be infected with latent TB,[20] creating a vast reservoir for future morbidity and mortality from active TB.

The Epidemiology of Tuberculosis in the United States

TB is also a well-recognized public health problem in the United States, accounting for 11,540 cases in 2009, or a rate of 3.8 per 100,000 person-years.[21] This represents a 11.4% decline from the previous year and was the lowest since national reporting began in 1953. Foreign-born and racial or ethnic minorities make up an increasingly larger

proportion of cases, with 57% of active TB cases occurring among the foreign born.[22] Rates of TB continue to be higher among the foreign-born compared to US-born even more than 20 years after entry into the US.[23] The proportion of TB cases that are HIV-infected has been decreasing since 1993, despite increasing proportions tested for HIV.[24] Nevertheless, HIV infection remains a major risk factor for the development of active TB, and was a major determinant in the resurgence of TB between 1985 and 1992.[25] The incidence of MDR-TB has also been decreasing from 3.5% in 1991[26] to less than 1% in 2006.[27] However, drug-resistant TB remains a major source of concern among public health officials and the community, as exemplified by the 2007 Andrew Speaker case.[28]

Latent tuberculosis infection (LTBI) occurs when an individual is infected with the tuberculosis bacillus, but the infection is contained, does not progress to active disease, and is not contagious. An estimated 11 million persons in the US are infected with latent TB.[29] Without treatment, 5 to 10% of persons with LTBI are thought to eventually develop active tuberculosis,[30] with approximately half of cases occurring within 2 years of infection.[31] Treatment with isoniazid is 70-90% effective in preventing subsequent development of active TB.[32]

Over 100 years ago, the first tuberculin material was developed by Robert Koch. By the 1930s, tuberculin skin testing (TST) had been developed into a screening method to identify those with latent tuberculosis infection (LTBI). For the past four decades, treatment of LTBI as identified by the TST has been an essential component of

tuberculosis control in the US.[33] However, with the decline of the prevalence of TB infection in the US,[13] the TST has had a corresponding decrease in the positive predictive value for LTBI.[34, 35] For this reason, testing for LTBI with the TST is only currently recommended for those at high risk for TB.[31]

The Epidemiology and History of Tuberculosis in the US Military

The history of programmatic screening for tuberculosis in the US military began in World War I. Prior to that, TB patients were sent to sanatoriums or were discharged from service, but no screening program to exclude them from service was in place. In World War I, Colonel Bushnell developed and advocated for a clinical examination which would identify those with active tuberculosis in order to avoid accessing infectious cases into military service.[36] The goal of this program was to not only prevent communicable diseases among soldiers, but also to reduce the administrative burden and costs of separating these soldiers as well as future disability costs. Bushnell's efforts involved hundreds of clinicians and led to 11,020 (0.8%) of potential accessions being rejected from service.[37] Despite these efforts, TB was still the leading cause of disability during the war, accounting for 552 discharges per 100,000 person-years and large economic losses to the US government after the war of over 1 billion US dollars.[38] The rate of active TB in the US military during the war was a staggering 1,200 per 100,000 person-years.

As a result of the recognition of the substantial burden of disease due to tuberculosis in the military, a chest x-ray (CXR) screening program was begun in World War II to better

identify the tuberculous infected. This led to a similar proportion of rejections from service (0.86%)[39] as that seen in the First World War. Although the rate of active TB was 10 times lower than those seen in World War I, it was still quite high, with 124 cases per 100,000 person-years. It was noted that most of the risk of TB during military service reflected the overall risk in the United States. Overseas service did not increase the risk of tuberculosis unless the soldier had been a prisoner of war (POW).[40] After the war, TB control program leaders expressed interest in moving beyond the chest x-ray due to its unsatisfactory performance in identifying TB. In particular, Esmond Long of the Phipps Institute noted not only variability between radiologists, but more importantly, the unsatisfactory performance characteristics of the chest x-ray as a screening tool: "…in order to exclude all infected men, it would have been necessary to exclude also many men…who did not have tuberculosis."[40] He pointed to the next development in tuberculosis control, the use of the TST, suggesting that, "The hope for substantial improvement in screening efficiency rests on the use of…the tuberculin skin test."[40]

Soon after World War II, US military forces were involved in the Korean conflict. Despite high rates of active TB in the local Korean population, no excess TB among military forces was observed. Leedham observed that "In spite of widespread exposure to tuberculosis in the Far East, American troops came away practically unscathed."[41] His evidence for this was that TB continued to decline in US forces, and no differences in rates of TB were noted between deployed and US-based forces. In the Vietnam era, variable conversion rates among deployed service members were reported, but the only one using the TST (rather than the less reliable TB tine) reported that 1% of Sailors and

Marines had a TST conversion after deployment.[42] MDR-TB was increasingly being noted in the US during the Vietnam conflict, and there was again concern that TB was being imported from Vietnam, and particularly drug-resistant TB. However, the rate of MDR-TB among deployed US service members was found to be similar to the general US population.[43] Several outbreaks of TB were noted and documented in sailors during the 1960s, but these were due to activation of latent infection while on the ship and subsequent onboard transmission within the closed environment of Navy ships,[44-47] rather than exposures in Vietnam or other endemic regions. Also during this time, a collaborative arrangement between the US Navy and the Public Health Service resulted in the Navy Recruit Study, which provided some of the seminal data supporting the use of the TST and mapping the geography of tuberculous and non-tuberculous infection in the US.[2, 9, 12, 48-50]

The most recent publications describing the epidemiology of active tuberculosis in the military up to the mid-1990s consist of three service-specific studies.[7, 51, 52] These studies described the incidence of active TB up to 1996. Parkinson found that cases in the Air Force were found mainly among retirees and children rather than active duty.[52] White found that the rate of hospitalization for TB in the Navy was 2.2 per 100,000 person-years in 1994.[7] Although Camarca found a slightly higher rate of TB in the Army in 1996 of 5.1 per 100,000 person-years, this was still three times lower than the rate seen among a similar age group in the US at that time.[51] The current risk of active tuberculosis (TB) in the active duty US military is thought to be lower than the age-adjusted US rate of 5.0 per 100,000.[53]

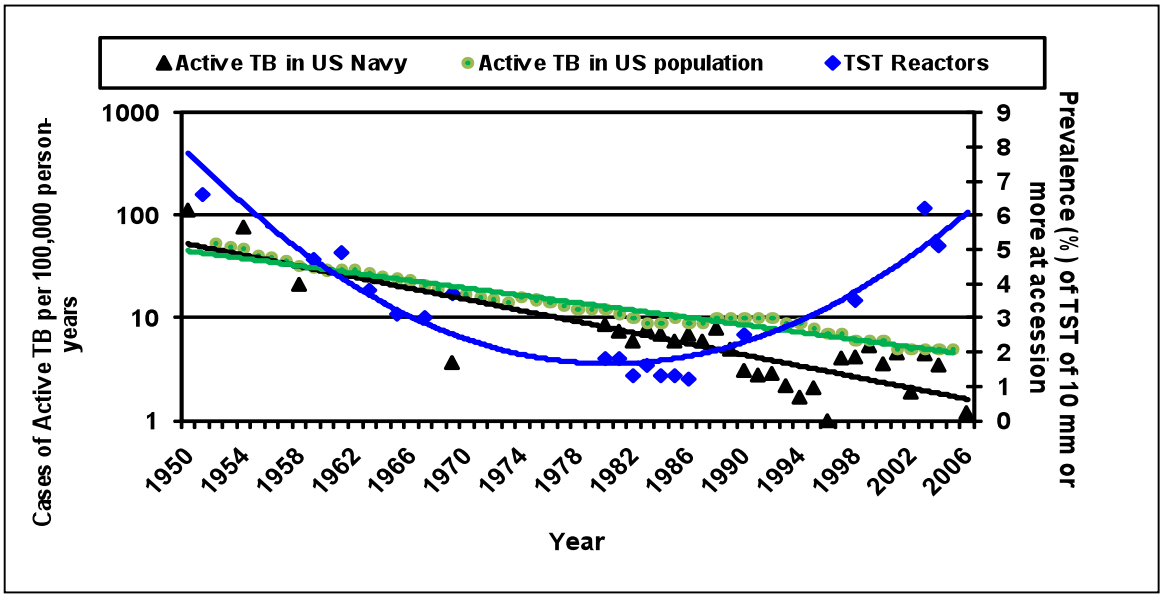
However, no recent data have been presented related to recent increases in deployments or other factors. Concerns regarding TB exposure have been raised in recent deployments to Iraq and Afghanistan, which are reported to have among the highest rates of active TB in the world.[54] Recent deployments to these endemic and hyperendemic areas have resulted in large numbers of potentially infected military service members and large-scale efforts aimed at preventing active TB. Furthermore, no assessments of prevention effectiveness of service-specific screening policies have yet been accomplished. Although no increase in active TB has been reported during that time interval, it is unclear whether this is attributable to prevention efforts or to a low level of exposure during deployments.

Latent Tuberculosis Infection in the US Military

Latent tuberculosis infection (LTBI) also poses a significant public health challenge to military forces. Of the more than 500,000 tuberculin skin tests performed each year among US military forces, most are either done at accession or before and after deployment. Figure 1, constructed from US Navy data contained in multiple sources,[1-10] superimposes the trend of prevalent TST reactions among new US Navy recruits and the incidence rates of active TB in the US Navy. It demonstrates that while the rate of TB in the US Navy continues to decline, the prevalence of TST reactors has fluctuated over time. The rate of active TB decreased from > 100 cases per 100,000 person-years in 1950 to < 1 per 100,000 in 2006. In contrast, over 5% of Navy recruits had TST reactions of 10 mm or more in the 1950s,[9] later decreasing to a nadir of 1.2% in

1986,[4] and then increasing again to 5.1% in 2005.[3] This suggests that although the risk of active TB is diminishing, the TST does not seem to accurately reflect these changes in risk.

Figure 1. Incidence of Active TB in the US Navy and Prevalence of Tuberculin Skin Test Reactors in US Navy Recruits, 1950-2006[1-10]



Furthermore, the absence of cases of active TB attributable to deployment suggests that TST conversions reported after deployment may not confer the same risk for subsequent development of active TB in this population as demonstrated in previous studies.[55] The only recent published study to directly assess deployment-related risk of TB infection was a cross-sectional study done in Dutch soldiers comparing the results of QuantiFERON® (QFT) and TST testing among recruits with soldiers returning from deployment to a TB endemic area.[56] This study found 6.2% prevalence of TST

reactions of at least 10 mm in recruits, but could not estimate prevalence in post-deploying soldiers because the sampling included only TST reactors for part of the study. No other study has assessed the risk of TST conversion in deployed military populations. Finally, no studies assessing the risk of LTBI or prevalent TST reactions have been reported among US Army recruits, which have a different demographic composition and prior exposure to TB than Navy recruits.[57]

Universal TB Testing Policies and False Positive TB Skin Tests

While TB skin testing in the US has shifted to a focus on persons at high risk due to the declining risk of infection,[31] testing and treatment for LTBI continues to increase each year in the military. While over 500,000 TB skin tests are performed and 9,000 service members are treated each year for this reason, many have questioned whether the majority of this low-risk population receives any preventive benefit from this therapy. The program is costly, time-intensive, and puts service members at risk for adverse drug events from therapy. Furthermore, preventive therapy for LTBI with isoniazid (INH) also involves the risk of well-known adverse drug events, including hepatitis and peripheral neuropathy.[58] Finally, the quality control, documentation, follow-up, and adherence to therapy for skin test reactors in military populations are unknown.

In addition to these limitations, the meaning of a "positive" or "negative" skin test is often still uncertain due to variable responses to the TST and the absence of a "gold standard" for the diagnosis of LTBI.[35, 59] This is because the TST has many sources of error and variability associated with its use. Due to the severity of the consequences of a false-negative result, more attention has typically been paid to factors that influence

sensitivity and false-negatives rather than specificity and false-positives.[60] The reasons for false-negatives have been well-described, and include host factors, particularly immunosuppression; product-related factors, such as improper storage or handling; and errors in administration, including errors during the injection, reading, or documentation of the test.[34, 60]

False-positive TST reactions are increasingly being noted, particularly in health care and prison settings where testing is common.[61-66] Typically, the most important factor associated with a high proportion of false-positive reactions is a low pre-test probability or prevalence of LTBI. This dramatically reduces the positive predictive value of the test to below 50% in low prevalence populations,[34, 35] such as the general US population or the US military. However, many other factors are associated with false-positive TST reactions, as summarized in Table 1.

Table 1. Potential Sources of False Positive Skin Test Results[67]

Product-related	**Host factors**	**Administration**	**Cross-reactivity**
Hot lots	Boosting	Wrong reagent used (e.g. Tetanus)	Non-tuberculous mycobacteria
Quality control	Biologic variability	Wrong amount used	BCG vaccine
Between-manufacturer variation (Aplisol®)		Not read correctly	
		Not documented correctly	
		Result not interpreted correctly	
		Intra-tester variation	
		Inter-tester variation	

Prominent among these is variability in administering the test, including errors in the administration, reading, and documentation of the TST;[68] and intra- and inter-tester variation.[69, 70] Another common reason for false positive TST results is cross-reactivity with non-tuberculous mycobacteria (NTM) or BCG vaccine. Product-related factors can also result in false-positive TST results, including "hot lots"[71], which have been attributed to particulate matter suspected to be undissolved antigen; and variation between manufacturers.[62, 72] Biologic variability resulting in boosting and reversion are also important causes of variability and misclassification.[59]

False positive TSTs affect not only individuals, but also result in pseudoepidemics of skin test conversion in populations. Numerous examples of pseudoepidemics of TB skin test conversions have been described.[61-63, 71, 73, 74] The factors responsible for these pseudoepidemics have included: errors in administration of the test dosing errors,[61, 66] errors in reading the TST,[65] particular "hot lots" of TST solution or other products variability,[62, 75] changes from one commercially available product to another, e.g. use of Parke-Davis Aplisol ® (Morris Plains, NJ) and the subsequently withdrawn Sclavo ® (Wayne, NJ) purified protein derivative (PPD) solutions,[62, 63, 74] or a combination of these factors.

Pseudoepidemics of Skin Test Conversions in US Military Populations

As shown in Table 2, frequent "pseudo-outbreaks" or "pseudo-epidemics" also occur in the military population. These cause confusion among medical providers, public health personnel and in the military and civilian communities. A large-scale, publicized contact

investigation of a case of active TB on the USS RONALD REAGAN revealed no further active TB cases, and only 12 active duty and 1 civilian contacts believed to be truly infected with latent TB.[76] The US Army Center for Health Promotion and Preventive Medicine (USACHPPM) was called to Afghanistan in 2005 to investigate a high (15%) reported risk of conversion. Again the results of this investigation pointed to a majority of false positives in the skin test procedure and with a particular commercial product.[77] Other examples of pseudo-outbreaks of skin test conversions had been previously reported in detention barracks,[78, 79] aboard ships,[80] and in deployments to Cuba[81] and Bosnia.[82]

Table 2. Pseudoepidemics of TST reactions in the US Army, 1983-2005[67]

Year	Population	Location	Reported % New Positive Reactors	% of Positives Confirmed on Repeat Testing	Active TB Cases Identified	Primary attributed cause(s) of outbreak
2005	Aviation unit	Afghanistan	15% (30 of 198)	4% (16 of 374)	0	Test administration and reading, use of Aplisol, prior positives not documented
2005	CA National Guard	TF Falcon, Kosovo	5.0% (75 of 1500)	5% (2 of 40)	0	Test administration and reading, use of Aplisol
2003	MN National Guard	TF Eagle, Bosnia	1.6% (19 of 1222)	--	0	68% (13 of 19) prior reactors; conversion rate not elevated
1996	Hospital Staff	TF Eagle, Bosnia	1.3% (1 of 80)	--	1	Conversion rate not elevated
1996	Prisoners and prison guards	Ft. Leavenworth, KS	2.6% (35 of 1346)	30% (9 of 30)	0	Use of Aplisol
1995	Military Police	Guantanamo Bay, Cuba	6.3% (81 of 1280)	0% (0 of 6)	0	33% (25 of 75) were prior reactors; foreign birth
1984	Prisoners and prison guards	Ft. Leavenworth, KS	9.1% (191 of 2106)	36% (62 of 172)	0	Increased surveillance, test administration and reading
1983	Medical students	Ft. Benning, GA	7.7% (5 of 65)	--	0	60% (3 of 5) had dominant reactions to PPD-B, indicating cross-reactions with non-tuberculous mycobacteria

These outbreaks further indicate the difficulties and expenses encountered in implementing a TB skin test screening program. Not only do these events cause alarm

and waste resources, but they also erode faith in the test among medical and non-medical personnel. Finally, they highlight the potential for false positives in the absence of recognized TB exposure, resulting in treatment costs and the potential for adverse drug events from subsequent therapy.

NON-TUBERCULOUS MYCOBACTERIA (NTM)

Non-tuberculous Mycobacteria as a Source of False Positive Skin Tests

False positive skin tests due cross reactions to NTM are not a new phenomenon and have been reported for over 60 years. Since the TST was initially used as a tool against bovine TB,[83] false positives were initially discovered in veterinary practice. As testing spread, "no-lesion reactors" were found, leading to substantial economic losses.[83] These were found to be associated with cross-reactions to avian and other mycobacteria. Cross-reactions to non-tuberculous or "atypical" mycobacteria were subsequently associated with false positives in humans in epidemiologic studies.[84-86] While the effect of NTM on tuberculin reactions is expected to be of little clinical significance in countries where the prevalence of TB infection is intermediate or high,[87] cross-reactions to NTM may be an important potential source of false-positives in areas where the likelihood of TB infection is very low and the likelihood of sensitization to NTM is increasing.[88] Both of these factors are true of the US population.[89, 90] False positive TSTs from cross-reactivity to non-tuberculous mycobacteria may particularly occur among those from the southern US[48] as well as in persons who have received BCG.[91] Such reactions result in lower TST specificity and positive predictive value, especially in persons who have a low probability of *M. tuberculosis* infection, including US-born recruits.[12] Although

only foreign-born recruits would be expected to have had BCG vaccination, a large proportion of soldiers (>40%) are from the southeast US,[57] and thus are expected to have a higher likelihood of sensitization to NTM.[48] Furthermore, the prevalence of sensitization to non-tuberculous mycobacteria (NTM) increased in the US civilian population from 11% in 1972 to 17% in 2000,[89] suggesting increased exposure to NTM within the US. The late Dr. George Comstock, one of the premier tuberculosis epidemiologists of his era, said in 1975 of Navy recruits, "The frequency of cross-reactions to tuberculin in this population is sufficiently great that the prevalence of true tuberculous infections among white recruits may already be approaching zero."[2] Increasing exposures to NTM, coupled with large decreases in *M. tuberculosis* exposures in the past several decades, makes it likely that non-tuberculous mycobacteria are responsible for an even larger proportion of TST reactions and conversions. Further exposure to NTM may occur during deployments and field training exercises due to contact with soil and potentially higher risk water systems.[92, 93] This may increase the likelihood of NTM as a potential source of false positives and discordance between TST and IGRA among US military populations, particularly among soldiers and Marines where these exposures are common.

The Battey Skin Test Antigen

Dr. Comstock remarked that "differentiating homologous and heterologous reactions to tuberculin…ranks with Koch's discovery of the tubercle bacillus in its implications for understanding the epidemiology of tuberculosis."[2] One of the primary agents used in assessing homologous and heterologous reactions is the Battey skin test antigen. The

Battey skin test antigen, or Purified Protein Derivative-Battey (PPD-B), is a test to aid in the differentiation of infection and sensitization to tuberculous mycobacteria from non-tuberculous mycobacteria. PPD-B is the purified protein derivative produced by Lewis Affronti[94] using the method described by Siebert[95] from Battey strain No. 100616. This strain has also been referred to as the Boone strain, and has been identified as *Mycobacterium intracellulare.* The Battey strains were isolated from patients admitted during the 1950s to the Battey State Hospital in Rome, Georgia, with tuberculosis-like disease that did not respond to chemotherapy.[96] The potency of each lot of PPD-B, diluted, is determined in four guinea pigs sensitized with the atypical organisms. Its administration is identical to the use of the closely-related TST, with 0.1 ml administered intradermally and read 48-72 hours later. Considerable human testing has been done with PPD-B, including the Navy Recruit Study (1958-1969),[12, 48] after which it was recommended as a companion antigen to the tuberculin skin test, or Purified Protein Derivative-Siebert (PPD-S) antigen, in order to differentiate between sensitization to tuberculosis or non-tuberculous organisms.[12] PPD-B has been employed for this purpose in large surveys in addition to the Navy Recruit study, including the National Health and Nutrition Examination Survey (NHANES) completed by the Centers for Disease Control and Prevention (CDC) in 1971[97] and 1999-2000.[13, 89] It has also been used in many other smaller epidemiologic studies.[98-103] Previous human experience with mycobacterial skin test antigens indicates that side effects are rare. Infrequently, nonspecific irritation may develop at a test site within 20 minutes of the injection, and subside within 24 hours. Highly sensitive individuals (less than 1%) may also experience local redness, warmth, discomfort, and itching as a result of vesiculation,

necrosis, or ulceration at the injection site for as long as a week. These reactions usually heal without any serious adverse events reported. Hundreds of thousands of doses of PPD-B have been administered in many studies throughout the world without report of serious adverse events.

The choice of PPD-B to assess the contributions of NTM on discordance between TST and the interferon gamma release assays (IGRAs) is based on the performance of various alternative antigens for NTM in previous studies.[84, 86, 100, 104] The Battey antigen seems to be the best choice for assessing the potential contribution of cross-reactivity NTM for several reasons. First, *M. intracellulare,* from which PPD-B is derived, has the greatest burden of disease of all the NTM according to these epidemiologic studies in the US.[48, 100] Second, PPD-B appears to serve as a better indicator for sensitization to a broader array of cross-reactive NTM than other purified protein derivative (PPD) products.[85, 86, 100] Third, it has the most prospective data to support its use.[12] Finally, it is the only other PPD for NTM that is currently available for use.

Evidence from the Navy Recruit Study

Figure 2. Relative sizes of reactions to PPD-S and PPD-B among Navy recruits (from the Navy recruit study sponsored by the US Public Health Service) [12]

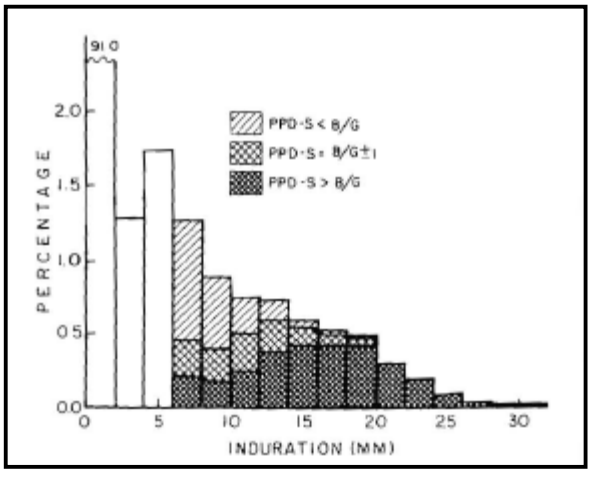


Figure 2 shows the frequency distribution of dual skin test reactions among Navy recruits.[12] The distribution of the PPD-S dominant reactions (darkly-shaded region)

looks much more like the typical normal frequency distribution of TST reactions seen with active TB than does the overall recruit TST frequency distribution. This led to the suspicion that the region with a dominant reaction to PPD-B (lightly shaded) was primarily attributed to cross-reactions from NTM. Some of the reactions which are non-dominant (within 1 mm) may be due to cross-reactivity as well.

Table 3 shows the results from long-term prospective follow-up of the same cohort of recruits initially examined with dual skin tests.[12] It demonstrates that those with an intermediate TST reaction (6-11 mm) and a larger PPD-B reaction were no more likely to develop active TB than those with smaller TST reactions (5 mm or less). The dual skin test results are seen to predict future risk of active TB in a long-term longitudinal study of military recruits. That is, those with PPD-B dominant reactions among those with TSTs in the 6-11 mm range were as unlikely to develop active TB as those with TSTs of 5 mm or less, demonstrating the value of dual skin testing in differentiating NTM from TB.

Table 3. Tuberculosis morbidity according to results of dual skin tests in Navy recruits (from the Navy Recruit Study sponsored by the US Public Health Service) [12]

TUBERCULOSIS MORBIDITY ACCORDING TO RESULTS OF DUAL TESTS IN 1,124,883 WHITE, NAVY RECRUITS

Dual Test Reactions (mm)	No. Tested	Cases of Tuberculosis (no.)	Cases of Tuberculosis (no./100,000 tested)
PPD-S 0–5	1,058,122	384	36.3
PPD-S 6–11	32,649	36	110.3
S < B/G	17,024	5	29.4
S = B/G ± 1	7,895	10	126.7
S > B/G	7,730	21	271.7
PPD-S 12–17	20,932	80	382.2
S < B/G	2,309	5	216.5
S = B/G ± 1	4,039	12	297.1
S > B/G	14,584	63	432.0
PPD-S 18+	13,180	49	371.8

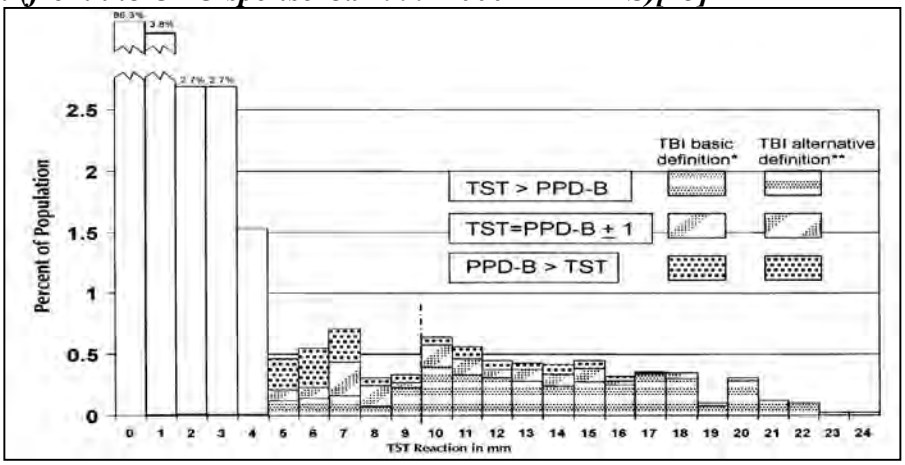
Definition of abbreviations: S = PPD-S; B/G = PPD of *Mycobacterium intracellulare* or *M. gause*.

Other Studies Involving the Use of Sensitin for NTM

As demonstrated earlier, the incidence of active TB has declined logarithmically in the intervening decades since the Navy recruit study was performed. This leads to a lower positive predictive value of the TST (or any diagnostic test for TB) and a larger proportion of TST reactions which may be result from cross-reactions to NTM.[34, 35] As a result, others such as von Reyn and Bennett have concluded that dominant skin test reactions of TST in the 12-14 mm range may be conservatively evaluated the same way as the 6-11 mm reactions.[13, 105] Studies by von Reyn provided more recent evidence of cross-reactivity to NTM as a potential source of TST reactivity. First, a study of dual skin testing among culture-confirmed TB and NTM subjects demonstrated that MTB-dominant skin test reactions were present in 18 (90%) of 20 patients with M. tuberculosis, whereas 15 (83%) of 18 nonanergic patients with *M. avium* infection had *M. avium*-dominant skin test reactions.[106] A similar study of culture-confirmed NTM and TB cases found that NTM-dominant skin tests had a specificity of 97% for discriminating disease due to *M. avium* from disease due to *M. tuberculosis*.[107] These studies suggested that dual skin testing could be useful in differentiating NTM from TB in certain settings. Subsequently, von Reyn also showed that among health care workers undergoing annual TST testing, NTM-dominant reactions, in this case defined as an NTM skin test of $\geq$ 3 mm larger than the TST reaction, were found in 10 of 20 subjects (50%) with 10-14 mm PPD reactions.[105] These reactions were more common among whites ($p = 0.046$), US-born ($p = 0.038$) and subjects without BCG immunization ($p = 0.004$), suggesting that cross-reactivity to NTM contributed to these reactions. This led them to conclude that infections with NTM are responsible for the majority of 5-14 mm

PPD reactions among US-born health care workers subject to annual tuberculin testing.[105] Figure 3 provides an updated frequency distribution of dual skin testing results done in the 1999-2000 NHANES by the CDC.[13] The dotted portions are the PPD-B dominant reactors, as defined by a PPD-B ≥ 2 mm larger than the TST. Although this proportion is low in the overall population whose TST reactions were 10-14 mm in size, the proportion of cross-reactions is expected to be larger in younger populations whose risk of previous TB infection is low.[13] The risk of sensitization to NTM, and its associated cross-reactivity to the TST, is also greater among those from the southeastern US than in the general population.[48, 89] As previously mentioned, more than 40% of soldiers originate from this area.[57] These studies further support the contributions of NTM as potential sources of false positives in a tuberculosis testing and as a source of discordance between the TST and IGRA.

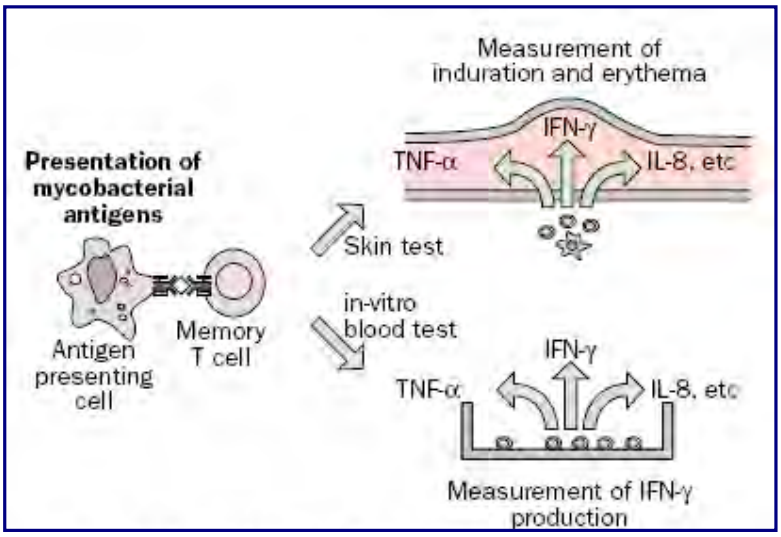
Figure 3. Estimated 1999-2000 US population TST reaction, compared with PPD-B reaction (from the CDC-sponsored 1999-2000 NHANES)[13]



The Development of the Interferon Gamma Release Assay (IGRA)

The QuantiFERON® (QFT) brand IGRA measures interferon-gamma released from lymphocytes in whole blood samples incubated with antigens to *Mycobacterium tuberculosis* (MTB),[11] as shown in Figure 4. The assay was developed by identifying regions in the *M. tuberculosis* genome that are absent in BCG and most NTM. These stretches of the genome are present in both *M. bovis* and MTB, but were deleted from BCG during in-vitro passage, and are called regions of differences (RD). The genes for ESAT-6 and CFP10, two of the three antigens contained in QFT, reside in one of these deleted regions, known as RD1. Since the peptide antigens used in QFT stimulate IFN-γ? responses in T-cells from individuals infected with *M. tuberculosis* but generally not from uninfected or BCG vaccinated persons without disease or risk for LTBI, the QFT is thought to have better specificity for MTB infection than the TST. There have been three generations of FDA-approved QFT products: the QuantiFERON®-TB (QFT-TB),

Figure 4. Comparison of in-vivo and in-vitro tests for infection with Mycobacterium tuberculosis [11]



QuantiFERON®-Gold (QFT-Gold), and Quantiferon® Gold-in-tube (QFT-GIT). The most recently developed QFT product, the QFT-GIT, was approved in October 2007 by the Food and Drug Administration (FDA) for use as an indirect test for *M. tuberculosis* infection (including disease). The addition of the TB7.7 (p4) antigen to the CFP10 and ESAT-6 RD1 antigens in QFT-GIT may add further sensitivity to the previous generations of the QFT test.[108, 109]

Like the QFT, the T-SPOT®.TB (T-Spot) is an IGRA test based on the RD1 antigens ESAT-6 and CFP10. The test has been used in Europe for several years, and was approved by the FDA for use in the US in August 2008. The T-Spot is a simplified variant of the enzyme-linked immunospot (ELISPOT) assay technique. The assay detects effector T cells that secrete interferon-gamma in response to stimulation by antigens specific for *M. tuberculosis*.[110, 111] The assay is also designed for use in patients at risk for LTBI or suspected of having TB disease.[112, 113] The T-Spot test used in this study will include the use of a stability agent (T-cell Xtend), which extends the window for processing from 8 to 32 hours after venipuncture. This makes the test more practicable for military use in a basic training setting.

Although the IGRAs were developed to reduce the cross-reactivity to BCG and most non-tuberculous mycobacteria seen with the TST, false positive and false negative results can also occur with IGRAs. Like the TST, immunosuppressed individuals may have reduced interferon-gamma (IFN-γ) responses, leading to false–negative results. Additionally, reduced lymphocyte activity can result in false negatives due to prolonged

specimen transport or improper specimen handling, including filling and/or mixing of blood tubes, or inability of the patient's lymphocytes to generate IFN-γ.[114] Furthermore, although fewer false-positive results from cross-reactive NTM are expected, *M. kansasii, M. marinum, and M. szulgai* also cross-react to IGRA RD1 antigens,[115] as shown in Table 4. This may lead to false-positive results with the IGRA tests as well.

Table 4. Antigen Expression in Mycobacteria[11]

Strain tested	Antigens		
	ESAT-6	CFP 10	MPT 64
Tuberculosis complex			
M tuberculosis	+	+	+
M africanum	+	+	+
M bovis	+	+	+
BCG substrain			
gothenburg	–	–	+
moreau	–	–	+
tice	–	–	+
tokyo	–	–	+
danish	–	–	–
glaxo	–	–	–
montreal	–	–	–
pasteur	–	–	–
Environmental strains			
M abcessus	–	–	–
M avium	–	–	–
M branderi	–	–	–
M celatum	–	–	–
M chelonae	–	–	–
M fortuitum	–	–	–
M gordonii	–	–	–
M intracellulare	–	–	–
M kansasii	+	+	–
M malmoense	–	–	–
M marinum	+	+	–
M oenavense	–	–	–
M scrofulaceum	–	–	–
M smegmatis	–	–	–
M szulgai	+	+	–
M terrae	–	–	–
M vaccae	–	–	–
M xenopi	–	–	–

–=not present in species/strain, +=present in species/strain.

The Use of the Interferon Gamma Release Assay

The CDC has published guidance on the use of the IGRAs as alternatives to the TST in the diagnosis of LTBI,[116, 117] whereas other countries such as the UK have suggested the use of IGRAs as an adjunct to the TST.[118] IGRAs are attractive for clinical use because of the need for only one patient visit, the absence of the booster effect, and the reduction in cross-reactivity to NTM and BCG.[114] However, there are significant potential disadvantages as well, including higher cost, laboratory burden, and variability in serial testing.[119] Most importantly, however, is that there is considerable uncertainty about the predictive ability of the IGRA tests, particularly in relation to the most important outcome, the subsequent longitudinal development of active TB.

The limitation to all evaluations of the IGRA is the lack of a gold standard for LTBI. The best current standard is the prospective development of active TB in cohort studies.[120] In practice, however, the IGRAs are routinely compared to the TST in cross-sectional evaluation studies, using active TB cases to assess sensitivity and low-risk populations to assess specificity. A meta-analysis of these studies by Menzies suggested that both IGRA tests had better specificity than and similar sensitivity as the TST,[114] and this was reiterated in an updated meta-analysis by Pai in 2008, using the most recent generations of these tests.[121] When comparing the two IGRAs, the evidence suggested that T-Spot had a similar but slightly lower specificity than the QFT or the TST, but with better sensitivity.[114, 121]

Analysis of Discordant Results between the TST and IGRA in the Diagnosis of LTBI

Although both the IGRA and the TST may be used in the diagnosis of LTBI, they do not give equivalent information and often have discordant results. Several studies have compared discordance both between one or both of the IGRAs "head-to-head," comparing discordant results between the QFT and the T-Spot and between each IGRA against the TST simultaneously.[122-129] The agreement between QFT and T-Spot is generally very good in simultaneous head-to-head comparison testing. Both tests are generally found to have a similar high specificity. However, Lee found greater sensitivity with the T-Spot (96.6%) than with the QFT (66.7%), as did Adetifa (using QFT),[127, 129] consistent with the findings from meta-analyses by Menzies[114] and Pai.[121]

In contrast to the concordance between the IGRAs, discordance between TST and IGRA is common. When an IGRA is compared to the TST in evaluation studies, discordant results are often found in 20-30%.[114] The analysis and interpretation of these discordant results is challenging because the results have been inconsistent, which may be largely due to the population studied. These varied results have led to differing interpretations in different populations. Arend, using the QFT and T-Spot, found both IGRAs to be insensitive to subjects with 15 mm or greater TST reactions during a contact investigation.[123] In contrast, Nienhaus reported better specificity for the QFT, with 86% of TST positive, IGRA negative discordance explained by BCG vaccination and 49% of TST positive, IGRA positive discordance explained by waning sensitivity due to

age.[122] Detjen found that the specificity of the IGRAs was overwhelmingly superior in a hospital-based population of bacteriologically-confirmed TB and NTM infections, using either QFT or T-Spot.[126] The inconsistent results and conclusions from these studies suggest that the causes of discordance between the IGRAs and TST are multifactorial and depend on the population studied, and that further study may elucidate these causes further. No head-to-head comparisons of the QFT and T-Spot IGRA products have been performed in military populations.

SCREENING STRATEGIES FOR LATENT TUBERCULOSIS INFECTION (LTBI)

Guidelines for High-Risk Groups and Targeted Testing

The CDC has published guidelines addressing different aspects of the diagnosis, treatment, and surveillance of active and latent TB, with the goal of reducing the burden of this disease and eventually eliminating it.[31, 130, 131] Special emphasis is placed on the importance of active TB case-finding and on use of targeted testing for latent TB infection (LTBI). CDC guidelines specifically state that "…targeted tuberculin testing programs should be conducted only among groups at high risk and discouraged in those at low risk."[31] The CDC guidelines clearly consider the following settings as potentially high-risk:

1. Contacts of an active TB case[130]
2. Hospitals and health-care settings[132]
3. Correctional and detention settings[133]
4. Other congregate settings (homeless, substance abuse treatment centers)[134]
5. HIV-infected persons[134]

6. Immigrants and refugees entering the US[134]

Many have argued for increasing emphasis particularly on the treatment of LTBI among foreign-born persons, since they now account for the majority of cases in the US, and a quarter of whom have resided in the US for more than 5 years.[135] Others have argued that more attention should be paid to reducing the global burden of disease, where money would be more effectively and justly spent.[136, 137] The Institute of Medicine's 2000 report, *Ending Neglect: The Elimination of Tuberculosis in the United States,* also called for aggressive efforts in implementing targeted testing programs, with particular emphasis on the identification and treatment contacts of cases of active TB and foreign-born individuals.[138]

The Evaluation of Targeted Testing Programs for LTBI in US Populations

As seen above, universal screening for tuberculosis in the US is no longer recommended, in favor of a targeted approach.[31, 138] In some settings, such as immigrants, prisons, and hospitals, exposure to tuberculosis is somewhat homogeneous and testing may be targeted based on association with a high-risk setting, although health-care workers are increasingly being recognized as having heterogeneous exposures.[132] Other settings also have a more heterogeneous population in which targeted testing of selected persons within that population may be more appropriate. Targeted testing programs have been implemented and evaluated using predictive models in several of these settings, including contact investigations,[139, 140] pediatric populations,[141, 142] and university entrance.[143] Bailey used case, contact, and environmental exposure characteristics to predict which TB contacts were most likely to have a positive TST result.[139] Aissa

recently used a similar predictive model to reduce investigations by 26% without affecting disease control efforts.[140] Two studies in pediatric populations assessed the ability of a questionnaire with only four or five questions to identify those at highest risk.[141, 142] Both had a prevalence of about 1% TST reactions and found 80-90% sensitivity and specificity with various combinations of questions. US colleges also have a variety of practices, but many target only international students and those in specific programs such as nursing.[144] The only study to assess questionnaire screening in order to target testing in a university population was done among college students in Virginia with a 2.3% prevalence of TST reaction.[143] Although begun as a 33 question survey, this study showed that using only the two criteria of: 1) foreign birth, and 2) close contact of a patient with TB, resulted in a sensitivity of 81.6% and a specificity of 91%. This study suggests applying this approach to a military recruit population, using a predictive model to target testing among those identified as higher-risk by a risk factor questionnaire.

Current Options for TB Screening in the US Military

Military populations are not typically considered inherently high-risk within the CDC guidelines, and the epidemiology of TB in the military supports this conclusion. The military does follow the CDC guidance for testing and treating those high-risk individuals within the other categories listed above. Additional medical risk factors which increase the risk of active TB and thus would warrant testing for LTBI (such as diabetes, silicosis, or other immunocompromising conditions)[31] are exclusions from military service and are very rare in military populations.[145] However, the long history of concern for TB

transmission in military forces has also resulted in the implementation of more generalized, widespread TB testing programs in military populations. Currently all the military services conduct universal TB testing at accession, despite the fact that the majority of recruits do not have any of the risk factors identified above.

In recent years, the Armed Forces Epidemiological Board (AFEB), now part of the Defense Health Board (DHB), has provided expert civilian recommendations to the military on TB screening policy. In 2000, the AFEB recommended that the services provide screening (not necessarily universal testing) to military service members at accession and periodically afterwards, but there was ambiguity in how this was to be performed.[146] They also recommended research priorities, including validity studies and cohort longitudinal studies involving interferon-gamma blood testing, studies of factors for noncompliance with therapy, and cost-effectiveness studies. Further, quality assurance measures were recommended and the services were encouraged to harmonize screening policies. Although each service has implemented the same type of universal testing at accession using the TST, they all have different requirements for screening during deployment and overseas stationing.

Problems with Universal TB Testing Strategy

In summary, there are several major problems with the universal testing strategy currently followed by all US military services at accession. First, the major public health and TB experts, such as CDC, American Thoracic Society (ATS), Infectious Diseases Society of America (IDSA), and World Health Organization (WHO), all recommend

against universal testing in favor of targeted testing. Second, all existing TB tests have a low positive predictive value in a low prevalence population such as the US military. Third, there are many sources of error and variation which make TB testing problematic, and may result in false-negative and particularly in false-positive results. The false-positives have been found to result in resource-intensive and anxiety-provoking pseudoepidemics of TST conversions. Treating false positives also results in adverse events such as hepatotoxicity and neurotoxicity. There is the potential for further harm from labeling someone as a TST converter (with or without treatment) if they are later exposed but testing and treatment is deferred due to being a prior positive. Considerable resources are spent in universal TB testing programs, including money, personnel, and time. Finally, there are numerous opportunity costs that should be considered. These include the diversion of resources from other public health programs as well as the diversion of resources away from other potentially more effective tuberculosis control strategies. It also includes the potential for undermining TB disease prevention efforts by overtreating a low-risk population, which may lead to poor risk communication, failure to adhere to TB or other communicable disease control efforts, and loss of faith in public health interventions.

Adherence to anti-tuberculous therapy

For a screening program based on subsequent preventive therapy to be effective, adherence factors for therapy must also be considered. Estimates of adherence to therapy for preventive tuberculosis medications are extremely variable. Three studies of adherence in US universities reported a range of 15-58% of students who met CDC

criteria for therapy and actually began it, and a range of 5-46% completion of a 6-month course of therapy.[152-154] A TB clinic at a major medical center found that only 19% completed 9 months of therapy, and almost two-thirds dropped out within 3 months.[155] They found that low perceived benefit and fear of venipuncture were the strongest predictors of failure to complete therapy. Other important factors reported have included race, ethnic group, and country of origin.[156] These factors have not been studied in a military population, but have an impact on both the program evaluation and effectiveness.

Cost-effectiveness of screening policies

Finally, the cost involved in screening for tuberculosis in different populations has been subject to debate. The cost-effectiveness of testing for LTBI has been largely determined by the population studied and its pre-test prevalence. For example, testing of contacts of active TB cases has generally been found to be cost-saving.[157, 158] Results of studies among immigrants, however, have conflicted about whether testing, even using chest x-ray, is efficient or effective, and have shown that other screening tests such as TST or IGRA are only marginally, if at all, better.[159-161] Studies comparing the economic impact of targeted testing with universal testing in children have agreed that targeted testing is not only more efficient but also probably cost-saving.[162, 163] Finally, a recent cost-effectiveness analysis looking at TB testing in association with travel suggested that a single test post-travel was warranted under conditions of a substantial risk of infection and good adherence to therapy.[164]

It has also been suggested that the interferon-gamma release assays may be more specific and thus more cost-effective tests. Several cost-effectiveness analyses of the IGRAs have been performed with different results, which again have been largely determined by the prevalence of LTBI in the population studied. Diel performed two cost-effectiveness analyses, one of QFT and one of T-Spot in which he found that testing of contacts of active TB cases was cost-effective, similar to TST.[165, 166] Wrighton-Smith found similar results, suggesting that sequential testing of TST followed by IGRA may be a more cost-effective strategy.[167] On the other hand, Oxlade found that IGRA was the least cost-effective strategy for testing of immigrants.[157] Little research has been done looking at the effect of IGRA on the cost-effectiveness of targeted testing programs in other heterogeneous, low-prevalence populations.

RESEARCH PROPOSAL

Overall Research Question

The overall objective is to assess the feasibility, cost, and potential impact of using a targeted testing approach and two IGRA as an alternative to the universal application of the TST to identify LTBI among military recruits. The central hypothesis is that targeted testing by use of the questionnaire followed by the TST or an IGRA, using either QFT or T-Spot, will reduce the amount of unnecessary testing previously performed among low-risk recruits without significantly affecting the capture of cases of higher-risk recruits.

Objective 1. Retrospective analysis of active TB surveillance in the US military.

Rationale

An analysis of TB epidemiology and surveillance is critical for the background and justification of the problem in assessing the burden of active TB disease in the US military. It will be used to assess the distribution and determinants of active TB and the potential impact of alternative testing strategies on active TB incidence.

Approach

To better define the incidence of active TB disease in the US military, a descriptive study using existing health surveillance databases will identify cases of active TB. Cases will be defined from reportable medical events and hospitalizations for all types of TB (International Classification of Diseases-9th Revision-Clinical Modification codes 010-018) among all active component military service members from 1990 to 2007. The Defense Medical Surveillance System (DMSS) will be used to obtain the cases and population denominators stratified by the categories of interest listed below.

Incidence rates using person-time will be calculated with 95% confidence intervals. This information will provide stratified rates of active TB per 100,000 person-years over time according to reporting type (reported cases or hospitalized cases), 5-year age groups, occupation, country of birth, branch of service, and other demographics. The margin of error will range between 10-30% during the study given that the cases range from 10-60 cases per year and the population ranges from 1.4 to 2 million, with the larger margin of error in the later part of the study. The descriptive analysis will analyze active TB data in

comparison with historical rates and the age-adjusted US rates reported by CDC. Completeness and case confirmation will be assessed by use of military laboratory and pharmacy data. A confirmed laboratory case is defined as having a culture, Polymerase Chain Reaction, or other molecular-based test positive for MTB. Cases will be suggested from pharmacy data by the use of 3 or 4 drug therapy including the drugs isoniazid, pyrazinamide, ethambutol, and rifampin. Sensitivity analysis will include looking at laboratory-confirmed cases, laboratory and pharmacy–confirmed cases, cohort effects, effect modifiers, missing data, and period effect. Period effects assessed will include post-1998 to look at the influence of changes in reporting systems, and post-2001 to look at the effect of increasing deployments to high-prevalence TB areas. Linkage will be accomplished using social security numbers (SSNs) within a 12-month interval of the date of diagnosis.

Objective 2. Nested case-control study to analyze risk factors for active TB

Rationale

A nested case-control study will examine the relative importance of deployment and overseas stationing as independent risk factors for active TB in active duty military service members, as compared to the importance of risk factors existing prior to accession. This objective will also examine the strength of association of these risk factors for the development of active TB disease in multivariable models. Finally, these risk factors will be used to inform the screening questionnaire by including factors known to be associated with the development of active TB.

Approach

A nested case-control analytic study with incidence density sampling will be performed to assess risk factors for the development of active TB, with cases as defined in objective 1. Four controls per case (from the cases described above) will be selected at random from the population, matched on year of entry into service and length of service. The estimated numbers are 600 cases and 2400 controls. This will provide a very precise estimate of the measure of effect, with 90% power to detect a 5% difference given 10% prevalence of exposure among controls and 15% among cases.

Analysis will be performed by the use of bivariate and multivariate epidemiological methods, using matched odds ratios and conditional logistic regression for matched data. The linkage with active TB cases will allow observational analysis of the risk factors for activation. Cases will be identified in the datasets described in Objective 1, and sensitivity analyses will likewise include similar data elements. A subset of cases and controls will be studied further by surveying these cases to verify case status in order to reduce misclassification. We will also obtain additional information on risk factors not available in administrative databases, including prior TB diagnosis and treatment, and risk factors during military service, with special emphasis on latent TB diagnosis and treatment, exposures prior to accession, civilian travel, and military deployment and stationing.

Objective 3. A cross-sectional comparison study of TB screening procedures.

This study will be performed among Army recruits to simultaneously assess the different tests, including: 1) the questionnaire, 2) QFT, 3) T-Spot, and 4) TST, and 5) PPD-B. The two component of this study are:

a. Evaluate a screening questionnaire designed to target testing among recruits at higher risk for TB infection by determining which questions are most predictive of TB test results.
b. To identify factors associated with positive test results and with discordance in TST and IGRA results. Skin testing with the PPD-B will be used to specifically examine the association of cross-reactivity to NTM with test discordance.

Rationale

1. To evaluate targeted testing for LTBI in the US military.

There are several ways in which this protocol hopes to build upon and address the gaps in the work done in previous studies. First, the major focus of the study is on the scientific evaluation of a targeted approach using a questionnaire to target higher risk recruits. Targeted testing has been the recommended approach by the CDC for several years, and is considered the standard of care.[31, 138] The development of a questionnaire-based approach in order to perform targeted testing in this proposal is consistent with the literature described above in pediatric and university populations, as well as in contact investigations, which have performed similar evaluations of targeted testing approaches using screening questionnaires.[141-143] This study will aid medical policy makers in

determining which questions are most predictive of what is judged to be LTBI in the recruit setting.

2. To analyze discordance between the TST and IGRA

Since the T-Spot was recently approved by the FDA in August 2008, the question of which (if any) IGRA should be used is of interest to military policy makers. This study will complement Mazurek's study by comparing both the QFT Gold In-Tube and the T-Spot, whereas Mazurek's published study included only QFT-TB and QFT-Gold (the first and second generation QFT products). Furthermore, this study will examine the discordance of each of the IGRA tests and the TST in this low-risk population, with emphasis on the assessment of the potential effects of cross-reactivity to NTM. Although both the IGRA and the TST may be used in the diagnosis of LTBI, they often have discordant results in many populations as discussed above. The causes of discordance between the TST and IGRA are of particular importance when discordance is extreme,[168] which may be the case in military populations. First, limitations in the use of the TST have been specifically demonstrated in military populations, as evidenced by several documented pseudoepidemics of false positive TSTs,[67] suggesting a source of discordance. Second, in Mazurek's study of Navy recruits, 11 of 15 (73%) of the highest risk individuals—whose country of birth had a rate of active TB of > 100 per 100,000 person-years and who had TST reactions of at least 15 mm—had negative QFT-Gold tests.[3] There are several explanations for these discordant results, including the use of RD1 antigens in the IGRA, which might result in a greater specificity, but it is also possible that the TST may have greater sensitivity, or that the IGRAs may detect

unresolved or more recent infections.[168] Mazurek stated that both higher IGRA specificity and lower sensitivity were possible explanations for this discordance, but did not conclude that either explanation was correct.[3] This study will specifically assess the association of NTM with discordance between the TST and IGRA in the military recruit population, in order to suggest cross-reactivity to NTM as a possible source of these discordant results in a low prevalence population, rather than MTB.

3. To examine cross-reactive mycobacteria as a potential source of discordance between the TST and IGRA

There have been few studies which assess the contribution of NTM as a potential source of discordance between the TST and IGRA,[3, 126] and no studies have been performed using the Battey antigen. This effect may be important in settings where the prevalence of TB is low and the prevalence of NTM sensitization is high.[87, 114] In a recent meta-analysis, Menzies suggested that an area of recommended research was "more data…to understand discordant tuberculin skin test and IGRA reactions, including…the role of nontuberculous mycobacteria."[114] In an article reviewing the future research agenda for LTBI, Pai also recommends further study of the effect of NTM on IGRA performance.[169] The military population is an excellent resource to explore NTM as a potential source of TST/IGRA discordance, since BCG and waning sensitivity to TST due to age are uncommon in military recruits.

The study will be performed among Army recruits for several reasons. First, there are differences between the services in demographics (including foreign birth), education, health behaviors, socioeconomic status, and other factors that may modify the risk of

LTBI between the services.[57, 170, 171] Obtaining data on Army recruits will complement previous Navy [3] and Air Force (unpublished) studies by providing a more complete representation of LTBI risks among military populations. Furthermore, since the Army has a lower proportion of foreign-born than does the Navy, the pre-test probability of TB infection may be lower, which may in turn lead to a greater proportion of false positives with either the TST or the IGRA. These false positives could provide a source of discordance between the IGRA and TST that may be explainable by cross-reactions to NTM.

Approach

This study will contribute to the assessment of the sources of discordance between the TST and IGRAs by accounting for cross-reactivity to non-tuberculous mycobacteria using the Battey skin test antigen. Other than culture-proven NTM disease, there are few methods besides the use of PPD-B for determining the association of cross-reactivity to NTM with TST and IGRA discordance. The trends of declining incidence of TB and increasing sensitization to NTM in the US is likely to be reflected in US military recruits, suggesting that much of the discordance seen between the TST and the IGRAs may be explainable by differences in reactivity to NTM.[67] It is also important to understand NTM as a potential source of discordance in military populations at risk for future soil and water exposure to NTM during deployments and field exercises. This study will also complement Mazurek's analysis of cross-reactivity to NTM as a potential source of discordance by using PPD-B, instead of the no longer marketed first generation QFT-TB assay.

This will be a cross-sectional comparison study designed to evaluate a questionnaire to target testing, and it will evaluate NTM as a potential source of discordance between the TST and IGRA. The participants will be recruited from an Army basic training site at Fort Jackson, South Carolina. TST induration will be interpreted relative to risk, in accordance with published guidelines.[31] The questionnaire will be used to develop predictive models for TST reactivity according to CDC criteria, consistent with the previous literature.[139-143] Predictive models for IGRA positivity and for TB-dominant skin test reactions using dual skin testing will also be developed. Risk factors will be evaluated using individual odds ratios and predictive values one variable at a time, followed by analysis of multivariate models using unconditional logistic regression. The primary variables to be evaluated include foreign birth, race/ethnic background, and contact with a case of active TB. Other factors to be evaluated include demographics, exposure assessment, BCG vaccination, as well as prior TB diagnosis, treatment, and skin testing.

Test discordance will initially be categorized as "a positive TST result and a negative IGRA result," or "a negative TST result and a positive IGRA result," or similar discordance between the QFT and the T-Spot.[3] Test agreement will be assessed using the kappa coefficient κ. Stratification, bivariate and multivariate analyses will be used to identify factors associated with TST and IGRA results and discordance. To examine the association of cross-reactivity to NTM with test discordance, results of Battey skin testing will be used. This will include the use of the Battey and dual skin test results as a

stratifying variable and in multivariate analysis of discordance between IGRAs and TST. Dual skin testing (DST) incorporates the results of the TST and the Battey antigen into a single outcome, which will be interpreted using the dominant reaction as defined in the CDC-sponsored NHANES in 1999-2000.[13] To evaluate test specificity, recruits assessed to be at low risk for *M. tuberculosis* exposure will be assumed to be uninfected. Estimates of specificity and indeterminate results will be compared using McNemar's test for correlated proportions. Sample size calculations based on previous Navy recruit data resulted in a necessary sample size of 1783 in a multivariate model to evaluate the questionnaire. A sample size of 20 discordant TST/IGRA specimens was calculated as necessary to evaluate the association of NTM with test discordance.

The primary variables to be evaluated include foreign birth, race/ethnic background, and contact with a case of active TB. Other factors to be evaluated include demographics, exposure assessment, BCG vaccination, as well as prior TB diagnosis, treatment, and skin testing. Although simple models involving bivariate and multivariate associations and predictive values from these three variables are of primary interest, additional models may also be constructed using variables obtained from the questionnaire that are found to minimize prediction error and maximize predictive accuracy using unconditional logistic regression. In these models to evaluate additional variables, variable selection will be guided by the c-statistic, which is the area under the receiver operating characteristic (ROC) curves, as an indicator of overall performance of the prediction model, as done in previous analyses of TB contacts.[139, 140] The c-statistic and ROCs are proposed in the context of predictive modeling using logistic regression. The ROC curve is typically

used to evaluate clinical utility for both diagnostic and prognostic predictive models. This curve assesses how well a test or model discriminates or separates individuals into two classes, such as diseased and nondiseased.[172] The c-statistic is based on the ranks of the predicted probabilities and compares these ranks in individuals with and without disease. The curve may also be used to estimate an optimal threshold for clinical use, such as that which maximizes both sensitivity and specificity. The optimal threshold, however, should also be a function of the relative costs of misclassifying diseased and nondiseased individuals.[173]

The study will also enable an assessment of the comparative performance of the questionnaire, QFT, and the T-Spot relative to the TST of the proportion of test results which were positive by each test; the proportion of TST results which had concordant test results; and the proportion and nature of TST results which had discordant test results. Associations will be explored between the epidemiological data and all combinations of test results (both concordant and discordant) to characterize factors associated with discordant and concordant results. Particular attention will also be paid to evaluation of those situations where discordant results occurred, in order to determine the degree and patterns of discordance found between alternative testing regimens.[3] Participants found to have discordant results will be characterized based upon the Battey test result, exposure and demographic information obtained from the questionnaire. In summary, the planned analyses include:

1. Estimate the proportion positive by each test.
2. Estimate the proportion of indeterminate or failed tests for each test.

3. Estimate test specificity by assuming that recruits at low risk for *M. tuberculosis* exposure are uninfected. Estimates of specificity and proportion of indeterminate results will be compared using McNemar's test for correlated proportions.
4. Estimate the proportion discordant and concordant, and calculate test agreement using kappa (κ) coefficient.
5. Perform stratified analysis to analyze heterogeneity and factors associated with discordance. Factors associated with TST positives will be analyzed after stratifying by IGRA result. Likewise, factors associated with positive IGRA will be stratified by TST result.
6. Perform bivariate and multivariate analyses to identify factors associated with TST and IGRA results and discordance. Discordant TST positive, IGRA negative and discordant TST negative, IGRA positive results will be analyzed separately in bivariate and multivariate analysis. The Pearson χ^2 test and multivariate unconditional logistic regression will be used to compare frequencies of test results among different groups of study recruits.

The association of discordance with sensitization to NTM will be examined by the use of dual skin testing using the dominant reaction. Dual skin testing (DST) incorporates the results of the TST and the PPD-B into a single result, which will be interpreted using the dominant reaction as defined in the CDC sponsored NHANES in 1999-2000.[13]

The categorization of the dominant reactions is shown in Table 5 below. If the Battey skin test has ≥ 2 mm greater induration than the TST, it is considered the dominant reaction. Therefore, all TST reactions of 15 mm or more (regardless of PPD-B reaction size) and those

with TST reactions of 10-14 mm but with reactions to PPD-B that are not 2 mm or more greater in size will be considered TB dominant reactions. In contrast, those TST reactions of 10-14 mm but with a PPD-B that is ≥ 2 mm larger in size than the TST will be considered NTM dominant reactions. Any TST reaction of <10 mm will also be considered negative, unless other risk factors defined by CDC guidelines are present.[31]

Table 5. Guide to categorization of dominant skin test reaction.

TST Induration Size	**TST ≥ PPD-B +2 (TB dominant)**	**TST=PPD-B ± 1 (Non-dominant)**	**PPD-B ≥ TST + 2 (NTM dominant)**
0-9	NEG	NEG	NEG
10-14	POS	POS	NEG
15+	POS	POS	POS

Objective 4. Cost-effectiveness Analysis of TB Screening Policies

Rationale

Cost-effectiveness analysis will be used to determine the costs and benefits of current and alternative TB screening policies at time of accession. Emphasis will be placed on these two components:

a. Allocative efficiency—Estimate costs and effects of the two programs: universal testing vs. targeted testing.
b. Technical efficiency—Perform stratified analyses comparing TST and IGRA as the confirmatory test to assess technical efficiency.

Approach

Using decision analytic techniques, cost-effectiveness analysis will be performed to assess the costs and benefits from alternative TB screening policies. Of particular interest will be status quo (universal mass screening), no screening, and targeted testing based on questionnaire. We will further estimate the effectiveness of TST and IGRA testing to accurately identify LTBI and the subsequent effect on prevention effectiveness.

The health outcome measured in this analysis is cases of active TB prevented. The estimates of the inputs of the health outcomes are shown in Table 6. The comparative summary measure is the incremental cost per case of active TB prevented. The time frame for the screening program is 1 year. The analytic horizon includes all of the future costs to the military associated with long-term health effects from cases of active TB that occur within the 1-year time frame. A societal perspective is taken, in which only the costs and benefits associated with the screening strategies which accrue while the service member is on active duty are included. From this perspective, the cost of the health outcomes is measured using the cost-of-illness approach, which includes direct medical and nonmedical costs, and costs associated with lost productivity. Cost-of-illness estimates for the health outcomes will be obtained from the TRICARE management agency and the published literature, as shown in Table 7. The analysis is also done from the health-care system perspective in which productivity losses are excluded, and from a societal perspective to estimate the long-term impact of these policies in the US.

Table 6. Inputs for Health Outcomes

Variable	**Estimate Source**
Prevalence of LTBI	Objective 3, medical literature
Risk Factor Questionnaire (RFQ) positive	Objective 3
Specificity of TST, QFT, T-Spot	Objective 3
Sensitivity of TST, QFT, T-Spot	Literature
Prevalence of T-Spot positive	
Prevalence of TST positive	Objective 3
Prevalence of QFT positive	Aim 4
Probability of follow-up for positive test	Literature
Probability LTBI therapy given	Literature
Probability LTBI therapy completed	Literature
Risk of progression to active TB if negative test	Literature
Risk of progression to active TB if positive test	Literature
Probability of active TB prevented by LTBI therapy	Literature

Table 7. Inputs for Cost Estimates

Variable	**Estimate Source**
RFQ administration	Published literature
TST administration	Literature
TST follow-up reading	Literature
QFT administration	Literature
CXR	Literature
Follow-up visit for positive test	Literature
9 months LTBI therapy	Literature
Evaluation and treatment of tuberculosis disease	TRICARE
Hospitalization for active TB	TRICARE
Productivity losses	Recruit Command

The purpose of sensitivity analysis is to determine the effect of changes in parameter estimates on the decision result. Sensitivity analyses are performed either to answer

specific policy questions or when uncertainty exists about the parameter estimates. The range of variables used in the sensitivity analyses for this study is based on medical literature and the results from the previous objectives. Sensitivity analyses will be performed one variable at a time and with several parameters simultaneously. The following variables are considered for the sensitivity analyses in this study: disease prevalence and incidence, efficacy of anti-tuberculous therapy, adherence to therapy, the cost of the testing, the proportion screening positive, the cost of the disease, and the discount rate. Because of the number of variables for which uncertainty exists, we will create "best-case" and "worst-case" scenarios to provide a realistic range of the impact that these screening strategies would have on morbidity from active tuberculosis.

SUMMARY AND PUBLIC HEALTH SIGNIFICANCE

Tuberculosis is a disease of public health importance in the United States and throughout the world because of its impact on human health and its transmissibility. Military personnel may have exposures to *Mycobacterium tuberculosis* infection while in congregate settings or during deployment to areas where TB is endemic. Although the incidence of TB in the US military continues to decline, it still has the potential to cause disease during military service, as well as future morbidity and mortality after the termination of service.

All three services are currently reviewing TB screening policy in light of the recent licensure of the QFT and the T-Spot. However, it is uncertain whether the current data supporting the use of QFT or T-Spot are generalizable to a low-prevalence military population. More importantly, the current US military policy of universal testing is not the standard of care for LTBI diagnosis, as evidenced by recommendations from the CDC and Institute of Medicine for targeted testing.[31, 138] CDC guidelines recommend targeted testing of only those with known risk factors for TB, specifically stating that "targeted tuberculin testing programs should be conducted only among groups at high risk and discouraged in those at low risk."[31] The challenge for the US military is to mitigate the risk of TB in those few recruits in our population with these risk factors, without exposing low-risk recruits to unnecessary therapy. Based on current data, it is expected that targeted testing through use of a questionnaire will greatly reduce the amount of testing while still allowing the capture of these higher-risk recruits, but the services have been reluctant to move to this policy without scientific evaluation.

This study is an evaluation of TB epidemiology and TB control policies in the US military. It will retrospectively examine TB epidemiology and prospectively evaluate a targeted testing approach in order to provide an evidence base with which to change policy. It will assess risk factors for active and latent TB infection in the US military, with emphasis on whether risk factors exist prior to or after accession into military service. The study will provide an evaluation of the utility of a questionnaire designed to target testing in US military recruits, as well as the sources of discordance between the TST and the newer IGRA diagnostic tests. Finally, the series of estimates obtained from the study will inform a cost-effectiveness analysis of a targeted testing strategy and the use of IGRAs for LTBI diagnosis. This information will be used to assess and guide military policy of testing military service members at accession.

This study will also estimate the prevalence of skin test sensitization to NTM and TB antigens in this population and assess the potential contribution of NTM as a source of discordance between the TST and the IGRAs. No studies have been performed using the Battey antigen to evaluate cross-reactivity to non-tuberculous mycobacteria as a source of discordance between the IGRA and TST. This study will complement Mazurek's previous study of the IGRA among Navy recruits by using this antigen to further assess the association of discordance with cross-reactivity to NTM.[3] Furthermore, it will compare both commercially-available IGRAs, the QFT and the T-Spot, whereas Mazurek's study assessed only QFT-Gold (an earlier generation of the QFT product). Since the T-Spot was recently approved for use by the FDA in August 2008, the question

of which (if any) IGRA is superior for military use should be answered. This study will examine the discordance of each of the IGRA tests side-by-side in this low-risk population, including assessment of the association with cross-reactivity to NTM.

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Chapter 2—Active Tuberculosis Surveillance in the United States Military, 1990-2007: Approaching Elimination

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Abstract

Objective

The US military may be at increased risk for tuberculosis (TB) infection and transmission due to congregate settings and overseas service. The purpose of this descriptive study was to examine the epidemiology of active TB in the active component US military in the context of the changing epidemiology of TB in the US and from recent conflicts:

Methods

Surveillance data were examined among all active component US military service members from 1990 to 2007.

Results

The rate of confirmed active TB in the US military decreased from 1.44 per 100,000 person-years in 1998 to 0.29 per 100,000 person-years in 2007, a decline of 80%.

Conclusion

The incidence of active TB in the active component US military is low and continues to decline. Continued vigilance for active TB and targeted testing and treatment at entry into service for latent TB infection may allow the elimination of TB in this population.

Introduction

Tuberculosis (TB) is a well-recognized public health problem in the United States, accounting for 12,898 cases in 2008, or a rate of 4.2 per 100,000 person-years.[1] However, the slowing decline of TB incidence makes it increasingly unlikely that the US will meet its goal of eliminating TB, defined as a rate of less than 1 per one million person-years [2, 3] in the foreseeable future. To speed the decline of TB in the US, increased attention is being paid to certain populations at higher risk for TB infection and subsequent active disease. Foreign-born and certain racial and ethnic groups make up an increasingly larger proportion of cases in the US, with 59% of active TB cases occurring among foreign-born persons in 2008.[1]

The military represents a segment of the US population that may be at increased risk for TB infection and transmission due to overseas service in endemic areas and potential residence in congregate settings. For this reason, surveillance for active TB in US military forces has existed since at least World War I. In both World War I and World War II, the risk of TB during US military service reflected the general risk in the United States, and overseas service did not seem to increase the risk of tuberculosis unless the Soldier had been a prisoner of war (POW).[4] In fact, lower rates of TB disease were seen among Soldiers serving in the European theater than among those in the continental US,[5] an early example of the "healthy warrior effect,"[6] a phenomenon analogous to the "healthy worker effect."

After World War II, the decline of active TB was rapid and dramatic in both US civilian and military populations. Despite high rates of active TB in the local populations of Korea and Vietnam, no excess TB in deployed military forces during these conflicts.[7, 8] Although several notable outbreaks of TB were documented among Sailors during the 1960s, including the well known outbreak aboard the USS Byrd,[9] these were due to activation of latent infection while on the ship and subsequent onboard transmission within the closed environment of Navy ships,[9-12] rather than new exposures in Vietnam or other endemic regions. The most recent data from the 1980s and 1990s continued to suggest that the incidence of active TB was low and declining in US military populations.[13-15]

Although these previous data have suggested that the US military is a low incidence population, no data have been presented related to recent changes in TB epidemiology or recent conflicts. Concerns regarding TB exposure have been raised by military leaders in relation to recent conflicts in Iraq and Afghanistan, countries where TB is endemic and hyperendemic, respectively.[16] No increase in active TB has been reported during that time interval, but no surveillance data have been presented to evaluate. The purpose of this study was to examine recent trends in the incidence of and risk factors associated with active TB in the low-incidence population of the active component US military.

Methods

This was a descriptive study of population-based surveillance data for cases of active TB among all active component military service members from 1990 to 2007. Institutional

Review Board approval was obtained at the Uniformed Services University of the Health Sciences (USUHS). Hospitalizations and reportable medical events (RME) among US military Service Members and other beneficiaries are reported to the Armed Forces Health Surveillance Center (AFHSC), and complete data were available starting in 1990.[17] The AFHSC provided the data from administrative databases, including hospitalizations and reported cases of TB, demographics, and other characteristics. Independent variables analyzed in this study included foreign birth, age, sex, race/ethnic group, service component, rank, occupation, HIV status, occupation, and length of service. Laboratory data were obtained from the Navy and Marine Corps Public Health Center (NMCPHC) from all military treatment facilities between 2004 and 2006.

Cases were identified from records of reportable medical events and hospitalizations for all types of active TB (International Classification of Diseases-9-Clinical Modification [ICD-9-CM] diagnosis codes 010-018) among all active component military service members from 1990 to 2007. However, differences were noted in the definition of reported TB cases between military and civilian reporting systems. Cases from US military reportable medical event surveillance are reported as "probable" if they are clinically compatible with TB, have acid-fast bacilli in a clinical specimen, and do not have a positive culture or nucleic amplification test.[18] Cases are reported as "confirmed" if they have symptoms clinically compatible with TB and have a positive culture or nucleic amplification test. In contrast, cases reported by states to the Centers for Disease Control and Prevention (CDC) are counted only if they are "verified."[19] Case verification is a more stringent requirement than simple reporting, and requires that

state and local health departments verify each reported case according to clinical and laboratory criteria, likely resulting in a more specific case definition.

Several case definitions are used in presenting the data for this analysis as part of the sensitivity analysis. A more strict case definition of only confirmed RME cases is employed since it is a more specific definition, is the most comparable with US national rates[19], and because it captures non-hospitalized cases. However, an alternate case definition of all hospitalization and RME TB diagnoses is also used, since this definition is more sensitive and is more comparable with historical US military TB literature.[13-15] Furthermore, RME data were not available and reasonably complete in the US military until 1998, midway through the period studied.

Incidence rates of active TB were calculated by use of mid-year population as the denominator. Associations of independent variables with active TB were examined by comparing rates among those with various risk factors as well as trends over time. Stratum-specific incidence rates were calculated as the total number of cases captured divided by the total person-years at risk. Person-time at risk was calculated using the mid-year population of active component US military service members for each stratum, obtained from the Defense Eligibility Enrollment Reporting System (DEERS). All statistical analyses were conducted using SAS 9.1 (SAS Institute, Cary, NC). Significance was defined as $p<0.05$ using a two-tailed test. Rate ratios and 95% confidence intervals were calculated using Poisson regression models. Direct standardization was performed to compare age-adjusted rates of active TB between US

military and civilian populations, using the US military population for each year as the standard population.[20]

Results

Of the 643 cases of active TB found in DMSS records from 1990 to 2007, 134 were found only in the reported medical events (RME) data source and 419 only in inpatient medical records; 90 (14%) were found in both data sources. The ICD-9-CM codes of the cases are listed in Table 8. As mentioned previously, the RME data were only complete for the latter half of the time period (since 1998), which is why there were only half the number of RME cases as hospitalized cases. During the period from 1998 to 2007, the numbers of cases identified were similar, with 195 hospitalized cases and 187 RME cases (309 total cases), 73 (23.6%) of which were found in both. A smaller proportion of extrapulmonary cases was found in RME surveillance (3%) compared to those found among inpatient records (16%), $\chi^2=23.6$, $p<0.001$. No increase in active TB incidence was seen after the initiation of conflicts in Afghanistan in 2001 or Iraq in 2003. Overseas service data were available for 28 of the 33 cases (84.8%) since 2003. Of the 28 confirmed cases since 2003, 12 (42.9%) had been stationed overseas prior to active TB diagnosis, 7 (25%) had been deployed in support of military operations, 4 (14.3%) to Iraq and none (0%) to Afghanistan.

Figure 5 compares trends in TB incidence between different data sources to facilitate comparisons with historical military surveillance data (hospitalization) and current US surveillance data ("verified" reported cases). The rate of active TB incidence decreased

between 1990 and 2007 using any data source. The decreasing trend for all of these was highly significant ($p<0.001$). Both RME and inpatient cases showed consistent trends and magnitude beginning in 1998. A spike in incidence from the 1998 outbreak of active TB on the USS Wasp is notable in all data sources.[21] As seen in Table 9, the rate of confirmed cases decreased from 1.44 per 100,000 person-years in 1998 to 0.29 per 100,000 person-years in 2007, a decline of 80%. Figure 6 shows a comparison of the rate of confirmed pulmonary TB in the US military with the age-adjusted rate of pulmonary TB in the US. From 1998 to 2007, the rate of confirmed pulmonary TB in the active component US military was 0.16 times the rate of the age-adjusted rate in the US population (95% CI: 0.13, 0.19).

Table 10 shows the incidence rates of active TB by selected characteristics. All racial or ethnic groups except American Indian/Alaskan Natives had higher rates of active TB than self-reported whites/Caucasians. Of the 59 confirmed cases since 1998 with known country of birth, 29 (49%) were foreign-born and 30 (51%) were US-born.

Prior skin test results were unavailable. Other factors considered were HIV status, length of service, rank, and military occupation, but denominators were unavailable for these factors, precluding the calculation of rates. None of the confirmed cases had a positive HIV test despite biennial testing. Twenty-seven (27) of 89 confirmed TB cases (30%) with known length of service had < 1 year of active service, 11 (13%) 1-2 years of service, 15 (17%) 3-4 years, 21 (24%) 5-9 years, and 15 (17%) 10 or more years of

service. Seven of ninety (8%) were officers. Five (6%) worked in health care, two (2%) worked in the Military Police, and the remainder (92%) worked in low-risk occupations.

Data on drug susceptibility were available from 2004-2006. Of the 28 culture-positive specimens over this time period, 25 had some drug susceptibility data available. None of the cases had MDR-TB. Two of 24 (8.3%) were resistant to isoniazid, 0 of 25 were resistant to rifampin, 0 of 16 were resistant to pyrazinamide, 0 of 25 were resistant to ethambutol, and 1 of 23 (4.3%) was resistant to streptomycin.

Discussion

The current rate of active TB is very low, and it was much lower than that seen in the age-adjusted rate among the US population (rate ratio=0.16) over the same time interval. The low rate in the US military meets the Healthy People 2010 goal of a rate of less than 1 per 100,000 person-years [22] and approaches the defined goal of elimination of TB in the US of 1 per 1 million person-years.[2, 3, 23] Furthermore, active TB continued to decline in the US military despite large-scale operations in the TB-endemic countries of Afghanistan (beginning in 2001) and Iraq (beginning in 2003). This study had similar findings as other recent studies describing the epidemiology of active tuberculosis in the military up to the mid-1990s, including low and declining incidence rates of active TB, and associations with foreign birth and ethnic groups.[13-15] As expected, the risk factors for TB exhibited in the US military were also similar to those seen in the civilian US population, particularly foreign birth, race, and ethnic group.[19, 24, 25]

The control of TB in low incidence populations presents different challenges than in higher incidence populations.[26] Outbreaks of TB have been described in other low-incidence populations and were attributed to lack of expertise resulting in delays in diagnosis and incomplete follow-up.[26, 27] Such outbreaks can already be seen in the US military, such as those on the USS Wasp and the USS Ronald Reagan.[21, 28] Understanding the epidemiology and transmission patterns of active TB in very low incidence populations is important in order to avoid increased transmission and resultant outbreaks. Surveillance is therefore a critical component of successful TB control, providing information necessary to target prevention efforts; to inform control measures, policies, and program evaluation; and to measure progress towards TB elimination.[29]

A major limitation of this study was the potential for misclassification of TB diagnosis. Unfortunately, examining each medical record to obtain a "gold standard" diagnosis of TB was not feasible due to geographic dispersion of these records worldwide. Another important limitation of this study is the difference in classification of cases between the civilian and military surveillance systems. Overcounting of military TB cases is possible since cases are not subject to the verification process routinely done for civilian cases, and many may not be true cases of active TB. Conversely, undercounting of military TB cases is possible when only confirmed cases are considered. To estimate the magnitude of this underreporting, we used the proportion of verified cases reported to CDC that were culture-negative (20%).[19] The revised estimate of active TB in the US military after accounting for this underreporting is 0.81 per 100,000 person-years, which is still much lower than the age-adjusted US population rate (rate ratio=0.19).

US military tri-service reporting guidelines only include pulmonary TB as reportable,[17] so extrapulmonary TB is underreported. There is also confounding from demographic differences between the services, in particular the long-standing relationship that the Navy has had with the Philippines.[30] Standardization by these factors, particularly foreign birth, was also desired, due to its profound confounding effect on TB epidemiology in the US, but this was not possible because no reliable denominators were available. Cases may also be incompletely captured due to less complete reporting overseas, seeking care outside the military health care system, or discharge from service prior to development of TB, although the number of these cases is expected to be small. The impact of large outbreaks of active TB aboard US Navy ships may have a dramatic impact on surveillance trends, as noted in the 1998 USS Wasp outbreak.[21] Although less common in recent years, the closed environment of a Navy ship has caused numerous outbreaks in the past, most recently in 2006.[9-12, 28, 31]

To achieve elimination, TB control efforts in the US military must focus on all of the four prioritized strategies recommended by the US Advisory Committee on the Elimination of Tuberculosis (ACET): 1) prompt detection and reporting of TB, 2) protecting close contacts of patients with contagious TB, 3) targeted testing and treatment of LTBI, and 4) identifying high-risk settings for TB transmission and mitigating that risk.[29] Continued vigilance is also critical to avoid autochthonous transmission of TB in this population by maintaining awareness and expertise in dealing with active TB both in garrison and when serving overseas. Eighty years ago, Frost advised that, "…as the cases become fewer and

fewer, …the protection thrown around these infective cases and their immediate contacts [should not] be relaxed, but steadily and progressively increased."[32] This includes education of providers and public health personnel in diagnosis, treatment, and follow-up investigation of TB.

Improvement of TB surveillance procedures is also a high priority, including the alignment of military and civilian surveillance procedures, such as reporting extrapulmonary TB and verification of all reported TB cases. DNA fingerprint surveillance is another uniquely valuable method of monitoring for transmission of TB in low-incidence populations that should be applied in military cases, particularly to document potential overseas service-related TB transmission. Alignment of civilian and military TB surveillance could be accomplished through the use of the RVCT (Report of a Verified Case of Tuberculosis).[19] The use of the RVCT would not only enhance comparability and confidence in TB rates and associated trends, but would also provide important additional information on TB case characteristics.

Finally, as most of the TB in the US continues to be activation of LTBI, continued emphasis should be placed on targeting high-risk groups for treatment of LTBI [33], particularly at entry into service. Treatment of LTBI with 9 months of isoniazid (INH) is 90% effective in preventing progression to active TB.[34] The US military encounters significant challenges in adherence to LTBI therapy, including frequent changes of station, deployments, and a young, healthy population reluctant to take medications. Efforts at better targeting and treatment of LTBI in order to improve completion among

high-risk individuals are warranted. One such method may be the use of the interferon-gamma release assay (IGRA). As the IGRAs are thought to be more specific than the TST,[35] they may be useful in focusing targeted testing by avoiding treatment of false positives. However, it is important to note that in a low-prevalence population such as the US military, IGRAs will still have a high proportion of false positives if performed universally among all service members. False positives for both the TST and IGRAs are greatly reduced when performed as recommended, by targeting testing of individuals at increased risk.[36] Logistics and cost should also be considered when deciding whether to use the TST or IGRA.

Several future studies are suggested by this descriptive study. A full record review of all active cases would help in assessing the validity of TB diagnosis in the different reporting systems, as well as with the general US population. Improved surveillance through use of the RVCT and DNA fingerprinting may also shed important insights into TB epidemiology in the US military. An evaluation of the LTBI program as implemented may also be useful in several respects, including adherence to therapy, risk of progression to active TB, and adverse events from LTBI therapy. Finally, an evaluation of the benefits and costs of the use of IGRAs for the diagnosis of LTBI as compared to the TST may be valuable to TB control efforts.

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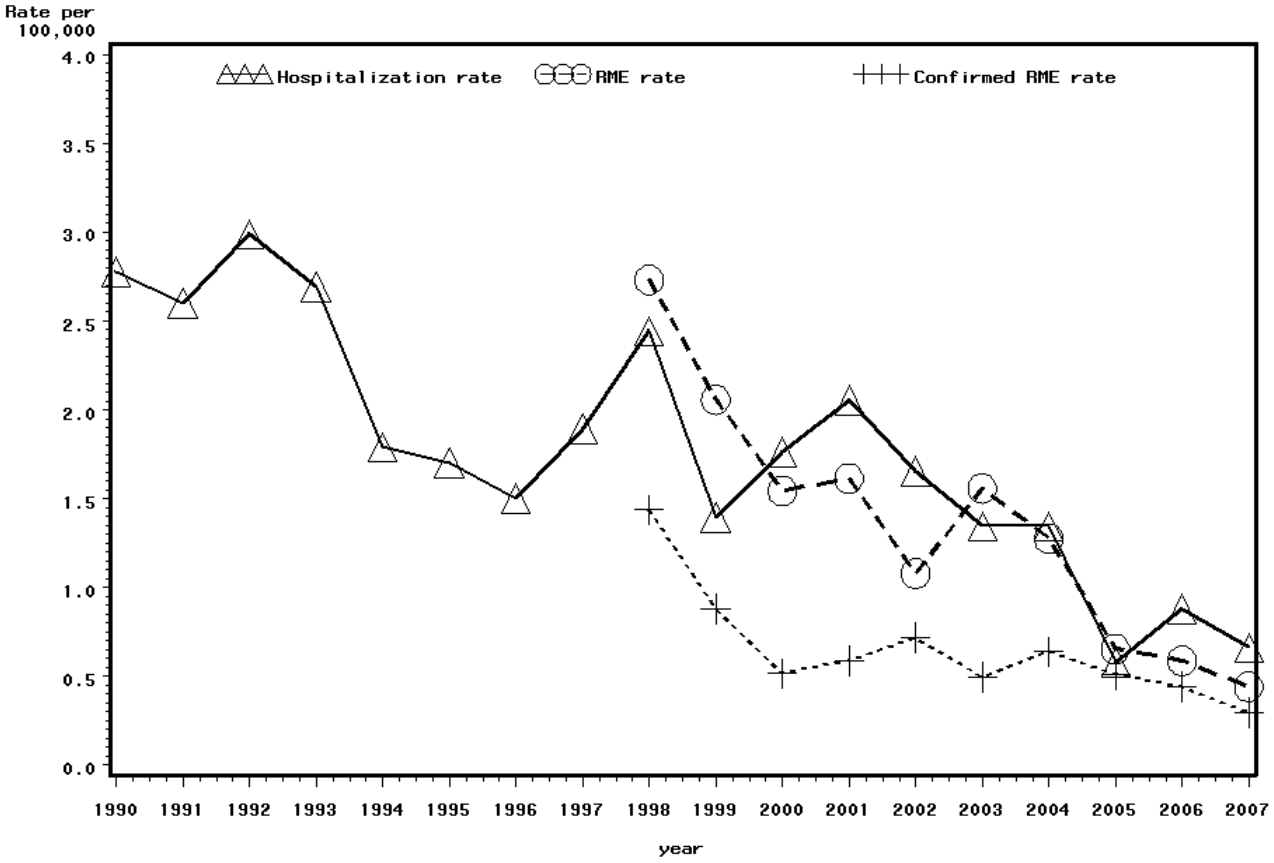
Table 8. ICD-9 Diagnosis codes of active TB cases in the active component US military, 1990-2007

Diagnosis	Reported	Hospitalized
PULMONARY	**217 (97%)**	**429 (84%)**
010 (Primary TB)	11 (4.9%)	14 (2.8%)
011 (TB of Lung)	206 (92.0%)	376 (73.9%)
012 (Other Respiratory TB)	0	36 (7.1%)
018 (TB, Miliary)	0	3 (0.6%)
EXTRAPULMONARY	**7 (3%)**	**80 (16%)**
013 (TB Meningitis)	1 (0.5%)	8 (1.6%)
014 (TB Peritonitis)	0	3 (0.6%)
015 (TB of Bone)	1 (0.5%)	40 (7.9%)
016 (TB of Genitourinary System)	0	5 (1.0%)
017 (TB of Organ)	5 (2.3%)	24 (4.7%)
Total	224 (100%)	509 (100%)

Note: There were 643 total cases, with 90 cases (14%) in both data sets

Figure 5. Comparison of the trends in incidence of active TB in the US military by data source, 1990-2007



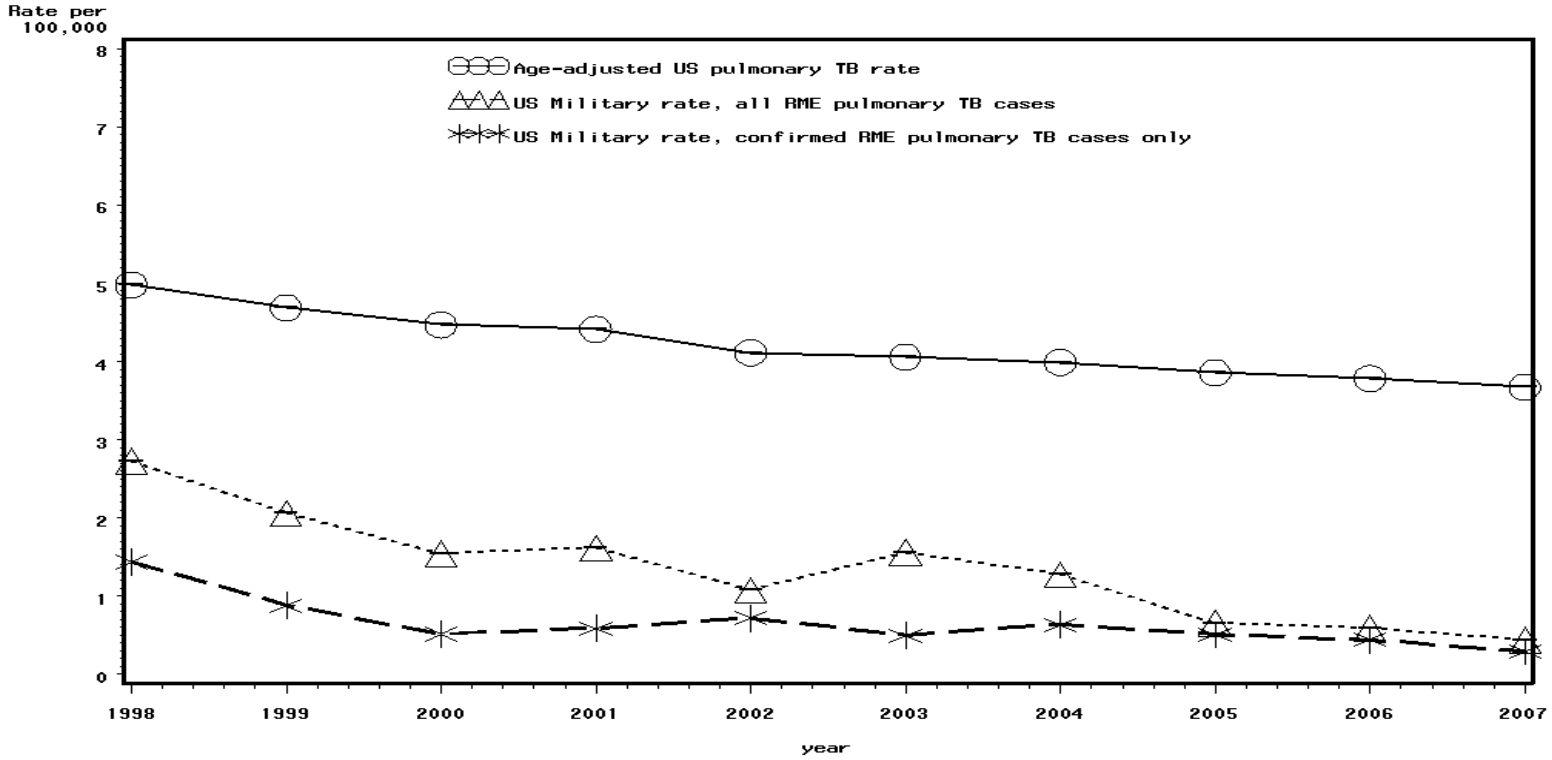
RME=Reportable Medical Events.
Note: χ^2 test for all three trends demonstrated $p<0.001$

Table 9. Incidence rates of active TB in the active component US military, by year

Year	*All RME and Hospitalized Active TB Cases, 1990-2007*					*Confirmed RME Cases Only, 1998-2007*				
	Number of cases	Incidence Rate (per 100,000)	Percent change in rate	Rate Ratio	95% Confidence Interval	Number of cases	Incidence rate (per 100,000)	Percent change in rate	Rate ratio	95% confidence interval
1990	57	2.78		(Ref)	(Ref)					
1991	52	2.60	-6.4%	0.94	0.65, 1.36					
1992	55	2.99	+14.9%	1.08	0.74, 1.56					
1993	46	2.69	-9.9%	0.97	0.66, 1.43					
1994	30	1.85	-31.2%	0.67	0.43, 1.04					
1995	28	1.83	-1.1%	0.66	0.41, 1.04					
1996	25	1.71	-6.9%	0.61	0.38, 0.98					
1997	41	2.88	+68.7%	1.04	0.69, 1.55					
1998	58	4.17	+44.9%	1.50	1.04, 2.16	20	1.44		(Ref)	(Ref)
1999	36	2.65	-36.6%	0.95	0.63, 1.44	12	0.88	-38.7%	0.61	0.30, 1.25
2000	36	2.65	+0.1%	0.93	0.63, 1.45	7	0.51	-41.6%	0.36	0.15, 0.85
2001	42	3.08	+16.4%	1.10	0.74, 1.65	8	0.59	+14.0%	0.41	0.18, 0.93
2002	31	2.23	-27.7%	0.80	0.52, 1.24	10	0.72	+22.5%	0.50	0.23, 1.07
2003	34	2.41	+8.0%	0.87	0.57, 1.32	7	0.50	-31.1%	0.34	0.15, 0.82
2004	31	2.20	-8.8%	0.79	0.51, 1.22	9	0.64	+28.6%	0.44	0.20, 0.97
2005	13	0.94	-57.0%	0.34	0.19, 0.62	7	0.51	-20.3%	0.35	0.15, 0.84
2006	15	1.10	+16.7%	0.40	0.22, 0.70	6	0.44	-13.3%	0.31	0.12, 0.76
2007	13	0.96	-13.3%	0.34	0.19, 0.63	4	0.29	-33.3%	0.20	0.07, 0.60
Total	643	2.34	-65.5%*			90	0.65	-79.6%*		

RME: Reportable Medical Events *Overall % change 1990-2007

Figure 6. Comparison of the Incidence of Confirmed Active Pulmonary TB in the Active Component US Military with the Age-Adjusted Incidence of Verified Active Pulmonary TB in the US, 1998-2007



Rate ratio:										
(All cases):	0.55	0.44	0.35	0.37	0.26	0.38	0.32	0.17	0.16	0.12
(95% CI)	(0.40, 0.75)	(0.30, 0.64)	(0.23, 0.53)	(0.24, 0.56)	(0.16, 0.44)	(0.25, 0.58)	(0.20, 0.51)	(0.09, 0.33)	(0.08, 0.31)	(0.05, 0.27)
(Confirmed):	0.29	0.19	0.12	0.13	0.17	0.12	0.16	0.13	0.12	0.08
(95% CI)	(0.19, 0.45)	(0.11, 0.33)	(0.05, 0.24)	(0.07, 0.27)	(0.09, 0.33)	(0.06, 0.26)	(0.08, 0.31)	(0.06, 0.28)	(0.05, 0.26)	(0.03, 0.21)

Table 10. Incidence of active TB in the active component US military by selected characteristics, 1990-2007

Factor	*All RME and Hospitalized Active TB Cases, 1990-2007*				*Confirmed RME Cases Only, 1998-2007*			
	Number of cases	Incidence Rate (per 100,000)	Rate Ratio	95% Confidence Interval	Number of cases	Incidence rate (per 100,000)	Rate ratio	95% confidence interval
Sex								
Male	550	2.28	1 (Ref)	1 (Ref)	77	0.91	1 (Ref)	1 (Ref)
Female	93	2.53	1.11	0.89, 1.38	13	0.68	0.75	0.43, 1.31
Race								
White	236	1.32	1 (Ref)	1 (Ref)	25	0.27	1 (Ref)	1 (Ref)
Black	199	3.74	2.83	2.34, 3.42	23	0.96	3.12	1.77, 5.50
Asian/PI	110	11.12	8.41	6.70, 10.54	31	5.50	18.57	10.96, 31.45
Hispanic	74	2.78	2.10	1.62, 2.73	10	0.78	2.49	1.20, 5.19
Other	11	5.08	3.84	2.10, 7.03	0	0	0	*
AI/AN	3	0.94	0.71	0.23, 2.22	0	0	0	*
Unknown	10	2.24	1.69	0.90, 3.18	1	0.40	1.27	0.17, 9.36
Age group								
<20	78	3.63	1 (Ref)	1 (Ref)	15	1.32	1 (Ref)	1 (Ref)
20-24	199	2.18	0.60	0.46, 0.78	30	0.65	0.49	0.26, 0.91
25-29	131	2.20	0.60	0.46, 0.80	15	0.51	0.39	0.19, 0.79
30-34	96	2.16	0.59	0.44, 0.80	11	0.52	0.44	0.18, 0.86
35-39	77	2.10	0.55	0.42, 0.79	11	0.58	0.36	0.20, 0.95
40+	65	2.49	0.68	0.49, 0.95	8	0.55	0.42	0.18, 0.98
Service								
Army	272	2.85	1 (Ref)	1 (Ref)	39	0.80	1 (Ref)	1 (Ref)
Navy	210	2.78	0.97	0.81, 1.17	30	0.82	1.02	0.64, 1.65
Air Force	92	1.29	0.45	0.36, 0.57	10	0.28	0.35	0.17, 0.70
Marines	69	2.15	0.75	0.58, 0.98	11	0.63	0.78	0.40, 1.52

* = not calculated due to small cell size RME=Reportable Medical Event

Chapter 3—Active Tuberculosis and Recent Overseas Deployment in the US Military

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Medical Subject Headings: Tuberculosis epidemiology, Nested Case-Control Study, Deployment medicine
Abbreviations: TB, tuberculosis; MDR-TB, multi-drug resistant tuberculosis; HIV, Human Immunodeficiency Virus; TST, tuberculin skin test; BMI, body mass index; LTBI, latent tuberculosis infection

Abstract

Background: The risk of active TB resulting from military deployment to endemic areas is unknown. It has typically been assumed that the risk of TB approximates the risk among local nationals in that country. This nested case-control study assesses the putative association of overseas deployment with active tuberculosis among active component US military service members.

Methods: Deployment histories and other exposures among 578 active TB cases and 2312 controls matched on year of entry into service and length of service between 1990 and 2006 were compared in 2009 using multivariate conditional logistic regression. Multiple imputation methods were used to account for missing data.

Results: The matched odds ratio of active TB for military deployers as compared to non-deployers was 1.18 (95% confidence interval: 0.91, 1.52). A statistically significant association with deployments of 90-179 days was found, but this was inconsistent with the overall negative result. Significant associations were seen with foreign birth and non-white racial or ethnic groups. Overseas stationing in Korea was also found to be associated with active TB.

Conclusions: We found no strong or consistent association between active TB and deployment, but an association was seen with long-term residence in TB endemic countries (Korea). The strongest risk factors for active TB in the US military population

were found to exist prior to accession into military service. These conclusions were robust in sensitivity analysis.

Introduction

The US military has a long history of dealing with tuberculosis (TB) as a communicable disease threat to its deployed service members. However, the amount of increased risk for TB resulting from overseas service has been uncertain. During World War I and World War II, high rates of disease existed in the military, but this was primarily due to the presence of high rates of TB within the US prior to accession into service rather than overseas exposure.[1-3] Similarly, despite high rates of active TB in the local Korean population during the Korean War, no excess TB in military forces was observed.[4] During the Vietnam conflict, multi-drug resistant tuberculosis (MDR-TB) was increasingly being noted in the US, and there was again concern that TB was being imported from Vietnam, particularly drug-resistant TB. However, the rate of MDR-TB among deployed US service members was found to be similar to the general US population.[5] Concerns that service members may become exposed to TB have been raised in recent deployments to Iraq and Afghanistan,[6] where TB is endemic and hyperendemic, respectively.[7]

The rate of active TB in the US military is known to be lower than that of the general US population due to the "healthy warrior effect," which is analogous to the healthy worker effect.[8] Despite the potential increase in exposure to TB from deployment to endemic areas, the current rate of active TB in the active component US military based on surveillance data remains low, with a rate of 0.7 verified cases per 100,000 person-years from 1998 to 2006 (Mancuso JD, Uniformed Services University, Bethesda, MD,

unpublished manuscript), compared to an age-adjusted US rate of 4.3 per 100,000 person-years.[9] Another major determinant of this difference between the epidemiology of TB in US military and civilian populations is the role of foreign birth and immigration status. Although the current proportion of foreign born in the US military is unknown, precluding formal standardization, it is believed to be approximately 5%.[10] This may be an underestimate since these data are not uniformly captured for all service members. Rates of TB in both military and civilian US populations continue to decline.

Long-term civilian travelers are often considered to have a risk of TB infection similar to the local population,[11] although some have questioned this.[12] TB control policies in the US military are based largely on the risks to long-term civilian travelers, which are poorly understood.[13] However, the actual contact of US military service members with potentially infectious TB cases in deployed settings may be very limited, as many US service members have limited or no contact with local nationals outside US military installations. Thus, their resultant risk of TB infection may be much lower than most long-term travelers. Nevertheless, there is a concern that deployments to endemic and hyperendemic areas such as Iraq and Afghanistan could result in large numbers of potentially infected military service members. US military services routinely perform widespread testing with the tuberculin skin test (TST) to identify and treat latent TB infection. For example, until late 2008 the US Army tested each soldier within a year prior to deployment, then twice upon return from deployment,[13] resulting in over 500,000 tests performed each year in the Army alone. Although some units have noted unusually high proportions of TST converters after returning from deployment,

subsequent investigation of these units has suggested that the excess cases are attributable to false-positive skin tests rather than TB transmission.[14]

Rates of active TB among US military service members in relation to recent deployments have not been examined. Although no increase in active TB has been reported since 2001, it is unclear whether this absence of cases is attributable to failure to recognize a relationship between cases and deployment, effective prevention efforts after deployment, or simply a low level of exposure during deployments. The purpose of this study was to determine whether deployment or being stationed overseas was associated with active tuberculosis among active component US military service members.

Methods

A nested case-control analytic study with incidence density or risk set sampling was performed in 2009 to assess risk factors for the development of active TB.[15] Cases were defined from reportable medical events and hospitalizations for all types of active TB (International Classification of Diseases, Ninth Revision, Clinical Modification codes 010-018). Cases were reported as confirmed if they had symptoms clinically compatible with TB and have a positive culture or nucleic amplification test. No spoligotyping or other genotyping was available for these isolates, nor were prior TST data. Cases were obtained from all active component military service members from 1990 to 2006, the underlying cohort in which this case-control study was nested. The risk set consisted of active component personnel who entered military service during the same month as the case, and who were still on active service and had never been diagnosed with active TB

at the time of the case. Four matched controls per case were selected at random from the risk set. There were 578 cases of active TB found in inpatient and reportable medical events (RME) data and 2312 matched controls. 494 (85%) of the cases were pulmonary, and the remainder were extrapulmonary.

The Defense Medical Surveillance System was used to obtain the data on medical encounters, demographics at time of entry into military service, deployment records, and other characteristics for both cases and controls.[16] Variables included foreign birth, citizenship, sex, race-ethnicity, and body mass index (BMI) at entry into military service. Also obtained were age, service, occupation and rank at the time of diagnosis of the case, prior infection with human immunodeficiency virus (HIV), stationing outside the US, and deployment. Data on stationing outside the US included location and length of residence. Deployment was defined as any event or activity that relates to duty in the armed forces that involved the movement or relocation of the service member into areas where military operations such as combat, peacekeeping, or humanitarian assistance are taking place,[17] such as Iraq, Afghanistan, Bosnia, and Kosovo. Deployment data included number of deployments, length of deployments, and deployment location. Overseas stationing was defined as being assigned to live at a fixed place of duty outside the United States, such as Western Europe, Korea, Guam, or Japan during peacetime. Deployment and overseas stationing were only defined as exposures if they preceded the date of onset of disease for both the cases and matched controls to avoid temporal bias. HIV testing is required on entry into service and then subsequently every two years

thereafter in all military services, so all service members had at least one HIV test performed.

Analysis was accomplished through the use of bivariate methods using matched odds ratios and 95% confidence intervals. Multivariate analysis was performed with conditional logistic regression for matched data using SAS 9.1 (SAS Institute, Cary, NC). Of the 2,890 cases and controls analyzed, only 1,478 (51.5%) were used in the traditional conditional logistic regression model using complete case analysis because of missing data. Variables were examined based on *a priori* biologic plausibility and selected for the model based on statistical significance with $p \leq 0.05$. As the variables of deployment and overseas stationing in Japan were of interest in the analysis, these were kept in the model even with $p > 0.05$. To further examine the diminished precision and potential bias resulting from missing data in complete case analysis,[18] multiple imputation was also used, as has been recently recommended.[19] Five imputations were performed using SAS procedures MI and MIANALYZE. A two-step imputation process was used, with the first imputation achieving a monotone missing data pattern using the Monte Carlo method, then a second to achieve full imputation using logistic regression. The variables used to impute missing data were TB case status, sex, race and ethnicity, age, rank, foreign birth, HIV status, stationing in Korea, stationing in Japan, and deployment status. Imputation was also performed separately for cases and controls to examine whether the joint distribution of exposures and covariates differed by case status. As no meaningful differences were seen, these results are not shown. Results from imputed and complete case analyses were compared, along with corresponding confidence intervals. Sensitivity

analyses included analysis of cohort effect using birth year, effect modifiers, restriction to only laboratory-confirmed cases, and period effects. Effect modifiers included all two-way interactions with foreign birth and prior deployment. Period effects included restriction to data from 2002 and later to look at the effect of increasing deployments in support of current US military operations in Afghanistan and Iraq.

Results

The bivariate (unadjusted) analysis of demographic data is presented in Table 11. The mean age at diagnosis was slightly higher in cases than in matched controls. There was no difference noted in the distribution of sex between cases and controls. Cases were more likely to be foreign-born (matched odds ratio [OR]=6.75, 95% confidence interval [CI]: 4.80, 9.50) and were more likely to self-report a racial or ethnic group other than Caucasian or "white." Notably, a large proportion of data were missing from the country of birth field (48%). Cases were less likely to be officers, and differences were noted by service, although only the odds ratio for Air Force personnel was statistically significant. Occupations expected *a priori* to have closer (higher) contact with the location population during deployment, such as medical, military police, and Special Operations personnel, did not have increased odds of TB. This was not affected by grouping the higher contact occupations together. HIV positive service members were more likely to be cases but only consisted of 6 cases. The mean BMI at entry into service was significantly less for cases than for controls.

Table 12 shows the bivariate (unadjusted) analysis of deployment- and stationing-related characteristics of cases and controls. Cases were equally likely to have been deployed (matched OR=1.18, 95% CI: 0.91, 1.52), and no association was seen with deployment to either of the current conflicts in Iraq or Afghanistan. It should be noted that the majority of deployments among cases and controls did not have a location recorded; 423 of 534 (79%) for the entire study period and 48 of 123 (39%) since 2002. A statistically significant association of deployments of 90-179 days was found, but this was inconsistent with the overall negative result. A dose-response relationship was observed with the number of deployments but this was not statistically significant, although the cell sizes were small. Overseas stationing or duration of overseas stationing was not associated with being a case, but cases were more likely to have been stationed in Korea (matched OR=2.24, 95% CI: 1.42, 3.52).

In Table 13, the bivariate (unadjusted) results are compared with the multivariate (adjusted) results using both complete case analysis and multiple imputation models. The results were similar in all three models, demonstrating that risk factors present prior to accession were more strongly associated with TB than military-related factors. TB risk factors present prior to accession included foreign birth, non-white racial or ethnic groups, and age. HIV positivity was associated with being a case in the bivariate and multiple imputation models, but the odds ratio could not be estimated in the complete case analysis due to the small number of HIV cases and controls. The only association between TB and military service was overseas stationing in Korea. There was no association with ever having deployed, number of deployments, duration of overseas

stationing, or overseas stationing in locations other than Korea. One association was found with deployments of 90-179 days in length, but not for any other duration of deployment. Cases were not associated with sex, rank, service, occupation, or BMI in the multivariate models (data not shown).

Sensitivity analyses were performed to examine whether these conclusions were robust to alternative assumptions (data not shown). Restriction to only laboratory-confirmed cases or cases with documented prescriptions to therapy did not alter the results, nor did restriction to the period post-2001 after the beginning of the current conflicts. Restriction to only US-born personnel did not significantly affect the results (data not shown). Cohort effects (birth year) were not significantly associated with being a case, although these effects may have been obscured by the effect of age and by matching cases and controls by year of diagnosis. No effect modification was identified in the two-way interactions studied. In particular, there was no evidence of the hypothesized interaction between deployment and branch of service.

Discussion

The major finding from this study was that overall, no strong or consistent association was found between deployment and active TB. There were other elements of the analysis that suggested some risk from deployment, however, including the dose-response relationship with number of deployments (although not statistically significant) and the statistically significant association with deployments of 90-179 days. Because of the limited statistical power of these data, a cautious interpretation of these findings is

warranted. Furthermore, it is unclear whether the overall lack of association is because US military forces generally have a low risk of active TB resulting from deployments, or because the US military TB control program is effective at preventing active TB resulting from deployment. What is clear, however, is that active tuberculosis among active component military is strongly associated with risk factors present prior to accession and unrelated to military service. These factors include foreign birth, racial and ethnic group, age, and HIV infection, similar to those identified in three previous service-specific studies.[20-22] Stationing in Korea was the only service-related risk factor found to have a strong and consistent association with active TB in this study. These associations were robust and were not altered in sensitivity analyses.

The strong associations seen with risk factors existing prior to accession suggest a need for continuous surveillance for TB during the service member's military career, beginning and focusing on the time of accession. The strong association with foreign birth suggests that these higher-risk individuals may need to be better targeted for interventions at accession, including LTBI diagnosis and treatment. Although all military services currently test for LTBI at entry into service, therapy is not universally accepted or adhered to. The services should consider ways to improve both initiation and adherence to therapy, particularly among higher-risk recruits such as those who are foreign-born.

No previous studies have examined the effect of recent US military deployments on incidence of active TB. However, these findings are similar to those found in previous

conflicts, including World War II,[2] Korea,[4] and Vietnam.[5] In these conflicts, deployment and overseas service did not increase the risk of active TB, with the important exception of those service members who had been taken as prisoners of war. However, active TB has been diagnosed among local and third-country nationals who work on military bases in deployed settings, and service members may be exposed to these cases, particularly in detainee or health care settings.[6, 23] Continued surveillance of service members is critical in identifying any changes in exposures or risks for TB resulting from rapidly changing environments and situations. Individual-level exposures to TB should be considered for each service member returning from deployment.

The association of TB with service in Korea may be explained in several ways. First, the rate of active TB has been higher in Korea (88 per 100,000 person-years in 2006) than in other areas service members are stationed, such as Japan (22 per 100,000 person-years in 2006).[7] Second, there may be greater exposure to active TB in Korea than in other overseas assignments or deployments. This may be due cultural differences among service members living in closer contact with the civilian population in Korea, including spouses or significant others and their families. Similarly, rates of TB higher than those found in the US population were recently reported among Peace Corps volunteers living in close contact with populations in endemic countries.[24] Third, pre- and post-assignment TST testing is done in Korea. This may introduce ascertainment bias either from better recognition of true active TB cases or from miscoding and misreporting of LTBI as active TB. Finally, since service members have been serving in Korea for many years and in Afghanistan and Iraq only since 2001 and 2003, respectively, the amount of

follow-up time at risk is much greater for exposures in Korea. Insufficient data were available to differentiate between these possible mechanisms.

Limitations of this study include limited power to detect meaningful differences, the potential for misclassification and missing data due to the nature of the administrative databases used. The limited power to detect an association with active TB is a result of the small number of active TB cases since 2003 and the development of TB in soldiers early in their careers before they have a chance to deploy. Misclassification was assessed by examination of these TB cases against other data sets, such as pharmacy and laboratory data. This showed that a substantial proportion of TB cases were actually LTBI or other "rule outs" which were misclassified as active TB. This was particularly true of hospitalization data, where the positive predictive value may be as low as 43% (Mancuso JD, Uniformed Services University, unpublished data). Thus, the number of active TB cases reported here is probably an overestimate. This misclassification did not vary by country of birth or deployment, so these differences are likely non-differential, attenuating the estimates of effect. Missing data were common particularly in the case of country of birth, which was missing in 48% of the study population, although this proportion was similar between cases and controls. If the service member's country of birth impacted whether the data were missing (i.e., missing not at random), imputation techniques would result in a biased estimate of effect. However, since the complete case analysis results were similar to the multiple imputation results, this effect is likely to be small. Other sources of misclassification are also possible. Deployment and overseas service records were often incomplete or misclassified, leading to error in these estimates.

For example, the location of deployment was not recorded in 79% of cases and controls, which attenuated the effect of the estimates of deployment to specific countries such as Afghanistan or Iraq.

Temporal trends certainly impacted the data, in the context of declining incidence of TB in the US over this time period,[25] but these were largely accounted for by matching of cases and controls on entry into service. There may be unmeasured or residual confounding from TB exposures and risk factors existing prior to or during military service, such as exposure to an active TB case in a family member or other close contact. More complete information on deployment location and exposures would also enable more precise estimates for individual countries of deployment and deployment activities. Information about several other possible confounders that we would have liked to obtain through record review included diagnosis or treatment of latent or active TB prior to entry into military service. This was not practicable because of the worldwide dispersion of these medical records. Future studies may be indicated to address the issues of unmeasured confounding and missing or misclassified information by obtaining more complete and precise information on TB exposures and other confounders. Cases may be incompletely captured due to deployment, seeking care outside the military, or discharge from service prior to developing TB, although these differences are expected to be small. Finally, the results of this study may not be generalizable to higher incidence populations due to the selection of a healthy, mostly HIV-negative military population.

Overall, the strongest risk factors for developing active TB while on active military service were those existing prior to entry, including foreign birth. This suggests that more intensive efforts at targeting these higher risk individuals at time of entry into service for LTBI therapy may be warranted. A moderately strong association was seen with overseas stationing in Korea, a country with an established higher incidence of TB and where service members often have regular, recurrent close contact with local nationals. Continued surveillance for both active and latent TB among service members stationed in Korea is also therefore also warranted. Although several limitations of the data were noted, no strong or convincing association was found with deployment. Nevertheless, due to the potential for transmission demonstrated by prior outbreaks,[26-29] TB skin test conversions,[14] and contact with active cases during deployment,[6] surveillance of deployed service members for active and latent TB should continue in order to mitigate these risks. In all of these situations, testing should be targeted based on individual risk factors and exposures in order to maximize benefit and minimize risk.[30] Greater efforts at promoting adherence to LTBI therapy among those who are at increased risk are also warranted, particularly among the foreign-born at time of entry into service.

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Table 11. Comparison of Demographic Characteristics of Cases of Active TB and Matched Controls, Active Component US Military, 1990-2006*

Factor	*Cases (n=578)*		*Controls (n=2312)*		*Bivariate Model*	
	No.	%	No.	%	Matched odds ratio	95% confidence interval
Mean age	28.6 (SD 8.1)		27.6 (SD 7.7)		1.04	1.02, 1.05
Sex						
Male	502	87	2000	87	1 (Ref)	
Female	76	13	312	13	0.97	0.74, 1.27
Race/ethnic group						
White	208	36	1487	64	1 (Ref)	
Black	179	31	473	20	2.74	2.17, 3.46
Hispanic	66	11	184	8	2.49	1.81, 3.42
Asian	102	18	79	3	8.95	6.40, 12.53
Other	23	4	89	4	1.91	1.18, 3.09
Country of birth						
US	194	34	1096	47	1 (Ref)	
Foreign	121	21	104	5	6.75	4.80, 9.50
Unknown	263	46	1112	48	0.99	0.69, 1.41
Rank						
Enlisted	549	95	2033	88	1 (Ref)	
Officer	29	5	279	12	0.38	0.25, 0.56
Service						
Army	241	42	929	40	1 (Ref)	
Navy	191	33	583	25	1.21	0.92, 1.59
Air Force	91	16	540	23	0.61	0.44, 0.84
Marines	55	10	260	11	0.77	0.54, 1.10
Occupation						
Infantry	19	3	67	3	1 (Ref)	
Other combat arms	20	3	96	4	0.75	0.37, 1.49
Medical	24	4	98	4	0.88	0.44, 1.74
Police	10	2	56	2	0.65	0.28, 1.51
Special Operations	0	0	2	0	*	*
Linguist	0	0	3	0	*	*
Student	2	0	44	2	0.16	0.04, 0.73
Aviation	34	6	137	6	0.91	0.48, 1.72
Other	468	81	1800	78	0.95	0.56, 1.61
HIV positive	6	1	2	0	12.0	2.42, 59.5
Mean BMI (N=819)	22.9 (SD 3.3)		23.8 (SD 3.3)		0.93	0.88, 0.98

* = cell sizes too small to estimate (contains zeros)

Note: Controls matched on date of entry into service and length of service

Table 12. Comparison of Deployment and Other Characteristics of Military Service of Cases of Active TB and Matched Controls, Active Component US Military, 1990-2006

Factor	*Cases (n=578)*		*Controls (n=2312)*		*Bivariate Model*	
	No.	%	No.	%	Matched Odds Ratio	95% confidence interval
Deployment						
Any	116	20	418	18	1.18	0.91, 1.52
Iraq	11	2	52	2	0.79	0.37, 1.70
Afghanistan	0	0	4	0	*	*
Number of deployments						
0	462	80	1894	82	1 (Ref)	
1	100	17	372	16	1.14	0.88, 1.50
2	11	2	37	2	1.32	0.64, 2.69
3	3	1	6	0	2.20	0.54, 8.88
4	2	0	3	0	3.39	0.45, 25.3
Total duration of deployments						
0	462	80	1894	82	1 (Ref)	
1-89 days	25	4	108	5	0.97	0.61, 1.53
90-179 days	38	7	104	5	1.58	1.04, 2.38
180-365 days	45	8	173	7	1.10	0.76, 1.58
>365 days	8	1	33	1	1.05	0.46, 2.37
Station overseas						
Any	112	19	429	19	1.08	0.82, 1.41
Korea	33	6	65	3	2.24	1.42, 3.52
Japan	22	4	70	3	1.32	0.78, 2.23

* = cell sizes too small to estimate (contains zeroes)
Note: Controls matched on date of entry into service and length of service

Table 13. Selected Risk Factors for Developing Active TB Among Active Component US Military Service Members, 1990 to 2006

Factor	*Unadjusted (bivariate) model*		*Adjusted (multivariate) model using complete case analysis** (N=1487)*		*Adjusted (multivariate) model using multiple imputation** (N=2890)*	
	Matched odds ratio	95% confidence interval	Matched odds ratio	95% confidence interval	Matched odds ratio	95% confidence interval
Deployment	1.18	0.91, 1.52	1.02	0.61, 1.71	1.30	0.95, 1.77
Stationed in Japan	1.32	0.78, 2.23	0.90	0.41, 1.98	1.38	0.77, 2.48
Stationed in Korea	2.24	1.42, 3.52	3.41	1.50, 7.75	2.53	1.51, 4.26
Foreign birth	6.75	4.80, 9.50	4.77	3.23, 7.05	3.78	2.77, 5.21
Not white	3.34	2.74, 4.06	3.17	2.30, 4.37	2.56	2.05, 3.16
Age (per year)	1.02	1.02, 1.05	1.02	0.97, 1.06	1.04	1.00, 1.05
HIV	12.0	2.42, 59.5	*	*	14.9	2.46, 90.0

* = cells sizes too small to estimate (contains zeroes)
** = adjusted for all the other variables listed in the model
Note: Controls matched on date of entry into service and length of service

Chapter 4—A Robust Evaluation of Commercially-Available Diagnostics for Latent Tuberculosis Infection

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Running head: Analysis of Discordance between TST and IGRAs

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Abstract

Introduction

The objective of this study was to provide the most complete methodology in understanding the interpretation of the commercially-available tests for latent tuberculosis infection (LTBI) among a heterogeneous, low prevalence US population. These included the tuberculin skin test (TST) and the two interferon-gamma release assays (IGRAs), QuantiFERON®-TB Gold In-Tube (QFT) and T-SPOT®.TB (T-Spot).

Methods

This was a cross-sectional comparison study among 2,017 military recruits at Fort Jackson, SC from April to June 2009. Several tests were performed simultaneously, including: 1) a risk factor questionnaire, 2) QFT, 3) T-Spot, 4) TST, and 5) Battey skin test (BST) using purified protein derivative from the Battey bacillus (PPD-B). Specificity was estimated among low-risk recruits. BST results were used to examine the association of non-tuberculous mycobacteria (NTM) cross-reactivity with test discordance. Bivariate and multivariate analyses were used to identify factors associated with discordance.

Results

Specificity estimates were 98.8% for the TST using a 10 mm induration cutoff or 99.4% using a 15 mm cutoff. The specificity of both the T-Spot and QFT was 98.8%. Agreement between TST and T-Spot was 96.9% (kappa=0.38); agreement between TST and QFT was 96.2% (kappa=0.26). Of the 95 with a positive TST or IGRA, only 12 (12.6%) were positive to all

three tests; 21 (22.1%) were positive to at least two of the tests. Strong associations between TST positive, IGRA negative discordance and BST reaction size were seen.

Conclusions

This study provides the most robust, complete evaluation of commercially-available diagnostics in a population representative of the low-prevalence seen in the United States seen to date. Factors associated with TST-positive, IGRA-negative discordance were similar for both T-Spot and QFT, including both NTM sensitization and BCG vaccination. There was modest agreement between TST and IGRA, but for the majority of positive results, the three tests identified different people. This suggests that in heterogeneous, low-prevalence US populations, the majority of positives resulting from any of the three commercially-available diagnostic tests will be false positives.

Introduction

The tuberculin skin test (TST) is prone to false positives following vaccination with Bacille Calmette-Guérin (BCG) (1) and sensitization to non-tuberculous mycobacteria (NTM) (2). Interferon gamma release assays (IGRAs), including the QuantiFERON®-TB Gold In-Tube (QFT) and T-SPOT®.TB (T-Spot) tests, were designed to be more specific and have other logistic advantages over the TST (3). For these reasons, military services are considering the use of IGRAs as an alternative to the TST in recruit populations. However, there are also important drawbacks to the use of IGRAs, including higher cost, laboratory burden, and variability in serial testing (4). Most importantly, however, is the relative lack of long-term, longitudinal data which demonstrate that IGRAs predict risk of progression to active tuberculosis (TB), as is available with the TST (5, 6).

There is no gold standard in evaluating the performance of the IGRAs in comparison with the TST other than the long-term progression to active TB in cohort studies (7). As these data are scarce, the IGRAs are routinely compared in practice to the TST in cross-sectional evaluation studies, using active TB cases to assess sensitivity and low-risk populations to assess specificity (3, 8). In these studies, significant discordance is often found between IGRA and TST results. In a study of Navy recruits, 11 of 15 (73%) of the highest risk individuals—whose country of birth had a rate of active TB of >100 per 100,000 person-years and who had TST reactions of at least 15 mm—had negative QFT-Gold tests (9). There are several explanations for these discordant results, including the use of region of difference one (RD1) antigens in the IGRAs, which might result in greater specificity. However, it is also possible that the TST may have

greater sensitivity, that the IGRAs may detect only unresolved or more recent infections (10), or that TST and IGRAs provide complementary measures of immune response (11).

One common explanation for this discordance is that the TST has a greater proportion of false-positive results due to cross reactivity induced by NTM. False-positive TST results due to cross reactions following NTM exposure and infection have been well-known for over 60 years (12). While the effect of NTM sensitization on tuberculin reactions is expected to be of little clinical significance in countries where the prevalence of *M. tuberculosis* infection is intermediate or high (13), cross-reactions due to NTM may be an important potential source of false-positives in areas where the likelihood of *M. tuberculosis* infection is very low (14). The late Dr. George Comstock remarked in 1975 that "the frequency of cross-reactions to tuberculin in this [Navy recruit] population is sufficiently great that the prevalence of true tuberculous infections among white recruits may already be approaching zero (15)." False-positive TSTs from cross-reactivity may be particularly common among those from the southeast US (16). Furthermore, the prevalence of sensitization to NTM in the US civilian population increased from 11% in 1972 to 17% in 2000 (17). Military recruits are an excellent population to explore NTM sensitization as a potential source of TST/IGRA discordance, since BCG and waning sensitivity to TST due to age are uncommon.

The impact of cross-reactivity on TST results has been previously investigated by comparing results of skin tests performed with purified protein derivative (PPD) made from *M. tuberculosis* and several NTMs, including a strain known as the Battey bacillus (PPD-B). PPD-B is a skin test antigen made from *Mycobacterium intracellulare* in a manner similar to how the TST is

made from *M. tuberculosis,* hereafter called the Battey Skin Test (BST). The BST has been used as an aid in the differentiation of reactivity to *M. tuberculosis* from reactivity to non-tuberculous mycobacteria in the Navy Recruit study (2, 16) and the National Health and Nutrition Examination Survey (NHANES) (17-19). It has also been used in many other smaller epidemiologic studies (20-25). The objective of this study was to examine agreement between the three commercially-available diagnostic tests for LTBI (TST, QFT, and T-Spot) in a heterogeneous, low prevalence US population, and to examine the role of NTM sensitization as a source of discordance between TST and IGRAs. The impact of NTM sensitization on discordance is investigated by comparing TST results with BST.

Methods

Regulatory Information

The PPD-B non-tuberculous mycobacterial skin test antigen was used under an Investigational New Drug (IND) sponsored by the Uniformed Services University (USU) in Bethesda, MD. The Infectious Diseases Institutional Review Board at USU provided approval and oversight of the study. The PPD-B was prepared from the same stock concentrate used in the NHANES and Navy surveys (16, 18), having been obtained from the Center for Biologics Evaluation and Research (CBER) at the Food and Drug Administration (FDA). The product was prepared in standard 0.1 mcg/mL doses by the Aeras Global TB Vaccine Foundation in Rockville, MD. After dilution and filling of vials, animal testing for potency and general safety was performed according to FDA regulations (26, 27).

Study Design

This cross-sectional comparison study among Army recruits at Fort Jackson, SC consisted of five elements: 1) a TB risk factor questionnaire (RFQ), 2) TST, 3) BST, 4) QFT, and 5) T-Spot.

Study Methodology/Procedures

The initial study procedures included recruitment, consent procedures, and eligibility determination, including a urine human chorionic gonadotropin (HCG) test to screen female participants for pregnancy. Exclusion criteria included severe reaction to a previous skin test, pregnancy, age less than 18 years, vaccination with a live virus vaccine within 28 days, and current major viral infection. All Soldiers undergoing medical processing in the morning were recruited for the study; Soldiers were not approached in the afternoon due to logistical difficulties with specimen processing later in the day.

Risk Factor Questionnaire (RFQ)

The RFQ contained questions about demographics, TB exposure, work history, location of residence, and other factors shown in Table 14. This questionnaire was developed from the risk factors previously identified in the military and non-military literature (28-33), as well as other factors considered candidates for causal relationships with LTBI.

Interferon-Gamma Release Assays

Blood for QFT and T-Spot was collected at the time of routine phlebotomy for recruit in-processing. Personnel performing IGRA assays were blinded to all patient data. QFT was

performed according to package insert instructions, including incubation and centrifugation within the prescribed times (34). QFT tubes containing approximately 1 ml of blood were incubated for 16 to 24 hours at 37ºC within 12 hours of blood collection. After incubation, the tubes were centrifuged and shipped cold overnight to the US Air Force School of Aerospace Medicine (USAFSAM) at Brooks City-Base, Texas (732 or 39.4% of the valid samples) or the Centers for Disease Control and Prevention (CDC) in Atlanta, GA (1118 or 60.4% of valid samples). Testing was completed with the aid of a Triturus automated ELISA workstation (Grifols USA, LLC, Los Angeles, CA). An Access 2003 database (Microsoft Inc, Redmond, Washington) developed by CDC was used to capture raw optical densities from a Triturus automated ELISA workstation and calculate QFT results. The software performs a quality control assessment for each ELISA, generates a standard IFN-γ concentration curve, and provides a test result for each subject. Blood for T-Spot was collected into 8 ml sodium heparin tubes, mixed gently, and shipped overnight at room temperature to the Oxford Immunotec, Inc. Laboratory in Marlborough, MA. T-Spot was performed per package insert instructions (35), except for the addition of T-cell Xtend (Oxford Immunotec, Ltd., Oxfordshire, UK) immediately prior to peripheral blood mononuclear cell (PBMC) recovery. T-cell Xtend (25 µl/ml of blood) was used to increase the window for processing from 8 hours up to 32 hours.

Skin testing

All personnel involved in placement and reading of the skin test were trained and monitored to strictly adhere to standard operating procedures (SOPs). SOPs were based on published methods for skin test administration and interpretation (28, 36). Subjects had both TST and BST placed by study personnel. The Mantoux technique was used to intradermally administer 0.1 mL (5

TU) of Tubersol® tuberculin PPD (Sanofi Pasteur Ltd., Toronto, Ontario, Canada) and 0.1 mL (0.01 mcg) of PPD-B at the same setting. One skin test was placed on each forearm. A random number table for each recruitment day determined the placement of PPD to each arm. The transverse diameter of induration at each skin test site was measured 2 days after PPD injection. Participants and those administering and reading the skin tests were blinded to which skin test antigen was administered on each arm.

Definitions

Recruits were categorized as to risk as follows: 1) "high-risk" if they reported household contact with someone with TB or immigration within prior 5 years, prior diagnosis or treatment of TB; 2) "moderate risk" if they did not meet criteria for being "high-risk" but reported casual contact with someone with TB, immigration more than 5 years ago, overseas travel of > 1 month duration; or having resided, worked, or volunteered for more than 1 month in a homeless shelter, prison, drug rehabilitation unit, hospital, or nursing home; or 3) "low risk" if they did not report any of the risks resulting in "moderate risk" or "high risk" classification. The TB prevalence reported by the World Health Organization in 1990 was used to estimate exposure risk by country using groups of: 1) less than 20 per 100,000, 2) 20 to 100 per 100,000, and 3) greater than 100 per 100,000 (9, 37). Test specificity was estimated by selecting recruits at low risk for *M. tuberculosis* exposure and assuming them to be uninfected. An indeterminate test was defined as a borderline result for the T-Spot or a low mitogen or high nil response for the QFT. An invalid test was defined as those with insufficient blood, misplaced or dislodged caps, an insufficient number of PBMCs recovered, or other laboratory errors. Test discordance was categorized as "TST-positive / IGRA negative" or "TST-negative / IGRA positive" for both the

QFT and the T-Spot. When comparing the reaction size of the TST and BST, the dominant skin test was defined as having an induration size at least 2 mm greater than the non-dominant skin test (2, 18).

Statistical Considerations

The proportion of recruits with a positive TST, T-Spot, and QFT were compared, along with the proportion of indeterminate and invalid results for each test. The proportions of discordant and concordant results were also measured, as well as test agreement using kappa (κ) coefficient. Estimates of specificity, proportion positive, and proportion of indeterminate results were compared using McNemar's test for correlated proportions. Factors associated with discordance were evaluated using standard chi-square bivariate statistics, stratified analyses, and multivariate analysis. Prevalence ratios were directly estimated for both bivariate and multivariate analyses. As the log-binomial model failed to converge due to numerical instability, Poisson regression with robust variance estimation was used to calculate multivariate prevalence ratios (38). The variables evaluated are listed in Table 14.

Discordance between TST and IGRA was further assessed using associations between demographic and exposure variables as well as the results of the BST. TST positive / IGRA negative discordance was assessed separately from TST negative / IGRA positive discordance. The comparison group used for both of these analyses was the group of concordant negatives.

Results

Figure 7 depicts subject participation and follow-up in a flow chart. Of the 3,095 recruits approached from April 1 to June 11, 2009, 2,697 were eligible to participate in the study, of which 2,017 subjects (75%) enrolled. Characteristics of study participants are shown in Table 14, and are similar to the overall recruit population, except for a greater proportion of female recruits in this study. Thirty-eight recruits withdrew prior to blood collection or completion of skin testing; 30 of these were for administrative reasons unrelated to the study. TST results were available for 1,978 (99.9%) of the remaining 1,979 participants, and T-Spot and QFT results were available for 1,888 (95.4%) and 1,835 (92.7%), respectively. TST induration was detected in 122 participants and ranged from 2 to 80 mm. No significant digit preference was identified on inspection of the histogram of reaction size (data not shown).

Using the 1457 low-risk recruits with results available for all three tests to estimate specificity led to estimates of 98.8% for the TST when using a 10 mm criteria for positive (95% CI: 98.1%, 99.3%), or 99.4% (95% CI: 98.8%, 99.7%) when using a 15 mm criteria for positive as recommended by the CDC for persons at low risk of exposure (39). The specificity of the IGRAs was 98.8% for the T-Spot (95% CI: 98.1%, 99.3%); and 98.8% for the QFT (95% CI: 98.1%, 99.3%). None of these differences were statistically significant.

Table 15 shows the outcome of testing by test type. The proportion of subjects with10 mm or greater TST reaction was significantly larger than the proportion of subjects with positive QFT results ($p = 0.009$) or the proportion of subjects with positive T-Spot results ($p = 0.002$). However, 20 of 67 (30%) recruits with 10 mm or greater TST reactions did not have identifiable

risk factors for TB infection. When using a risk stratified interpretation (RSI) for TST as suggested by the CDC (39), a similar proportion of positive results were observed among all three tests, 2.4% for the TST and 2.1% for both the T-Spot and QFT. A greater proportion of QFT samples had invalid results (6.5%) as compared to T-Spot (3.4%) ($p<0.0001$). Stratifying test results by risk category, an assessment of exposure-response was restricted to recruits who had all three tests completed (n=1,820). All three tests showed a dose-response relationship with increasing TB exposure which was highly significant (Table 16).

Analysis of agreement was limited to subjects who had positive or negative results for all three tests (n=1,783) and excluded subjects with indeterminate results by any test. 95 (5.3%) had a positive result to at least one of the three tests. Of these, only 12 (12.6%) were positive to all three tests; 21 (22.1%) were positive to at least two of the tests. Modest agreement between TST and the two IGRAs was seen, with kappas of 0.38 and 0.26 (Tables 17, 18). The proportion of TST-positive / IGRA-negative discordance was roughly twice that of TST-negative / IGRA-positive discordance. Although there was slightly better agreement between T-Spot and QFT, as shown in Table 19 (kappa=0.39), substantial discordance was still evident. In contrast, the agreement seen when using different blinded readers of the skin test under good quality control mechanisms was good for the TST (kappa=0.76, data not shown).

Figures 8 and 9 show the relative reaction sizes of the TST and the BST for two groups. First, the group of concordant TST and IGRA positives is shown in figure 8. Only one of this group out of 12 (8.3%) had a BST dominant reaction (at least 2 mm larger than the TST), with the

remainder having a dominant TST reaction. In contrast, the group of discordant TST positive, IGRA negative (shown in figure 9) had a greater proportion with a dominant BST reaction (12 of 39, or 30.8%). Of the specimens only positive to one test, T-Spot positives rarely also had a BST reaction of 5 mm or greater (3 of 14, or 21%), as did QFT positives (3 of 21, or 14%). In contrast, 29 of 39 (74%) positives to TST only had a similar BST reaction size. However, of those positive to two or more of the three tests, 15 of 21 (71%) also had a BST reaction size of 5 mm or greater.

The associations of TST positive, IGRA negative discordance with participant characteristics of BCG vaccination history, TB prevalence in the country of birth or residence, and BST reaction size are of large magnitude and similar in bivariate analysis for both the T-Spot and QFT (Table 20). Although these effects are attenuated in the multivariate analysis, the associations are still very strong, particularly the associations with PPD-B reaction size. A strong dose-response relationship is also seen between increasing PPD-B reaction size and increasing prevalence of discordance. The dose-response relationship seen with increasing TB prevalence in the country of birth was not as strong. No relationship was seen with discordance and farm work, residence in the southeast US, or time since emigration.

No significant associations were seen between any variables and IGRA-positive / TST-negative discordance or T-Spot / QFT discordance (data not shown). There was no association of TST positive and invalid or indeterminate samples, nor were there differences in results between QFT samples sent to CDC compared to USAFSAM.

Discussion

This study provides the most comprehensive, robust evaluation of current commercially-available TB diagnostics to date. It examines the performance of these diagnostic tests in a population representative of the underlying US source population which is heterogeneous but generally at low risk for TB. The major findings of this study include very similar proportions of positives, estimates of specificity, and TB exposure dose-response relationships for the TST, T-Spot, and QFT. There was modest agreement and a large proportion of discordance between the three tests. Factors associated with TST positive, IGRA negative discordance were very similar for both T-Spot and QFT and strong associations were seen between discordance and sensitization to NTM as measured by BST reaction size. History of BCG vaccination was also independently associated with discordance. This suggests that a substantial proportion of discordance may be attributable to false-positive TST from cross-reactivity induced by NTM and BCG. However, the association of TST positive / IGRA negative discordance with TB prevalence in the country of birth or long-term residence, and the observed dose-response, also suggests that a portion of the discordance may be attributable to false-negative IGRA results. No associations were found with TST negative / IGRA positive discordance. However, the modest agreement seen between the three tests suggests that the majority of positives of any of the tests are false positives in low risk US populations.

As the military population provides an excellent sample of the low-risk, US-born population, the estimates of specificity found in this study should be very reliable. Although both IGRAs are generally found to have specificity higher than the TST, there was surprisingly little difference in specificity between TST and either IGRA seen in this study. These specificity estimates are

limited to recruits who had no history of BCG vaccination. Since BCG vaccination is generally only given in TB endemic countries, the inclusion of these subjects into specificity calculations would have introduced uncertainty as to whether positives should be attributed to false-positive reactions due to BCG vaccine or true-positives reactions from latent *M. tuberculosis* infection. The specificity estimates between TST and IGRA found in this study are similar to those found in previous studies of Navy recruits (9). Although the specificity of QFT is sometimes thought to be higher than that of T-Spot (3, 8), the specificity of the two tests were equivalent in this study. The strong dose-response relationships between TB exposure and positive TST and IGRA results were similar to those reported previously (3, 8).

While IGRAs and TST may be used in the diagnosis of LTBI, they do not give equivalent information and often have discordant results. Several studies have compared IGRAs with TST results and with each other "head-to-head" (40-47). The agreement between QFT and T-Spot is generally very good in simultaneous head-to-head comparisons. However, when an IGRA is compared to TST, discordant results are often found in 20-30% of subjects (8). Although the overall discordance seen in this study was low, with only 83 of 1783 discordant among any of the three tests (4.7%), a high proportion of discordance (87%) was seen among positives. The analysis and interpretation of these discordant results is challenging because the results have been inconsistent, which may be largely due to the population studied. These varied results have led to differing interpretations in different populations, with some concluding that the IGRAs have lower sensitivity (41), while others have concluded that the IGRAs have better specificity due to less cross-reactions to BCG vaccine and waning sensitivity due to age (40).

Few studies have examined the contribution of NTM as a potential source of discordance between the TST and IGRA, and none have used PPD-B. The specificity of the IGRAs was found to be overwhelmingly superior to TST in a hospital-based population with bacteriologically-confirmed NTM infections (44). NTM sensitization was found to be associated with discordance in a previous study of Navy recruits using the first-generation QuantiFERON®-TB test (9). The dose-response relationship between increasing BST reaction size and increasing prevalence of discordance seen in this study also suggests a strong association between NTM sensitization and discordance, which indicates that false-positive TSTs may be a strong contributor to this discordance. BCG vaccination was also strongly associated with discordance in this study.

A limitation of this study is the lack of a gold standard in determining the presence of *M. tuberculosis* infection, making it difficult to assess the true significance of discordance between TST and IGRAs. The significance of reactivity to PPD-B also has some uncertainty. Although it has been shown to assist in differentiating between LTBI and cross-reactions due to NTM (2, 16), PPD-B reactivity also may be due to cross-reactivity following *M. tuberculosis* infection (22, 48). Furthermore, there are other mycobacteria which contain RD1 antigens, such as *M. kansasii, M. szulgai,* or *M. marinum,* which may cause cross-reactions and false-positive reactions to both TST and IGRAs (3, 49). The PPD-B is known to react to these other NTM, although misclassification still occurs since its primary antigenic components are to *M. intracellulare* (22, 48). The prevalence of TB in country of birth or long-term residence was also strongly associated with discordance. This could be from residual confounding of BCG vaccination in these recruits, but it could also be the effect of increased TB exposure, especially

among those born in very high prevalence countries. This suggests that some of the discordance also may be attributable to false-negative IGRAs. There is potential for misclassification of several variables, particularly the recall of BCG vaccination among recruits, but also for other variables such as prior TB treatment and contact with a TB case. Finally, although the recruit population was selected as a representative sample of US populations, which are heterogeneous and low-risk, it may not represent the causes of test discordance in other higher risk populations.

This study is unique in applying the most robust methodology to date in evaluating the commercially-available diagnostics for LTBI, coupling the results from all three tests with the BST and risk factor analysis. No previous studies have employed such a comprehensive evaluation strategy. Furthermore, the study of diagnostics in this population is ideal and appropriate for study as a population that is representative and generalizable to the underlying low-prevalence US source population. In this trial, TST positive, IGRA negative discordance was strongly associated with NTM sensitization, suggesting NTM as a source of false positive TST tests. This study also suggests that the commercially-available TB diagnostics have similar results in heterogeneous US populations with low TB prevalence. The TST, T-Spot, and QFT resulted in similar proportions of positives, specificity, and dose-response relationships with TB exposure in these populations. Despite these areas of agreement, the three tests identified different people for the majority of positive test results. This suggests that in low-prevalence populations, the majority of positives resulting from any of the three commercially-available diagnostic tests are false positives. Given the similar specificities between TST and IGRAs seen in this and other studies, targeted testing should still be performed in this and other heterogeneous populations. The pre-test probability in these populations is very low, and the use

of risk factors such as foreign birth and contact with a TB case can be used to increase this probability, reducing the proportion of false positives.

Longitudinal follow-up to assess the long-term progression to active TB is suggested by this study to better understand the significance of this discordance. Exposure and sensitization to NTM should be considered in these assessments, including not only *M. intracellulare* but also other NTM as well. Applying the methodology used in this study to other populations (2, 18, 50) will also provide a more robust evaluation that may provide a more complete understanding of the test interpretation and discordance.

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We also thank Oxford Immunotec for performing the T-Spot test as an in-kind contribution.

Figure 7. Flow chart of study comparing the tuberculin skin test with two interferon-gamma release assays in 2009 at Fort Jackson, SC.

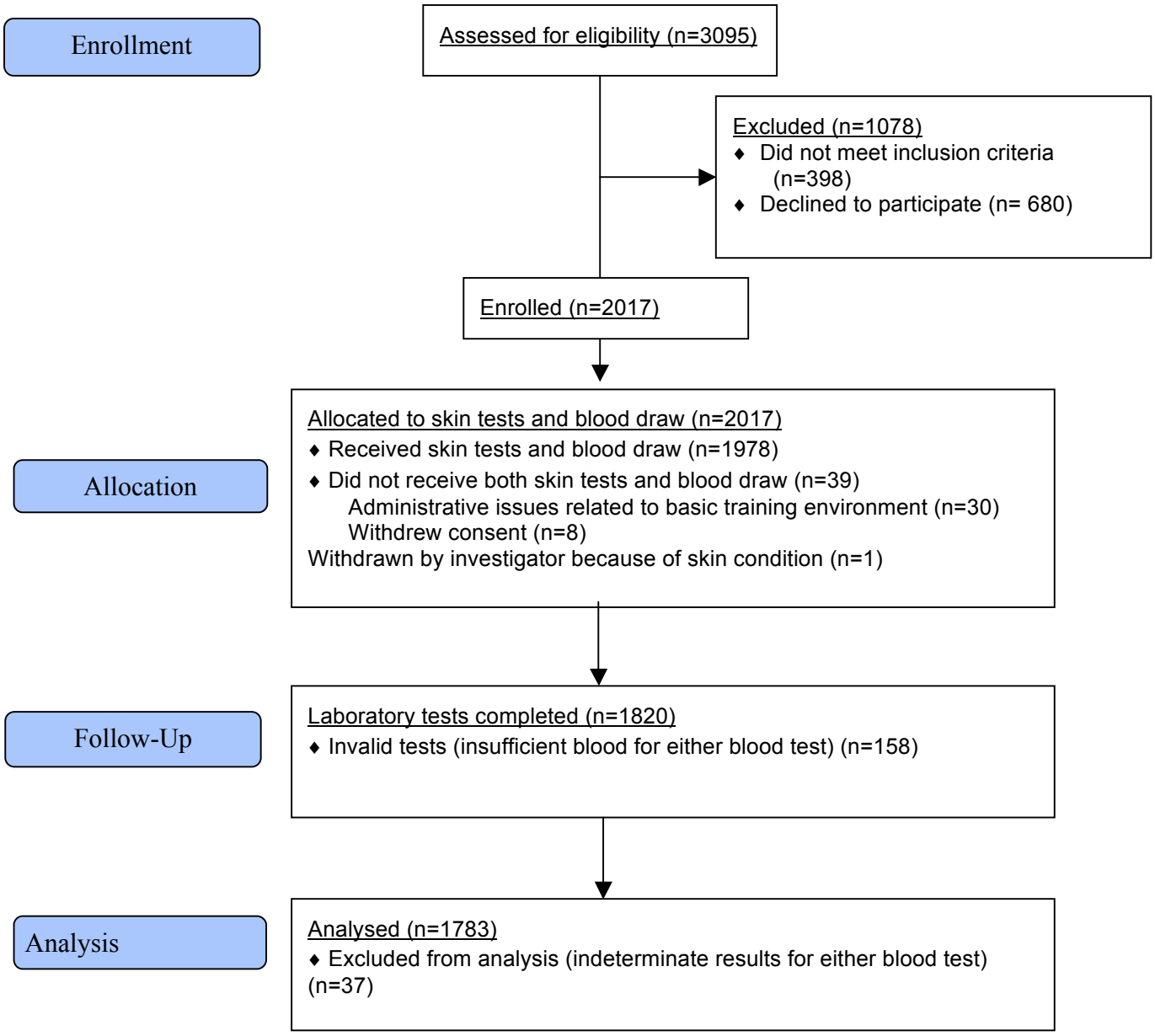


Table 14. Characteristics of Study Participants (n=1978)

Characteristic	Number*	Percent (%)
Sex		
Male	1294	65.5
Female	681	34.5
Age (SD)	1974	21.8 years (SD 4.6 years)
Race/ethnic group**		
White	1298	65.6
Black	459	23.2
Hispanic	221	11.4
Asian/Pacific Islander	117	5.7
Other	63	3.2
Prevalence of TB in country of birth		
<20 per 100,000	1873	94.7
20-100 per 100,000	35	1.8
>100 per 100,000	70	3.5
BCG vaccinated	69	3.5
Prevalence of TB among countries the subject lived in or traveled to for > 1 month		
<20 per 100,000	1811	91.6
20-100 per 100,000	62	3.1
>100 per 100,000	105	5.3
Contact with someone with TB		
In same household	24	1.2
Casual contact	73	3.7
Health care work	232	11.7
Lived or worked in congregate setting	120	6.1
Farm work or residence	383	19.4
Current Residence		
Northeast US	337	17.0
Southeast US	657	33.2
Western US	706	35.7
Other	278	14.1
Smoking		
Never	1480	75.1
< 1 pack per day	395	20.0
1+ pack per day	97	4.9
Education		
< 12	257	13.0
12	1095	55.4
13-15	468	23.7
16+	158	8.0
Prior TB treatment	45	2.3

Prior TB skin test performed	710	35.9
Prior positive skin test	24	3.4 (of those with a prior test)
Unknown result	54	7.6 (of those with a prior test)

** Note: some cells do not total to 1978 due to missing data*

***Note: Recruits could choose more than one group; Other includes 39 American Indian, 8 Bi- or multi-racial*

S.D.=Standard Deviation; TB=tuberculosis; BCG= Bacille Calmette Guerin Vaccine

Table 15. Outcome of Testing

TEST TYPE	*Positive*	*Negative*	*Borderline/ Indeterminate*	*Total*	*Invalid samples*
TST					
≥ 10 mm	67 (3.4%)	1911 (96.6%)	0	1978	0
≥ 15 mm	47 (2.4%)	1931 (97.6%)	0	1978	0
T-Spot	39 (2.1%)	1847 (96.9%)	21 (1.1%)	1907†	71 (3.6%)*
QFT	39 (2.1%)	1794 (97.0%)	17 (0.9%)	1850‡	128 (6.5%)**
BST	227 (11.5%)	1751 (88.5%)	0	1978	0

TST=tuberculin skin test using PPD-S
T-Spot = T-SPOT®.*TB* test
QFT=QuantiFERON® -TB Gold In-Tube test
BST= Battey skin test using PPD-B

† T-Spot had a borderline result for 21 subjects

* T-Spot was not completed for 50 subjects because an insufficient volume of blood was submitted; for 10 subjects because an insufficient number of peripheral blood mononuclear cells were recovered; and for 11 subjects because of laboratory or other errors.

‡ QFT was indeterminate for 15 subjects due to a low mitogen response and 2 subjects due to both a high nil and low mitogen response

** QFT was not completed for 89 subjects because the volume of blood in 1 or more of the 3 test tubes was < 0.5 ml or > 1.5 ml; for 34 subjects because of misplaced or dislodged tube caps; and for 5 laboratory or other errors.

Table 16. Test results stratified by risk

Test	*Low-risk (n=1486)*	*Moderate-risk (n=234)*	*High-risk (n=100)*
TST*			
≥10mm	**18 (1.2%)**	**17 (7.3%)**	**24 (24.0%)**
<10mm	1468 (98.8%)	217 (92.7%)	76 (76.0%)
T-Spot**			
Positive	**19 (1.3%)**	**7 (3.0%)**	**10 (10.0%)**
Negative	1450 (97.6%)	225 (96.2%)	89 (89.0%)
Indeterminate	17 (1.1%)	2 (0.9%)	1 (1.0%)
QFT***			
Positive	**19 (1.3%)**	**11 (4.7%)**	**7 (7.0%)**
Negative	1455 (97.9%)	220 (94.0%)	91 (91.0%)
Indeterminate	12 (0.8%)	3 (1.3%)	2 (2.0%)

Note: Only those with valid results for all three tests were used (n=1820)
TST=tuberculin skin test
T-Spot = T-SPOT®.*TB* test
QFT=QuantiFERON®-TB Gold In-Tube test
Risk Categorization:

1. Low-risk= No risk factors
2. Moderate-risk= Casual contact with an active TB case, immigrants more than 5 years since immigration, overseas travel > 1 month
3. High-risk=household contact of an active TB case, recent immigrants within 5 years, prior TB diagnosis or treatment, prior skin test positive

* χ^2(trend)=155.8, p<0.0001
** χ^2(trend)=33.0, p<0.0001
*** χ^2(trend)=24.8, p<0.0001

Table 17. Agreement between T-Spot and TST

	TST ≥ 10 mm	TST < 10 mm	Total
T-Spot positive	18 (1.0%)	16 (0.9%)	34 (1.9%)
T-Spot negative	40 (2.2%)	1709 (95.8%)	1749 (98.1%)
Total	58 (3.3%)	1725 (96.7%)	1783

T-Spot = T-SPOT®.*TB* test
TST=tuberculin skin test
% agreement = 96.9%
K (95% CI)=0.38 (0.25, 0.50)

Table 18. Agreement between QFT and TST

	TST ≥ 10 mm	TST < 10 mm	Total
QFT positive	13 (0.7%)	23 (1.3%)	36 (2.0%)
QFT negative	45 (2.5%)	1702 (95.5%)	1747 (98.0%)
Total	58 (3.3%)	1725 (96.7%)	1783

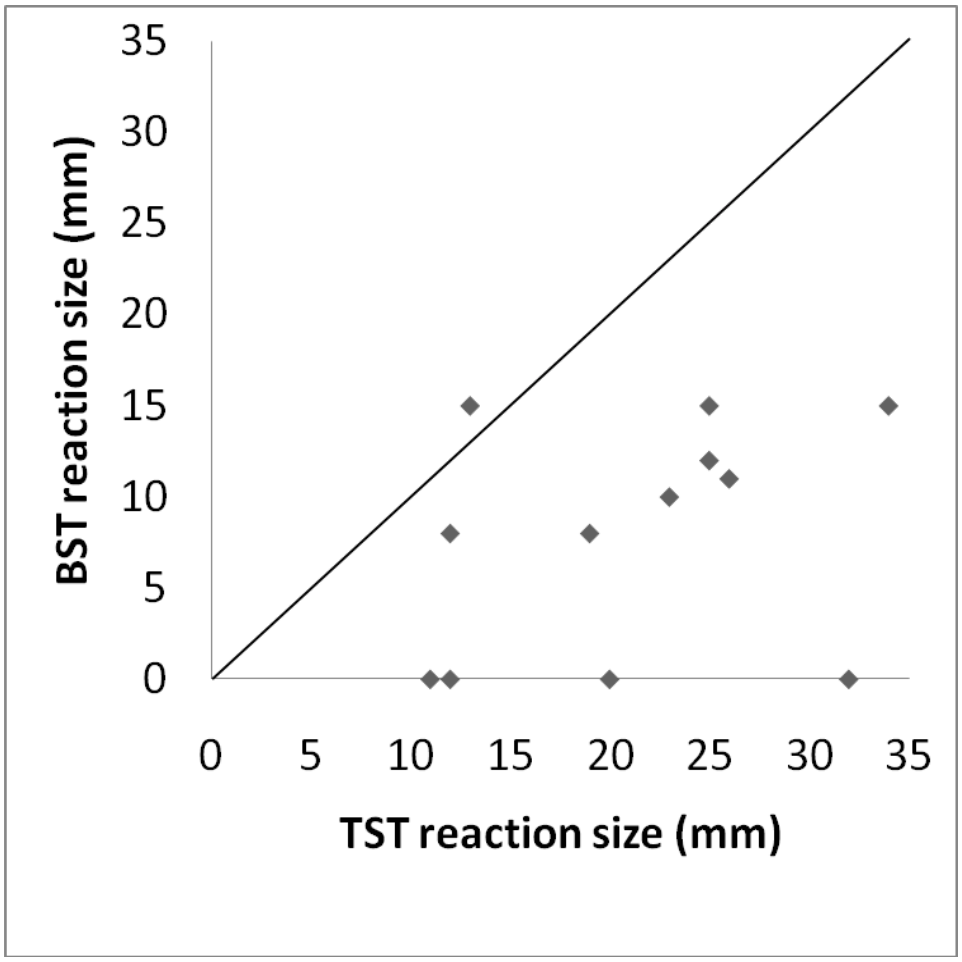
TST=tuberculin skin test
QFT=QuantiFERON®-TB Gold In-Tube test
% agreement=96.2%
K (95% CI)=0.26 (0.14, 0.38)

Table 19. Agreement between T-Spot and QFT

	QFT positive	QFT negative	Total
T-Spot positive	14 (0.8%)	20 (1.1%)	34 (1.9%)
T-Spot negative	22 (1.2%)	1727 (96.9%)	1749 (98.1%)
Total	36 (2.0%)	1747 (98.0%)	1783

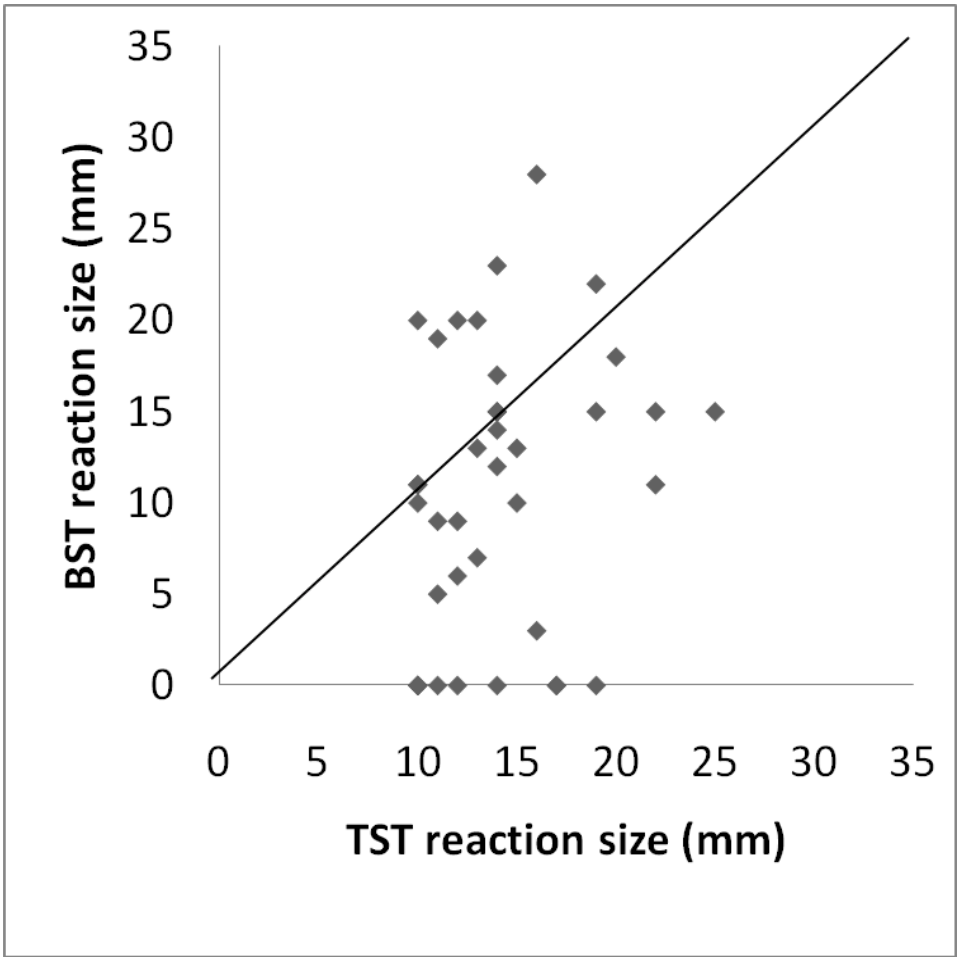
T-Spot = T-SPOT®.*TB* test
QFT=QuantiFERON®-TB Gold In-Tube test
% agreement = 97.6%
K (95% CI) =0.39 (0.24, 0.54)

Figure 8. Comparison of TST and BST reaction size among those with concordant TST and IGRA tests



TST=Tuberculin skin test
BST=Battey skin test
Positive TST=10mm or greater induration size

Figure 9. Comparison of TST and BST reaction size for those with discordant TST positive, IGRA negative tests



TST=Tuberculin skin test
BST=Battey skin test
Positive TST=10mm or greater induration size

Table 20. Associations between selected characteristics and discordance between positive TST (≥ 10mm) and negative IGRA

Characteristic	*Recruits with a negative QFT result*			*Recruits with a negative T-Spot result*		
	# with TST ≥10 mm (total)	Bivariate Prevalence Ratio (95% CI)	Multivariate Prevalence Ratio (95% CI)	# with TST ≥10 mm (total)	Bivariate Prevalence Ratio (95% CI)	Multivariate Prevalence Ratio (95% CI)
Age	45 (1721)	1.11 (1.06, 1.15)	*	40 (1724)	1.10 (1.06, 1.15)	*
Sex						
Male	28 (1123)	1.0 (REF)	*	25 (1115)	1.0 (REF)	*
Female	17 (596)	1.1 (0.6, 2.1)		15 (607)	1.1 (0.6, 2.1)	
Race/Ethnic Group						
White	13 (1161)	1.0 (REF)	*	13 (1152)	1.0 (REF)	*
Black	16 (385)	3.6 (1.8, 7.4)		15 (392)	3.3 (1.6, 6.9)	
Asian or PI	11 (49)	16.6 (7.7, 35.4)		7 (53)	10.5 (4.3, 25.2)	
Hispanic	8 (189)	3.7 (1.5, 8.7)		8 (189)	3.6 (1.5, 8.7)	
TB prevalence in country of birth or long-term residence						
<20 per 100,000	21 (1603)	1.0 (REF)	1.0 (REF)	19 (1602)	1.0 (REF)	1.0 (REF)
20-100 per 100,000	6 (48)	8.6 (3.6, 20.4)	3.2 (1.1, 9.7)	6 (48)	9.5 (3.9, 22.8)	4.6 (1.7, 12.5)
> 100 per 100,000	18 (70)	15.8 (8.8, 28.6)	5.0 (2.3, 10.9)	15 (74)	14.4 (7.6, 27.3)	5.4 (2.6, 11.4)
BCG vaccination						
No	28 (1682)	1.0 (REF)	1.0 (REF)	26 (1686)	1.0 (REF)	1.0 (REF)
Yes	17 (39)	18.5 (10.8, 31.8)	4.1 (1.8, 9.4)	14 (38)	17.7 (9.8, 31.9)	4.0 (1.9, 8.2)
PPD-B reaction						
0-4 mm	11 (1424)	1.0 (REF)	1.0 (REF)	10 (1426)	1.0 (REF)	1.0 (REF)
5-9 mm	7 (127)	6.8 (2.7, 17.3)	4.6 (2.0, 10.6)	5 (128)	5.4 (1.9, 15.6)	3.6 (1.2, 9.3)
10-14 mm	12 (134)	10.7 (4.8, 23.9)	5.4 (2.3, 12.5)	10 (133)	10.0 (4.3, 23.7)	5.0 (2.1, 12.2)
15-19 mm	8 (27)	29.8 (12.8, 69.5)	23.7 (9.9, 56.8)	9 (28)	34.9 (15.1, 80.9)	20.7 (8.3, 51.7)
20+ mm	7 (9)	57.1 (25.4, 128)	30.0 (10.4, 86.3)	6 (9)	57.4 (23.9, 138)	56.5 (24.5, 130)
Region of birth						
NE	11 (290)	1.0 (REF)	*	10 (290)	1.0 (REF)	*
SE	11 (586)	0.5 (0.2, 1.2)		11 (583)	0.6 (0.2, 1.3)	
West	12 (609)	0.5 (0.2, 1.2)		11 (611)	0.5 (0.2, 1.2)	
Other	11 (236)	1.2 (0.5, 2.8)		8 (240)	1.0 (0.4, 2.4)	

Farm work	40 (1377)	1.0 (REF)	*	35 (1385)	1.0 (REF)	*
No	5 (344)	0.5 (0.2, 1.3)		5 (339)	0.6 (0.2, 1.5)	
Yes						

* Multivariate models did not include variables with p>0.05

PPD-B= Battey skin test antigen, TST= tuberculin skin test, T-Spot = T-SPOT®.*TB* test, QFT=QuantiFERON®-TB Gold In-Tube test, IGRA=interferon-gamma release assay

Chapter 5—Comparative Effectiveness of Tuberculosis Screening Strategies in a Heterogeneous Population

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Abstract

Background

Testing for latent tuberculosis infection (LTBI) is only recommended among high-risk populations. Within heterogeneous exposure populations, high-risk groups may be targeted for testing using risk factors. The overall objective of this study was to identify risk factors for LTBI in a heterogeneous but generally low-risk population of US military recruits and compare the effectiveness of targeted testing strategies using the three commercially available diagnostic tests.

Methods

A cross-sectional comparison study was done among recruits undergoing Army basic training at Fort Jackson, SC from April to June 2009. Several tests were performed simultaneously, including: 1) a risk factor survey, 2) the QuantiFERON® Gold-in-tube (QFT), 3) T-SPOT®.TB (T-Spot), and 4) tuberculin skin test (TST). Prediction models used logistic regression to identify factors associated with LTBI. The characteristics of the three prediction models were compared, including sensitivity, specificity, and c-statistic.

Results

Use of a four variable model resulted in 79% sensitivity, 92% specificity, and c-statistic of 0.871 in predicting a positive TST. This model included birth in a tuberculosis endemic country, close contact with an active tuberculosis case, prior history of a positive skin test, and history of residence with a family member born outside the US. Targeted testing of only those with a positive response to one of these 4 questions would reduce testing by > 90%. Prediction models

for the QFT and T-Spot had similar specificities as the TST but had lower sensitivities and c-statistics.

Discussion

Targeted testing of heterogeneous populations such as military recruits will maintain a similar level of effectiveness as universal testing. It will also reduce false positives among low-risk individuals and the resultant waste of resources and risk of adverse effects from unnecessary LTBI treatment.

Introduction

Universal screening for tuberculosis (TB) in the US is no longer recommended; current practice favors a targeted approach. Centers for Disease Control and Prevention (CDC) guidelines recommend targeted testing of only those with known risk factors for TB, specifically stating that "targeted tuberculin testing programs should be conducted only among groups at high risk and discouraged in those at low risk" (1). Similarly, the Institute of Medicine has called for the development of targeted TB screening programs based on epidemiologic risk analysis (2). Targeted testing offers logistic and efficiency advantages over universal screening, as well as increasing the positive predictive value (PPV) of a positive test by selecting a higher prevalence population for testing (3). In relatively homogeneous exposure settings, such as immigrants, prisons, and hospitals, universal testing is still performed based on association with a high-risk setting, although health-care workers are increasingly being recognized as having heterogeneous exposures (4). Targeted testing has been implemented and evaluated using predictive models in several such heterogeneous settings, including contact investigations (5, 6), pediatric populations (7, 8), and university entrants (9). These have all found that targeted testing may dramatically reduce the amount of testing without negative effects on disease control efforts.

The US military has performed universal testing of recruits entering military service since the 1960s (10). However, the US military has a low risk for active TB, with a rate of 0.65 cases of confirmed pulmonary TB per 100,000 person-years from 1998-2007, a rate 84% lower than the age-adjusted US rate (unpublished manuscript, Mancuso JD, Walter Reed Army Institute of Research). A population with heterogeneous exposures to tuberculosis (TB) prior to accession

(i.e. entry into military service), the US military is challenged to mitigate the risk of TB in higher risk recruits without exposing low-risk recruits to unnecessary therapy. Targeted testing relies on identifiable risk factors and an assessment tool which can accurately predict latent tuberculosis infection (LTBI). IGRAs are thought to be more specific and have other logistic advantages over the TST (11). The purpose of this study is to provide an evaluation of the prevalence of LTBI and associated risk factors in a heterogeneous US Army recruit population using the tuberculin skin test (TST) and the commercially available IGRAs, the QuantiFERON® Gold-in-tube (QFT) and the T-SPOT®.TB (T-Spot). This is used to compare the effectiveness of targeted testing strategies in a heterogeneous population of US military recruits using commercially available diagnostic tests for LTBI.

Methods

Study Procedures

The study was approved in 2009 by the Uniformed Service University Institutional Review Board and conducted in the same year. Study procedures included informed consent of all subjects. This cross-sectional comparison study among Army recruits at Fort Jackson, SC, consisted of: 1) a TB risk factor questionnaire (RFQ), 2) TST, 3) QFT, and 4) T-Spot. Fort Jackson is the largest US Army basic training site, with over 40,000 recruits each year, and it is one of only two basic training sites where women are trained. Other than this difference, the recruits at Fort Jackson are generally representative of all Army basic training installations. A total of 2,017 subjects were enrolled in the study from April 1 to June 11, 2009.

The RFQ was developed from previous literature and validation studies (5, 6, 8-10, 12). The RFQ was done prior to all other TB testing, and subjects were encouraged to complete all fields. The RFQ took about 3-5 minutes for participants to complete. The primary variables of interest included foreign birth, race and ethnic background, and contact with a case of active TB (10). Other factors included demographic characteristics, foreign residence or travel, exposure assessment, Bacille Calmette-Guérin (BCG) vaccination, history of prior TB diagnosis or treatment, and prior positive skin test, as shown in Table 21. Infection with Human Immunodeficiency Virus (HIV) and other immunosuppressive conditions are disqualifying for entry into military service and therefore are not reported here. The TB prevalence reported by the World Health Organization in 1990 was used to estimate exposure risk in country of birth and during overseas travel or residence using groups of: 1) less than 20 per 100,000, 2) 20 to 100 per 100,000, and 3) greater than 100 per 100,000 (13, 14).

Blood for QFT and T-Spot was collected at the time of routine phlebotomy for recruit in-processing. Personnel performing IGRA assays were blinded to all patient data. QFT was performed according to package insert instructions, including incubation and centrifugation within the prescribed times (15). Testing was completed with the aid of a Triturus automated ELISA workstation (Grifols USA, LLC, Los Angeles, CA). Blood for T-Spot was collected into sodium heparin tubes, mixed gently, and shipped overnight at room temperature to the Oxford Immunotec, Inc. Laboratory in Marlborough, MA. T-Spot was performed per package insert instructions (16), except for the addition of 25 µl/ml T-cell Xtend® (Oxford Immunotec, Ltd., Oxfordshire, UK) immediately prior to peripheral blood mononuclear cell recovery to increase the processing window from 8 hours up to 32 hours.

All personnel involved in placement and reading of the skin tests were trained and monitored to strictly adhere to standard operating procedures (SOPs) based on published methods (17, 18). The Mantoux technique was used to administer 0.1 mL (5 TU) of Tubersol® tuberculin PPD (Sanofi Pasteur Ltd., Toronto, Ontario, Canada). The transverse diameter of induration at each skin test site was measured 2 days after administration.

Statistics and Data Analysis

SAS Version 9.2 was used for all analyses (SAS Institute, Cary, NC). A positive TST was defined by meeting induration criteria relative to risk categories described in published CDC guidelines (1). IGRA endpoints were defined by using established cutoffs from the manufacturer (15, 16). The RFQ was used to develop predictive models for positive responses to TST and IGRA (5-9). Factors associated with a positive result were evaluated using standard chi-square bivariate statistics. Fisher's exact test was use for analyses which had cell sizes with less than 5. Unadjusted and adjusted odds ratios were directly estimated for both bivariate and multivariate analyses using unconditional logistic regression.

Several different definitions of a positive RFQ were used in order to evaluate predictive value of different combinations of factors. Sensitivity, specificity, PPV and negative predictive value (NPV) were calculated for each RFQ variable response and for different combinations of variables. Missing data were rare (generally <1%), so no imputation techniques were necessary. Receiver operator curves (ROCs) were constructed by plotting sensitivity vs. 1-specificity for each probability level. Variables were selected for inclusion into the models based on prior

knowledge of risk factors for TB exposure and contribution to the predictive ability of the model. The contribution to the model was assessed by change in area under the curve (AUC), or c-statistic, when adding each predictor variable to the model. To validate the prediction model, the analysis was performed on a second set of samples obtained by bootstrap methods (19, 20). For this analysis, 1000 bootstrap samples of the same size as the original model were taken from the original data set with replacement. The AUC and estimates of the odds ratios were reported for the bootstrap validation data set estimates and compared to the original data set.

Results

Of the 3,095 recruits approached from April 1 to June 11, 2009, 2,697 were eligible to participate in the study, of which 2,017 subjects (75%) enrolled. Characteristics of study participants are shown in Table 21 and were similar to the overall recruit population. Thirty-eight recruits withdrew prior to blood collection or completion of skin testing; 30 of these were for administrative reasons unrelated to the study. TST results were available for 1,978 (99.9%) of the remaining 1,979 participants, and T-Spot and QFT results were available for 1,888 (95.4%) and 1,835 (92.7%), respectively. For comparability between the prediction models, this analysis was limited to subjects who had positive or negative results for all three tests (n=1,783) and excluded subjects with an indeterminate or invalid result by any test.

TST induration was detected in 105 participants (5.9%) and ranged from 2 to 80 mm. TST induration size of ≥ 10 mm was seen in 58 (3.3%). Of these, 20 (34%) had no epidemiologic risk factors for TB infection and had induration size of < 15 mm. There were 38 positive TSTs by RSI criteria (2.1%). Of note, 15 of the 38 (39%) had no identifiable risk factors other than an

induration size of 15 mm or greater. Similar proportions of positives were seen for the IGRAs, with 34 T-Spot positives (1.9%) and 36 QFT positives (2.0%).

The prevalence of demographic and exposure risk factors are shown in Table 21, along with corresponding bivariate associations with TST, QFT, and T-Spot test results as demonstrated by the prevalence odds ratio (OR) and 95% confidence interval (CI). Increased prevalence of a positive result was consistently seen with several factors, including: increasing age (likely due to the cohort effect at year of birth), race and ethnic groups, increasing TB prevalence in the country of birth, history of residence with a family member born outside the US, increasing closeness of contact with a TB case, history of a prior positive TST, BCG vaccination, and prior TB treatment. No associations were seen with sex, region of residence, or smoking status. Of recruits born in the US or other low-prevalence countries, 1.1% had a positive TST, compared to 1.6% with a positive QFT and 1.4% with a positive T-Spot; these differences were not statistically significant. As in previous studies, birth in a TB endemic country had a particularly strong association with a positive test result (6, 8, 9, 21, 22).

The multivariate associations of selected factors with positive TST or IGRAs are shown in Table 22. After adjusting for the other variables in the model, significant associations were found between a positive test and exposure to a TB case, TB prevalence in the country of birth, residence with a family member born outside the US, positive prior TST, and residence in a congregate setting such as homeless shelter, prison, or drug treatment facility. Other variables were kept in this model despite lack of statistical significance because of *a priori* knowledge and interest as in these variables as risk factors and predictors of TB infection. Removal of these

variables did not affect the conclusions or meaningfully alter the magnitude of association seen with the significant predictors in sensitivity analysis. To account for overfitting, validation of the models with bootstrap estimates were obtained. The estimates obtained via bootstrap were similar to those obtained in the original data set, although some predictors were no longer statistically significant and the AUCs were moderately lower.

The characteristics of the prediction models are shown in Tables 23, 24, and 25. As expected, the sensitivity, NPV, and AUC were seen to improve with increasing numbers of predictors, with corresponding decreases in specificity and PPV. A 4-variable model for prediction of a positive TST was selected as having the best bias-variance tradeoff, with a sensitivity of 79% (95% confidence interval [CI]: 63%, 90%), specificity of 92% (95% CI: 91%, 93%), and AUC of 0.871. As only 9.3% had a positive response to one of these 4 variables, targeted testing of only these positives would be expected to reduce testing by 90.7% (95% CI: 89%, 92%). In contrast, when all potential risk factors were included, 32.5% had at least one "positive" response, but this increased the sensitivity only slightly while dramatically lowering specificity. Sensitivity analysis was done using an outcome of TST $\geq$ 10 mm instead of TST meeting the criteria based on epidemiologic risk factors. This resulted in similar associations between risk factors and similar conclusions in the multivariable models, except for slightly lower sensitivity, specificity, and AUC of the models. The 4-variable model using this alternate TST endpoint resulted in 66% sensitivity, 93% specificity, and an AUC of 0.814.

Figure 10 compares the ROC curves for the performance of the full model for predicting positive results for the TST, QFT, and T-Spot. This graphically demonstrates the lower performance

characteristics of the RFQ in predicting an IGRA outcome as compared to TST. Tables 24 and 25 show the characteristics of the prediction models using the same combinations of variables for the QFT and T-Spot as used in Table 23 for the TST. Although the specificities and NPVs are similar to the TST, the sensitivity, PPV, and AUC are all considerably lower than the TST. For the 4-variable model, the QFT had a sensitivity of 44% (95% CI: 28%, 62%), specificity of 91% (95% CI: 90%, 93%), and AUC of 0.684. The T-Spot had very similar estimates, with a sensitivity of 44% (95% CI: 27%, 62%), specificity of 91% (95% CI: 90%, 93%), and AUC of 0.688.

Discussion

Risk factors for LTBI among US Army recruits were similar whether measured by the TST or one of the two commercially-available IGRAs. Prediction models were constructed using variables including birth in a country with a high prevalence of TB, close contact with an active TB case, history of living with a family member born outside the US, and history of a prior positive TB skin test. Use of these 4 variables resulted in 79% sensitivity, 92% specificity, and AUC of 0.871 in predicting a positive TB skin test. Targeted testing of only those with a positive response to one of these 4 questions would reduce testing by > 90%, increasing the efficiency of the testing program. Prediction models for the IGRAs had similar specificities and reductions in testing but had lower sensitivities and AUCs.

This is the first study to evaluate the comparative effectiveness of targeted testing using either IGRA as an endpoint in any population, as well as the first to compare targeted testing as a predictive tool using IGRA and TST criterion standards. Like previous studies using TST as the

outcome, birth in a TB endemic country was found to be a strong predictor of a positive test (7-9, 22). Close contact with a TB case, foreign-born family members, and prior positive TST have also been associated with LTBI in previous studies (7-9, 23). Other variables have also sometimes been associated with a positive TST, including travel (23), smoking (6), male sex (5, 9), health care work (4), and education (8), but these were not found to be important predictors of LTBI in this study. Race and ethnic group did not contribute meaningfully as predictors after adjusting for other factors. The only study to assess use of a questionnaire to target testing in a similar heterogeneous adult population was among college students in Virginia (9). That study showed that using only 2 variables of foreign birth and close contact with a TB patient resulted in a sensitivity of 81.6% and a specificity of 91%. Although our two variable model had lower sensitivity than this, we found comparable sensitivity and specificity using a 4-variable model. Two studies in pediatric populations also found that using 4 or 5 questions to identify high risk LTBI patients resulted in similar sensitivities and specificities as those seen in this study (7, 8). Prediction models of LTBI among contacts of active TB cases have had more modest reductions in testing, due to a higher pre-test probability of infection and less concern for false positives than for false negatives (5, 6).

This study has several important strengths. There was nearly complete follow-up (99.9%), reducing this common source of bias. The population was a good geographic representation of the underlying US source population in this age group, although healthier and free of most immunosuppressive conditions, including HIV. Also, the three forms of TB testing allowed direct comparisons of the effectiveness of targeted testing to predict LTBI as measured by each test, which has not been done previously.

There are also several limitations to this study. Most important is the lack of a gold standard in evaluating the presence of LTBI. The potential for false positive TSTs due to BCG, cross-reactivity to non-tuberculous mycobacteria, and other factors is well-known (24). The IGRAs are also known to have limitations in sensitivity and specificity (11), and it is uncertain whether the predictive capability of the IGRAs is better than the TST. There is no gold standard in evaluating the performance of the IGRAs in comparison with the TST other than the long-term progression to active TB in cohort studies (25). The small number of positive tests also may have led to less power to detect small differences in the groups studied, which may have led to false conclusions regarding the significance testing both in those considered significant and insignificant. Misclassification of exposures and outcomes were also possible due to measurement error, although the outcomes were probably better controlled in this study than they would be in practice. Finally, this study is not expected to be generalizable to higher risk populations such as those with HIV or other immunosuppressive conditions, or to other groups at higher risk for TB exposure, such as hospital workers or prison guards.

Interestingly, the RFQ in this study was better at predicting a positive TST than for either IGRA. This may have been somewhat biased by the use of CDC risk stratified interpretation (RSI), since the factors under evaluation were also correlated with a positive result on both the RFQ and the TST. Similarly, the use of a history of a prior TST positive may bias the prediction model in favor of the TST. Despite this, the RFQ still had superior sensitivity and specificity in predicting TST compared to the IGRAs even when discarding this as a risk factor. This is seen in the 3 variable model in Tables 23, 24, and 25.

An important implication of this study is that targeted testing of a heterogeneous population is feasible and effective using the TST or either IGRA, albeit with higher sensitivity of these predictors for a positive TST. The use of well-accepted, scientifically defensible risk factors to target testing presented in this study should assist in providing an evidence base for the implementation of targeted testing among recruits or other low prevalence populations. Targeted testing in this population would reduce testing by >90%, greatly reducing costs of the TB screening program and adverse events from therapy while still maintaining effectiveness. Furthermore, the potential for false positives among low-risk populations may also be decreased by reducing the testing of low-risk populations. More than 50% of all positives in this population are estimated to be false positives due to NTM and other factors (3, 26, 27). Targeted testing may therefore reduce treatment of false positives who derive no benefit from LTBI therapy but still incur the risk of adverse events.

Future studies suggested by this study include cost-effectiveness analysis of targeted testing in heterogeneous populations to determine the magnitude and relative cost-effectiveness of targeted testing programs for the IGRAs compared to the TST. Prediction models in other heterogeneous populations may also be considered, including health care workers, prison guards, long-term travelers to TB endemic countries and military service members deploying to TB endemic countries, as risk factors in these populations remain ill-defined by previous studies (28, 29). Finally, studies comparing the long-term rate of progression to active TB among TST and IGRA positives will allow a more accurate determination of LTBI status and risk of progression to active TB. This will ultimately be the most important criterion in comparing the performance characteristics and predictive ability of the different tests.

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Table 21. Association of Selected Factors with Positive TST, QFT, or T-Spot Among US Army Recruits at Entry into Military Service

Factor	*N*	*TST*		*QFT*		*T-Spot*	
		# positive (%)	OR (95% CI)	# positive (%)	OR (95% CI)	# positive (%)	OR (95% CI)
Age							
18-24	1438	20 (1.4%)	1 (Ref)	28 (2.0%)	1 (Ref)	22 (1.5%)	1 (Ref)
25-29	193	4 (2.1%)	1.5 (0.4, 4.5)	2 (1.0%)	0.6 (0.06, 2.1)	4 (2.1%)	1.4 (0.3, 4.1)
≥ 30	149	14 (9.4%)	7.4 (3.2, 15.7)	6 (4.0%)	2.1 (0.7, 5.3)	8 (5.4%)	3.7 (1.4, 8.7)
Sex							
Male	1160	23 (2.0%)	1.0 (Ref)	23 (2.0%)	1 (Ref)	27 (2.3%)	1 (Ref)
Female	621	15 (2.4%)	1.2 (0.6, 2.4)	13 (2.1%)	1.1 (0.5, 2.1)	7 (1.1%)	0.5 (0.2, 1.1)
Race/ethnic group*							
White	1175	8 (0.7%)	1 (Ref)	15 (1.3%)	1 (Ref)	16 (1.4%)	1 (Ref)
Black	408	12 (2.9%)	4.4 (1.6, 12.5)	10 (2.5%)	1.2 (0.8, 4.7)	7 (1.7%)	1.3 (0.5, 3.5)
Hispanic	203	7 (3.5%)	5.2 (1.6, 16.6)	10 (4.9%)	4.0 (1.6, 9.7)	7 (3.5%)	2.6 (0.9, 6.8)
Asian/Pacific Islander	106	13 (12.3%)	20.4 (7.6, 57.9)	5 (4.7%)	3.8 (1.1, 11.4)	6 (5.7%)	4.3 (1.4, 12.0)
Other	137	6 (4.4%)	6.7 (1.9, 22.3)	7 (5.1%)	4.2 (1.4, 11.1)	5 (3.7%)	2.7 (0.8, 8.0)
Prevalence of TB in country of birth							
<20 per 100,000	1687	19 (1.1%)	1 (Ref)	27 (1.6%)	1 (Ref)	24 (1.4%)	1 (Ref)
20-100 per 100,000	33	4 (12.1%)	12.1 (2.8, 39.5)	3 (9.1%)	6.1 (1.1, 21.7)	3 (9.1%)	6.9 (1.3, 24.7)
>100 per 100,000	63	15 (23.8%)	27.4 (12.1, 60.6)	6 (9.5%)	6.5 (2.1, 16.8)	7 (11.1%)	8.7 (3.0, 21.8)
Lived with family member who was not born in US							
No	1701	25 (1.5%)	1 (Ref)	26 (1.5%)	1 (Ref)	27 (1.6%)	1 (Ref)
Yes	79	13 (16.5%)	13.2 (6.5, 27.0)	10 (12.7%)	9.3 (4.3, 20.1)	7 (8.9%)	6.0 (2.1, 14.8)
Prevalence of TB in country the subject lived in or traveled to for > 1 month							
<20 per 100,000	1717	31 (1.8%)	1 (Ref)	33 (1.9%)	1 (Ref)	31 (1.8%)	1 (Ref)
20-100 per 100,000	25	1 (4.0%)	2.3 (0.1, 14.8)	0 (0%)	0 (0, 7.9)	0 (0%)	0 (0, 8.5)
>100 per 100,000	41	6 (14.6%)	9.3 (3.0, 24.6)	3 (7.3%)	4.0 (0.8, 13.7)	3 (7.3%)	4.3 (0.8, 14.7)
Contact with TB case							
None	1697	30 (1.8%)	1 (Ref)	27 (1.6%)	1 (Ref)	31 (1.8%)	1 (Ref)
Casual	65	5 (7.7%)	4.6 (1.4, 12.6)	6 (9.2%)	6.3 (2.0, 16.3)	2 (3.1%)	1.7 (0.2, 7.0)

In same household	20	3 (15.0%)	9.8 (1.7, 36.5)	3 (15.0%)	10.9 (1.9, 40.9)	1 (5.0%)	2.8 (0.1, 18.9)
Health care work							
No	1573	9 (4.3%)	1 (Ref)	32 (2.0%)	1 (Ref)	30 (1.9%)	1 (Ref)
Yes	209	29 (1.8%)	2.4 (1.1, 5.1)	4 (1.9%)	0.9 (0.2, 2.7)	4 (1.9%)	1.0 (0.4, 2.9)
Lived or worked in congregate setting							
No	1671	33 (2.0%)	1 (Ref)	31 (1.9%)	1 (Ref)	30 (1.8%)	1 (Ref)
Yes	112	5 (4.5%)	2.3 (0.9, 6.1)	5 (4.5%)	2.5 (0.9, 6.5)	4 (3.6%)	2.0 (0.7, 5.9)
BCG							
No	1722	22 (1.3%)	1 (Ref)	30 (1.7%)	1 (Ref)	25 (1.5%)	1 (Ref)
Yes	61	16 (26.2%)	27.5 (13.5, 55.8)	6 (9.8%)	6.2 (2.5, 15.4)	9 (14.8%)	11.7 (5.2, 26.4)
Treated for TB							
No	1739	33 (1.9%)	1 (Ref)	34 (2.0%)	1 (Ref)	31 (1.8%)	1 (Ref)
Yes	42	5 (11.9%)	7.0 (2.6, 18.9)	2 (4.8%)	2.5 (0.3, 10.4)	3 (7.1%)	4.2 (1.2, 14.5)
Prior TST positive							
No	1762	26 (1.5%)	1 (Ref)	34 (1.9%)	1 (Ref)	29 (1.7%)	1 (Ref)
Yes	21	12 (57.1%)	89.0 (34.5, 229)	2 (9.5%)	5.3 (0.6, 23.6)	5 (23.8%)	18.7 (6.4, 54.4)
Region of US							
Northeast	303	8 (2.6%)	1 (Ref)	7 (2.3%)	1 (Ref)	5 (1.7%)	1 (Ref)
Southeast	601	9 (1.5%)	0.6 (0.2, 1.7)	9 (1.5%)	0.6 (0.2, 2.1)	10 (1.7%)	1.0 (0.3, 3.8)
West	627	11 (1.8%)	0.7 (0.2, 1.9)	13 (2.1%)	0.9 (0.3, 2.7)	12 (1.9%)	1.2 (0.4, 4.3)
Other	252	10 (4.0%)	1.5 (0.5, 4.5)	7 (2.8%)	1.2 (0.4, 4.1)	7 (2.8%)	1.7 (0.5, 6.9)
Education							
<12 years	234	0 (0%)	0 (0, 1.0)	3 (1.3%)	0.6 (0.1, 1.9)	6 (2.6%)	1 (Ref)
12 years	989	16 (1.6%)	1 (Ref)	22 (2.2%)	1 (Ref)	16 (1.6%)	0.6 (0.2, 2.0)
13-15 years	421	13 (3.6%)	1.9 (0.8, 4.3)	10 (2.4%)	1.1 (0.4, 2.4)	7 (1.7%)	0.6 (0.2, 2.3)
16+ years	138	7 (5.1%)	3.2 (1.1, 8.5)	1 (0.7%)	0.3 (0.01, 2.0)	5 (3.6%)	1.4 (0.3, 5.7)
Smoking							
None	1322	34 (2.6%)	1 (Ref)	24 (1.8%)	1 (Ref)	30 (2.3%)	1 (Ref)
<1 pack per day	365	3 (0.8%)	0.3 (0.1, 1.0)	10 (2.7%)	1.5 (0.6, 3.3)	4 (1.1%)	0.5 (0.1, 1.4)
≥1 pack per day	90	1 (1.1%)	0.4 (0.01, 2.6)	2 (2.2%)	1.2 (0.1, 5.1)	0 (0%)	0 (0, 1.8)

**** Note: Soldiers were allowed to choose more than one race/ethnic group;***

OR=Prevalence Odds Ratio, TST=Tuberculin Skin Test, QFT=QuantiFERON® Gold-in-tube, T-Spot=T-SPOT®.TB

Table 22. Association of Selected Factors with Positive TST, QFT, and T-Spot Among US Army Recruits

Factor	TST		QFT		T-Spot	
	Adjusted OR (95% CI)	Bootstrap Adjusted OR (95% CI)	Adjusted OR (95% CI)	Bootstrap Adjusted OR (95% CI)	Adjusted OR (95% CI)	Bootstrap Adjusted OR (95% CI)
Close contact of active TB case	2.8 (0.4, 18.0)	1.4 (<0.01, 44.7)	4.1 (0.96, 17.6)	2.4 (<0.01, 70.1)	0.7 (0.06, 7.8)	<0.01 (<0.01, <0.01)
Prevalence of TB in country of birth: <20 per 100,000 20-100 per 100,000 >100 per 100,000	 1 (Ref) 4.3 (1.0, 18.2) 12.6 (4.8, 33.0)	 1 (Ref) 2.4 (<0.01, 31.2) 13.7 (2.2, 101)	 1 (Ref) 3.5 (0.93, 13.3) 3.0 (0.99, 8.8)	 1 (Ref) 1.5 (<0.01, 20.5) 2.7 (0.3, 26.0)	 1 (Ref) 3.5 (0.9, 13.8) 3.8 (1.3, 10.9)	 1 (Ref) 1.3 (<0.01, 20.5) 3.3 (0.4, 23.6)
Lived with family member not born in the United States	4.8 (1.8, 13.1)	5.2 (0.67, 37.3)	5.7 (2.2, 14.8)	5.6 (0.7, 31.5)	3.3 (1.2, 9.6)	3.1 (0.3, 22.9)
Prior TST positive	46.2 (15.1, 142)	125.2 (10.4, >999)	2.2 (0.4, 11.7)	0.2 (<0.01, 13.9)	9.5 (2.9, 31.3)	9.6 (1.04, 70.1)
Lived in shelter or congregate setting	4.4 (1.5, 13.0)	2.91 (0.23, 20.9)	3.0 (1.1, 8.3)	2.5 (0.3, 13.9)	2.4 (0.8, 7.2)	1.4 (0.1, 10.1)
Age ≥ 30 years	3.5 (1.5, 8.4)	3.42 (0.86, 13.2)	1.5 (0.6, 4.0)	1.2 (0.2, 5.5)	2.1 (0.9, 5.2)	1.8 (0.3, 7.0)
Travel ≥ 1month to a country with a TB prevalence of: <20 per 100,000 20-100 per 100,000 >100 per 100,000	 1 (Ref) 0.3 (0.02, 4.3) 2.2 (0.6, 8.0)	 1 (Ref) <0.01 (<0.01, 2.72) 2.0 (0.12, 15.6)	 1 (Ref) <0.01 (<0.01, >999) 1.0 (0.2, 4.3)	 1 (Ref) <0.01 (<0.01, <0.01) 0.4 (<0.01, 5.3)	 1 (Ref) <0.01 (<0.01, >999) 1.5 (0.3, 6.6)	 1 (Ref) <0.01 (<0.01, <0.01) 0.5 (<0.01, 8.7)
AUC (c-statistic)	0.88	0.84 (0.76, 0.91)	0.72	0.69 (0.62, 0.78)	0.74	0.70 (0.61, 0.79)

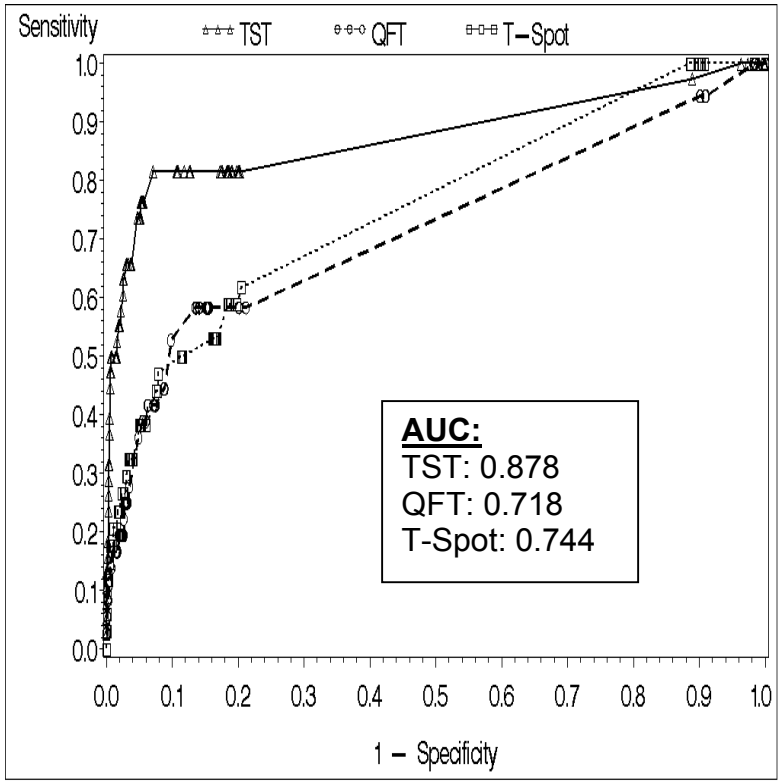
OR=Prevalence Odds Ratio, TST=Tuberculin Skin Test, QFT=QuantiFERON® Gold-in-tube, T-Spot=T-SPOT®.TB, AUC=Area Under the Curve

Table 23. Validity of Predictors of LTBI as measured by the TST among US Army Recruits

Factor	*Positive RFQ responses (n=1783)*	*# with positive TST (n=38)*	*Sensitivity*	*Specificity*	*PPV*	*NPV*	*AUC*
1. Close Contact of TB Case	20 (1.1%)	3	7.9%	99.0%	15.0%	98.0%	0.535
2. TB Prevalence ≥ 20 per 100,000 in Country of Birth	96 (5.4%)	19	50.0%	95.6%	19.8%	98.9%	0.730
3. Lived with Parent Not Born in the US	79 (4.4%)	13	34.2%	96.2%	16.5%	98.5%	0.652
4. Prior TST positive	21 (1.2%)	12	31.6%	99.5%	57.1%	98.5%	0.655
5. Age ≥ 30 years	149 (8.4%)	14	36.8%	92.3%	9.4%	98.5%	0.646
Combinations of these factors:							
1 and 2	113 (6.3%)	21	55.3%	94.7%	18.6%	99.0%	0.753
1, 2, and 3	124 (7.0%)	27	71.1%	94.4%	21.8%	99.3%	0.798
1,2,3, and 4 (selected model)	**166 (9.3%)**	**30**	**79.0%**	**92.2%**	**18.1%**	**99.5%**	**0.871**
1,2,3,4, and 5	282 (15.8%)	31	81.6%	85.6%	11.0%	99.5%	0.876
1,2,3,4,5, and Overseas Travel ≥ 1 month	318 (17.8%)	31	81.6%	83.6%	9.8%	99.5%	0.877
1,2,3,4,5, and Residence in Congregate Setting	367 (20.6%)	31	81.6%	80.7%	8.5%	99.5%	0.876
All risk factors*	579 (32.5%)	32	84.2%	68.7%	5.5%	99.5%	0.878

RFQ=Risk Factor Questionnaire
TST=Tuberculin Skin Test
PPV=Positive Predictive Value
NPV=Negative Predictive Value
AUC=Area Under the Curve (c-statistic)
* Risk factors include all of the previously mentioned risk factors, plus: health care work, casual contact with a TB case, and prior TB treatment

Figure 10. Receiver Operator Characteristics Curve for Predictors of LTBI as measured by TST, QFT, and T-Spot among US Army Recruits*



* Predictors included in the logistic regression model: Close contact with a TB case, casual contact with a TB case, TB prevalence in country of birth, history of living with parent born outside the US, prior positive skin test, prior TB treatment, history of living in a congregate setting, health care work

LTBI=Latent tuberculosis infection, TST=Tuberculin skin test, QFT=QuantiFERON® Gold-in-tube, T-Spot=T-SPOT®.TB, AUC=Area Under the Curve

Table 24. Validity of Predictors of LTBI as measured by the QFT among US Army Recruits

Factor	*Positive RFQ responses (n=1783)*	*# with positive QFT (n=36)*	*Sensitivity*	*Specificity*	*PPV*	*NPV*	*AUC*
1. Close Contact of TB Case	20 (1.1%)	3	8.3 %	99.0%	15.0%	98.1%	0.537
2. TB Prevalence ≥ 20 per 100,000 in Country of Birth	96 (5.4%)	9	25.0%	95.0%	9.4%	98.4%	0.600
3. Lived with Parent Not Born in the US	79 (4.4%)	10	27.8%	96.0%	12.7%	98.5%	0.619
4. Prior TST positive	21 (1.2%)	2	5.6%	98.9%	9.5%	98.1%	0.522
5. Age ≥ 30 years	149 (8.4%)	6	16.7%	91.8%	4.0%	98.2%	0.543
Combinations of these factors:							
1 and 2	113 (6.3%)	12	33.3%	94.2%	10.6%	98.6%	0.638
1, 2, and 3	124 (7.0%)	12	33.3%	93.6%	9.7%	98.6%	0.685
1,2,3, and 4 (selected model)	**166 (9.3%)**	**16**	**44.4%**	**91.4%**	**9.6%**	**98.8%**	**0.684**
1,2,3,4, and 5	282 (15.8%)	18	50.0%	84.9%	6.4%	98.8%	0.691
1,2,3,4,5, and Overseas Travel ≥ 1 month	318 (17.8%)	18	50.0%	82.8%	5.7%	98.8%	0.697
1,2,3,4,5, and Residence in Congregate Setting	367 (20.6%)	21	58.3%	80.2%	5.7%	98.9%	0.719
All risk factors*	579 (32.5%)	24	66.7%	68.2%	4.2%	99.0%	0.718

RFQ=Risk Factor Questionnaire
QFT=QuantiFERON® Gold-in-tube
TST=Tuberculin skin test
PPV=Positive Predictive Value
NPV=Negative Predictive Value
AUC=Area Under the Curve (c-statistic)
* Risk factors include all of the previously mentioned risk factors, plus: health care work, casual contact with a TB case, and prior TB treatment

Table 25. Validity of Predictors of LTBI as measured by the T-Spot among US Army Recruits

Factor	*Positive RFQ responses (n=1783)*	*# with positive T-Spot (n=34)*	*Sensitivity*	*Specificity*	*PPV*	*NPV*	*AUC*
1. Close Contact of TB Case	20 (1.1%)	3	2.9%	98.9%	5.0%	98.1%	0.509
2. TB Prevalence ≥ 20 per 100,000 in Country of Birth	96 (5.4%)	10	29.4%	95.1%	10.4%	98.6%	0.623
3. Lived with Parent Not Born in the US	79 (4.4%)	7	20.6%	95.9%	8.9%	98.4%	0.582
4. Prior TST positive	21 (1.2%)	5	14.7%	99.1%	23.8%	98.4%	0.569
5. Age ≥ 30 years	149 (8.4%)	8	23.5%	91.9%	5.4%	98.4%	0.577
Combinations of these factors:							
1 and 2	113 (6.3%)	11	32.4%	94.2%	9.7%	98.6%	0.633
1, 2, and 3	124 (7.0%)	12	35.3%	93.6%	9.7%	98.7%	0.667
1,2,3, and 4 (selected model)	**166 (9.3%)**	**15**	**44.1%**	**91.4%**	**9.0%**	**98.8%**	**0.688**
1,2,3,4, and 5	282 (15.8%)	18	52.9%	84.9%	6.4%	98.9%	0.708
1,2,3,4,5, and Overseas Travel ≥ 1 month	318 (17.8%)	18	52.9%	82.9%	5.7%	98.9%	0.712
1,2,3,4,5, and Residence in Congregate Setting	367 (20.6%)	19	55.9%	80.1%	5.2%	98.9%	0.711
All risk factors*	579 (32.5%)	21	61.8%	68.1%	3.6%	98.9%	0.744

RFQ=Risk Factor Questionnaire
T-Spot=T-SPOT®.TB
TST=Tuberculin skin test
PPV=Positive Predictive Value
NPV=Negative Predictive Value
AUC=Area Under the Curve (c-statistic)
* Risk factors include all of the previously mentioned risk factors, plus: health care work, casual contact with a TB case, and prior TB treatment

Chapter 6—Targeted and Sequential Screening Strategies for Latent Tuberculosis in a Heterogeneous Population

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Abstract

Background

There is uncertainty as to whether interferon-gamma release assays (IGRAs) are more cost-effective tests in the diagnosis of latent tuberculosis infection (LTBI) than the tuberculin skin test (TST). Furthermore, no cost-effectiveness studies of testing for (LTBI) have incorporated both targeted testing and the use of IGRAs in heterogeneous, low-prevalence populations. The purpose of this study was to examine the cost-effectiveness of universal versus targeted and sequential testing strategies using TST versus IGRAs in a heterogeneous population of US military recruits.

Methods

Using a decision analytic model, incremental cost-effectiveness ratios were calculated in 2009 among nine potential strategies for screening recruits. A societal perspective was taken over a 20-year analytic horizon, discounting future costs at 3% annually. Sensitivity analyses were conducted to determine how changes in assumptions affected the estimates.

Results

Targeted strategies cost over $250,000 per case prevented, whereas universal testing strategies cost over $700,000 per incremental case prevented in base case and most sensitivity analyses.

Conclusions

In this heterogeneous, low-prevalence setting, the incremental cost-effectiveness ratios of all testing strategies were high. Sequential testing with both TST and IGRAs provided a poor incremental value compared to targeted and universal testing strategies. Targeted testing offered the best value in this population, although it was still relatively expensive compared to no testing. Targeted testing using TST was slightly more cost effective than targeted testing using Quantiferon Gold-in-tube® or T-SPOT.TB®, but these estimates were very sensitive to changes in model assumptions.

Background

Despite recent declines, tuberculosis (TB) remains a major cause of morbidity and mortality in the US, with 12,898 cases in 2008.[1] The cost of treating TB is also great, resulting in a cost of over USD 700 million in 1991 on direct medical expenditures, or 1.1 billion in adjusted 2009 dollars.[2] The US military is generally a young, healthy subset of the US population, and the risk of active TB in this population is lower than the age-adjusted general US population.[3-5] However, TB control in the military has many unique challenges, including deployments or service in TB endemic countries such as Korea, Iraq, or Afghanistan;[6] the potential for outbreaks in closed environments such as Navy ships;[7-11] and a sizeable proportion of foreign-born individuals serving in the military.[12-15] Concern about the potential for TB transmission in the US military has led to universal tuberculin skin test (TST) screening programs among military recruits, and periodically thereafter according to service.[16-18] In 2009, this resulted in more than 200,000 TSTs among US military recruits.

However, the policy of universal TB testing of all military recruits is inconsistent with current Centers for Disease Control and Prevention (CDC) and American Thoracic Society (ATS) guidelines, which recommends only targeted testing.[19] Furthermore, universal testing of the low prevalence US military population has led to pseudoepidemics of TST conversions from false positives,[20] risk of subsequent adverse events from isoniazid (INH) or other treatment for latent tuberculosis infection (LTBI),[21] and large health-care expenditures.[2] Targeted testing using a risk factor

questionnaire (RFQ) has been proposed among pediatric[22, 23] and university[24] populations, but there has been resistance to targeted testing in US military recruit populations.[25]

The recent development of the interferon-gamma release assay (IGRA), including both the QuantiFERON® Gold-in-tube (QFT) and T-SPOT®.TB (T-Spot), has further complicated potential policy changes. Although the currently available IGRAs may be more specific than the TST,[26, 27] there are concerns about their sensitivity to detect LTBI, particularly in military populations.[28] While many have advocated for the use of IGRAs, there is uncertainty as to whether either IGRA is a better test in the diagnosis of TB infection than the TST in this population, particularly given the long history of TST use and demonstrated association with subsequent active TB.[13, 29, 30] There is also uncertainty in how to apply the IGRAs, with some analyses suggesting that sequential testing with TST followed by IGRA may be more cost effective than either test alone.[31-34] There is additional uncertainty as to which IGRA (QFT or T-Spot) is more cost-effective.

This cost-effectiveness analysis (CEA) evaluates screening strategies for LTBI among a heterogeneous, low-prevalence population of US military recruits at time of accession. US military recruits are >85% male and typically aged 17-25. The aim of the evaluation is to determine which of the following LTBI screening strategies provides the best value in this population: 1) universal testing of all recruits with the TST (status quo), 2) universal testing with T-Spot, 3) universal testing with QFT, 4) sequential testing with

TST followed by T-Spot, 5) sequential testing with TST followed by QFT, 6) targeted testing with a risk factor questionnaire (RFQ) followed by TST, 7) targeted testing with RFQ followed by T-Spot, 8) targeted testing with RFQ followed by QFT, or 9) no screening. These screening strategies are summarized in Table 26. Each of these strategies is evaluated from the societal perspective using an incremental analysis that adheres to reference case guidelines.[35] This study is targeted to military medical and public health leaders in order to guide imminent and potentially costly policy decisions, but may be applicable to other heterogeneous groups such as university students and pediatric populations.

Methods

A decision analytic cost-effectiveness model was developed in 2009 using TreeAge Pro 2009 Suite (TreeAge Software, Inc., Williamstown, MA) to assess the costs and effects from alternative TB screening policies. Of particular interest was the question of who should be screened in the recruit setting: universal testing (status quo), targeted testing based on a positive RFQ, sequential testing with TST followed by an IGRA, or no screening. We further compared the efficiency of the TST (status quo) with both IGRA tests (T-Spot and QFT) for each universal, targeted and sequential testing strategy. The model incorporated several Markov processes, including the health states of latent TB infection, previous active TB disease, and death, consistent with previous approaches.[36, 37] Other elements incorporated into the model included initiation of therapy, adverse events during therapy, adherence to a full course of therapy, and efficacy of therapy.

The health outcome measured in this analysis was cases of active TB prevented. The parameter estimates of the inputs of the health outcomes are shown in Table 27. The base-case parameter estimates of LTBI prevalence and specificity of TST, T-Spot, and QFT were directly obtained from a study of 1,979 recruits entering the US military at Fort Jackson, SC from April to June 2009. All other estimates were obtained from the medical literature. Positive tests determined to be LTBI were treated in accordance with established guidelines.[19, 38] The comparative summary measure was the incremental cost per case of active TB prevented. The time frame for the screening program was 1 year. Estimates were presented for a cohort of 200,000 recruits, the approximate number of military recruits that enter US military basic training during a calendar year. Recruits were assumed to enter at an average age of 20, and the analytic horizon was 20 years.

A societal perspective was taken, in which all costs and benefits associated with the screening strategies are included. From this perspective, the cost of the health outcomes was measured using the cost-of-illness approach, which included direct medical and nonmedical costs and indirect costs associated with lost productivity. Cost-of-illness estimates for the health outcomes were obtained from the TRICARE management agency and the published literature, as listed in Table 28. Expected future cost estimates were discounted at an annual rate of 3%, in accordance with established US guidelines.[35] Costs were adjusted to 2009 US dollars (USD) using the consumer price index.[39] Death from other causes was estimated from published US life tables.[40] Secondary transmission of active TB within the population was not accounted for in the model due to uncertainty of these estimates and inability to obtain reliable estimates in the context of

extremely heterogeneous exposures and settings. Dominated interventions were excluded from consideration according to standard techniques. Interventions which were less effective and more costly than another intervention were considered to be strongly dominated. Interventions which had an incremental cost-effectiveness ratio that was higher than the next most effective intervention were considered to be weakly dominated.

The purpose of the sensitivity analysis was to assess the robustness of the findings by determining the effect of changes in parameter estimates on the decision result. Sensitivity analyses were performed either to answer specific policy questions or to examine the effect of uncertain parameter estimates. The range of variables used in the sensitivity analyses for this study was based on medical literature and the results from the previous objectives. Sensitivity analyses were performed one variable at a time and with several parameters simultaneously. The following variables were considered for the sensitivity analyses in this study: prevalence of LTBI, risk of progression to active TB, performance characteristics (sensitivity and specificity) of the TST, IGRA and RFQ, adherence to LTBI treatment, efficacy of anti-tuberculosis therapy, the cost of TST and IGRA testing, the cost of the disease, and the discount rate. Because of the number of variables for which uncertainty existed, “best-case” and “worst-case” scenarios were created to provide a realistic range of the impact that these screening strategies would have on morbidity from active tuberculosis.

Results

Base case estimates are presented in Table 29. Universal, targeted and sequential testing strategies had roughly equivalent effectiveness, preventing between 18 and 30 cases during a 20-year analytic horizon in the 200,000 person cohort as compared to no testing. However, targeted testing programs were much less costly than sequential or universal testing strategies. The choice of test (TST, T-Spot or QFT) had a relatively small impact on both costs and effectiveness whether using a targeted, sequential, or universal testing strategy.

The targeted strategies using RFQ followed by TST or IGRA were compared in the one-way sensitivity analyses presented in Tables 30 and 31. Estimates comparing universal and sequential testing with targeted testing strategies were robust, with minimal changes when the parameter estimates were varied. In contrast, the estimates comparing the use of TST, T-Spot, and QFT within the testing strategies were fairly sensitive to small to moderate changes in model assumptions. Estimates were particularly sensitive to changes in prevalence of LTBI, as shown in Table 30. The estimates from this sensitivity analysis may be useful in estimating cost-effectiveness in other populations. Incremental cost-effectiveness varied widely with the prevalence of LTBI, but at most plausible values the targeted approach offered the best value. Incremental cost-effectiveness only dropped below 100,000 USD per case prevented at extreme values, such as 10% LTBI prevalence or 0.5% annual risk of progression to active TB, as shown in Tables 30 and 31. These are values that might be seen in close contacts of an active TB case or in HIV-infected patients.[41] Uncertainty in the performance characteristics of the diagnostic tests affected the relative cost-effectiveness of the strategies in predictable ways.

The multi-way sensitivity analyses in Table 32 showed similar results as the one-way analyses. Estimates were very sensitive to small changes in sensitivity and specificity of the selected test. As no gold standard exists in the diagnosis of LTBI, the uncertainty of these characteristics suggests that the findings are not robust. Notably, the use of values from a systematic review of specificity of the TST (97%) and QFT (99%)[42] and an intermediate value for the T-Spot (98%) favored the use of T-Spot.

Discussion

This is the most complete analysis of the cost-effectiveness of TB screening strategies in a heterogeneous population. This includes consideration of all plausible strategies, including targeted, sequential, and universal testing, as well as comparisons between the two commercially-available IGRAs (T-Spot and QFT) and the TST. The current strategy of universal TST testing of military recruits is extremely costly, at over USD 700,000 incremental cost per case prevented. Universal testing with either IGRA or sequential testing with TST followed by an IGRA was also extremely costly. Targeted strategies using a risk factor questionnaire followed by a TST or either IGRA prevent nearly the same number of cases as universal testing strategies at greatly reduced costs.

Similar to previous studies[43], the relative cost-effectiveness between the targeted testing strategies was highly sensitive to small to moderate changes in model assumptions. In sensitivity analysis, changes in the parameters of prevalence of LTBI and risk of progression to active TB resulted in the greatest changes in cost-effectiveness.

However, at all but extreme ranges of the parameter estimates, the cost of preventing a case of active TB among US military recruits by any method was high, typically over 200,000 USD.

Although targeted testing is the approach recommended by the CDC and other leading health agencies,[19, 44] the cost-effectiveness of screening for tuberculosis in different populations has been the subject of substantial debate. The cost-effectiveness of testing for LTBI has been largely determined by the population studied and its pre-test prevalence, as was seen in this study in the sensitivity analysis. For example, testing of contacts of active TB cases has generally been found to be cost-saving.[31, 45] Studies in immigrants have reported that other screening tests such as TST or IGRA are only marginally better than chest x-ray, if at all,[36, 46-49] while others argue that testing for LTBI may be highly cost-effective.[50] This analysis found that targeted testing of US military recruits was more cost-effective than universal testing, as had been demonstrated in pediatric populations[22, 23] and long-term travelers.[37] Several studies have reported and advocated the use of IGRAs as a cost-effective method of testing TST-positive individuals (sequential testing)[31-34, 43, 51] or as a replacement for the TST.[33] In contrast, this analysis found that sequential testing strategies were dominated (that is, they were less effective and more expensive) by targeted and universal testing strategies under a wide range of assumptions. This study also found similar costs and effectiveness between the particular test chosen, including TST, T-Spot, and QFT. The incremental cost-effectiveness varied much more based on the testing strategy (universal, sequential, or targeted) than on the particular test chosen.

The differences in conclusions between this and previous studies are largely based on the assumptions in the models and the additional strategies considered in this analysis. This analysis incorporates estimates of prevalence and specificity of the TST and both IGRAs obtained from a study designed for this purpose; i.e. a large sample of 1,979 US military recruits in 2009. Previous analyses have been limited by the fact that few studies of the T-Spot have been done in low-prevalence populations to estimate specificity. Additionally, this model incorporated indirect costs into the analysis, which many of the previous studies did not. Finally, the low prevalence of LTBI in the US military recruit population greatly impacts the cost-effectiveness ratios of the interventions, as was seen in the sensitivity analysis. Similar to the findings from long-term travelers, all methods of screening will be expensive when testing a low-risk population.[37]

There are several limitations to this study, the most important of which is the absence of a gold standard to diagnose TB infection. In the absence of such a gold standard, any comparison of cost-effectiveness between TST and IGRA will be difficult and imprecise, since it is unclear whether the IGRAs or the TST are more accurate. Other estimates such as risk of progression to active TB also have uncertainty, particularly in a healthy, low-risk population. The small effect sizes of the active TB outcome seen in this study are another source of imprecision in the estimates. Secondary transmission was not included in the model, which will underestimate the cost-effectiveness of the interventions. There is considerable uncertainty regarding the rate of secondary transmission in any population, but especially in the heterogeneous set of exposures and

settings which exist in the US military. Added costs from retesting indeterminate or invalid IGRA tests were not considered in this analysis, which will underestimate the true costs of testing with IGRAs. Similarly, complete (100%) follow-up reading of TST was assumed, which may overestimate the benefits of TST testing in populations where 100% follow-up is not possible. Finally, demographic and health-related differences between US military services and the general US population may limit the generalizability of this study,[12] making comparisons with other higher risk populations difficult. However, cost-effectiveness of LTBI screening strategies have been well-studied among higher-risk populations [31, 33, 43, 47, 48]. Furthermore, the use of varying estimates of the prevalence of LTBI as shown in Table 30 may be useful in generalizing the estimates of this study to other low- to moderate-prevalence populations.

There are several ethical and distributional implications of this study. The most notable is the concern about singling out foreign-born minorities in targeted testing programs and the perception of coercion of treatment among military service members once identified as infected. However, more than 50% of the burden of TB disease in the US has been among the foreign-born since 2000,[52] and this proportion is increasing. Both the risk of TB and the benefit of LTBI treatment are largely borne by these individuals, so this approach may be scientifically and ethically justified. Furthermore, although it is always recommended in accordance with CDC guidelines,[19] LTBI therapy is not compulsory for US military service members. Another concern is that the US military may not be interested in the societal perspective or a 20-year analytic horizon, but instead in a direct cost perspective and a shorter, more typical 4 year enlistment-length horizon. However,

in addition to the concerns about TB transmission and outbreaks in congregate settings while on active duty, the military has a long history of experience with paying for active TB that was discovered while on active duty,[53-55] has commitments to service members who stay in longer than the initial enlistment up to and beyond the 20-year requirement for retirement, and is very interested in keeping its service members fit for active service and worldwide deployment.

The US military is at generally low risk for both prevalent LTBI at time of entry into military service, and for subsequent progression to active TB disease. Under such conditions, targeted testing appears to be a more cost-effective method of screening than either universal or sequential testing. Although the targeted TST strategy was found to offer the best value in this model, all targeted strategies had similar costs and effects, and the model was sensitive to relatively small changes in model assumptions. Targeted use of an IGRA may offer logistical advantages to the TST in a recruit setting, where reducing time away from training is a high priority. However, the IGRA also presents challenges in added costs and significant logistical requirements for the supporting laboratory. Improvements in cost, automation of test processing, and documentation will make these tests more feasible for training sites where existing laboratory infrastructure may not be robust. Finally, future studies investigating the ability of the IGRA to predict the longitudinal risk of active TB are necessary to provide further evidence as to whether the IGRA may be a better gold standard than the TST, as some have suggested.[56] Future studies investigating the risk of TB infection resulting from deployment are also

warranted, including assessments of the cost-effectiveness of strategies designed to prevent active TB in redeploying service members.

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Table 26. Summary of the nine potential screening strategies considered for use among US military recruits

Screening Strategy		*First Test*	*Second Test*	*Comments*
Universal testing				Current standard of practice in all US military services using the TST
1.	TST (status quo)	TST	None	
2.	T-Spot	T-Spot	None	
3.	QFT	QFT	None	
Sequential testing				IGRA would be performed only on TST positives, currently recommended in some European countries such as the UK
4.	TST/T-Spot	TST	T-Spot	
5.	TST/QFT	TST	QFT	
Targeted testing				TST or IGRA would be performed only on those with positive screening risk factor questionnaire
6.	RFQ/TST	RFQ	TST	
7.	RFQ/T-Spot	RFQ	T-Spot	
8.	RFQ/QFT	RFQ	QFT	
No testing				No testing of recruits
9.	None	None	None	

TST = Tuberculin skin test
QFT= Quantiferon Gold-in-tube
IGRA = Interferon-gamma release assay
RFQ = Risk factor questionnaire

Table 27. Parameter estimates for health outcomes in the decision analytic model

Parameter	*Base Case (Sensitivity Range)*	*Sources*
Overall prevalence of LTBI	2.0% (0.5%-20%)	*,[57]
Sensitivity		
TST	77% (70%-99.5%)	[42]
QFT	70% (60%-99.5%)	[42]
T-Spot	80% (80%-99.5%)	[42]
RFQ	60% (50%-99.5%)	*,[24]*
Specificity		
TST	98.9% (95%-100%)	*,[42]
QFT	98.8% (98%-100%)	*,[42]
T-Spot	98.7% (93%-100%)	*,[42]
RFQ	95% (90%-100%)	*,[24]
Adverse events from treatment of LTBI		
Severe hepatitis	0.001 (0.0005, 0.0018)	[21]
Incidence of active TB	0.1% per year (0.01%, 0.5%)	[41]
Treatment of LTBI		
Initiation	70% (50%-90%)	[45, 58, 59]
Adherence	70% (50%-90%)	[45, 58, 59]
Efficacy of full course of therapy	90% (70-100%)	[60]
Efficacy of partial course of therapy	22.5% (0%-50%)	[60]

TST = Tuberculin skin test
TB = Tuberculosis
LTBI = Latent tuberculosis infection
QFT = Quantiferon Gold-in-tube
RFQ = Risk factor questionnaire
* Base case estimates were obtained from a prevalence study performed at Fort Jackson, SC, April-June 2009

Table 28. Cost estimates for decision analytic model

Cost Parameters	*Base case (sensitivity range)*	*Source*
Direct Costs:		
TST administration and reading	$19.11 (10-30)	[50]
QFT	$47.47 (30-60)	[31, 61]
T-Spot	$55.00 (30-75)	Manufacturer
RFQ	$1.60 (1.00-5.00)	[23]
LTBI evaluation	$116.43 (40-200)	[45]
LTBI follow-up costs	$326.53 (100-500)	[50]
Active TB Hospitalization	$17,869 (7,000-35,000)	DoD
Active TB Contact Investigation	$4,896 (2,000-10,000)	[2, 22]
Major adverse reaction to Isoniazid	$10,730 (4,000-45,000)	[36]
Indirect Costs:		
Cost of recruit time	$35.85 per hour ($20-$50)	[62]
Screening visit (1/2 hour)	$17.93 (10-25)	
TST follow-up reading (1/4 hour)	$8.96 (5.00-12.50)	
LTBI evaluation (2 hours)	$71.74 (40-100)	
LTBI follow-up visits (8 visits)	$286.80 (160-400)	
Partial course of therapy (2 visits)	$71.74 (40-100)	
Adverse events indirect costs (2 weeks lost duty time)	$2,870 (1,000-5,000)	[36, 62]
Active TB indirect costs (2 weeks lost duty time)	$2,870 (1,000-5,000)	[36, 62]

TST = Tuberculin skin test
TB = Tuberculosis
LTBI = Latent tuberculosis infection
QFT = Quantiferon Gold-in-tube
RFQ = Risk factor questionnaire
DoD=TRICARE Management Agency, Falls Church, VA

Table 29. Cost-effectiveness of screening 20 year-old US recruits for latent TB infection over a 20 year interval

Strategy	*Expected costs per 200,000*	*Expected cases per 200,000*	*Incremental cost per 200,000*	*Incremental cases prevented per 200,000*	*Incremental cost per case prevented (ICER)*
None	$1,540,000	78			
RFQ followed by TST	$6,580,000	60	$5,060,000	18	$285,777
RFQ followed by QFT	$6,660,000	62	$80,000	-2	Dominated (strong)
RFQ followed by T-Spot	$6,840,000	60	$260,000	0	$369,273
TST followed by QFT	$13,620,000	58	$6,780,000	2	Dominated (weak)
TST followed by T-Spot	$13,760,000	54	$140,000	4	Dominated (weak)
Universal TST	$14,720,000	50	$960,000	4	$711,363
Universal QFT	$16,820,000	52	$2,100,000	-2	Dominated (strong)
Universal T-Spot	$18,560,000	48	$3,840,000	2	$3,347,268

QFT = Quantiferon Gold-in-tube
TST = Tuberculin skin test
RFQ = Risk factor questionnaire
IGRA = Interferon-gamma release assay
ICER = Incremental cost-effectiveness ratio

Table 30. One-way sensitivity analysis: estimated cost per case of TB prevented for each screening strategy by prevalence of LTBI

Prevalence of LTBI By Screening Strategy	0.5%	1.0%	*2.0%**	5.0%	10.0%	20.0%
None						
RFQ followed by TST	$1,021,649	$531,068	*$285,777*	$138,603	$89,545	$65,016
RFQ followed by QFT	D	D	*D*	D	D	D
RFQ followed by T-Spot	$1,198,028	$645,525	*$369,273*	$203,522	$148,271	D
TST followed by QFT	D	D	*D*	D	D	D
TST followed by T-Spot	D	D	*D*	D	D	D
Universal TST	$2,776,339	$1,399,697	*$711,363*	$298,383	$160,719	$93,568
Universal QFT	D	D	*D*	D	D	D
Universal T-Spot	$13,292,334	$6,662,290	*$3,347,268*	$1,358,255	$695,250	$363,748

LTBI=Latent tuberculosis infection
TST = Tuberculin skin test
RFQ = Risk factor questionnaire
QFT=Quantiferon Gold-in-tube
*=Base Case
D=Dominated

Table 31. One-way sensitivity analyses: estimated cost per case of TB prevented by other selected parameters

Parameters	*Values in sensitivity analysis*	*RFQ+ TST*	*RFQ+ QFT*	*RFQ+ T-Spot*	*TST+ QFT*	*TST+ T-Spot*	*TST only (Status quo)*	*QFT only*	*T-Spot only*
Annual risk of progression to active TB	0.5%	*$44,214*	*D*	*$66,664*	*D*	*D*	*$133,159*	*D*	*$684,023*
	0.01%	*$3,003,070*	*D*	*$3,829,779*	*D*	*D*	*$7,217,011*	*D*	*$33,315,505*
T-Spot: cost	$30	D	D	$272,296	D	D	D	D	$574,918
	$75	$285,777	D	D	D	D	$691,383	D	$6,833,058
T-Spot: sensitivity	70%	$285,777	D	D	D	D	$691,383	D	D
	95%	D	D	$248,966	D	D	D	D	$812,825
T-Spot: specificity	93%	$285,777	D	D	D	D	$691,383	D	$8,508,231
	99.5%	$285,777	D	$308,911	D	D	$715,122	D	$2,622,922
QFT: cost	$30	$285,777	D	$369,273	D	D	$711,376	D	$3,347,268
	$60	$285,777	D	$369,273	D	D	$711,376	D	$3,347,268
QFT sensitivity	60%	$285,777	D	$369,273	D	D	$711,376	D	$3,347,268
	95%	D	$244,514	D	D	D	D	$708,744	D
QFT specificity	95%	$285,777	D	$369,273	D	D	$711,376	D	D
	99.5%	$285,777	D	$369,273	D	D	$711,376	D	D
RFQ cost	$1	$278,987	D	$369,273	D	D	$722,194	D	$3,347,268
	$5	$324,257	D	$369,273	D	D	$650,074	D	$3,347,268
RFQ sensitivity	50%	$334,738	D	$423,418	D	D	$566,891	D	$3,347,268
	95%	$195,586	D	$269,532	D	D	D	D	$7,366,011
RFQ specificity	80%	$357,101	D	D	D	D	$584,398	D	$3,347,268
	99.5%	D	D	$259,176	D	D	$760,587	D	$3,347,268

TST = Tuberculin skin test
QFT = Quantiferon Gold-in-tube
RFQ = Risk factor questionnaire
LTBI = Latent TB infection
D=Dominated

Table 32. Multi-way sensitivity analyses: estimated cost per case of active TB prevented among US military recruits

Parameter	*Values in sensitivity analysis*	*RFQ+ TST*	*RFQ+ QFT*	*RFQ+ T-Spot*	*TST+ QFT*	*TST+ T-Spot*	*TST only (Status quo)*	*QFT only*	*T-Spot only*
Using Specificities from Systematic Review[42]:									
TST									
T-Spot	97%	D	D	$290,889	D	D	$886,062	D	$2,260,749
QFT	98%								
	99%								
Best Case T-Spot values:									
Cost									
Sensitivity	$30	D	D	$233,071	D	D	D	D	$435,490
Specificity	95%								
	99.5%								
Best Case QFT values:									
Cost	$30	D	$233,071	D	D	D	D	$435,490	D
Sensitivity	95%								
Specificity	99.5%								
Best Case RFQ values:									
Cost	$1	D	D	$175,453	D	D	D	D	$7,801,222
Sensitivity	95%								
Specificity	99.5%								

TST = Tuberculin skin test; QFT = Quantiferon Gold-in-tube; RFQ = Risk factor questionnaire; LTBI = Latent TB infection; D = Dominated

Chapter 7—Conclusion

Summary of Major Findings

The overall objective of the study was to evaluate the effectiveness of current and alternative forms of TB control program in the US military at accession into military service. There were five components to this evaluation: 1) analyze active TB epidemiology and surveillance trends; 2) assess independent risk factors for active TB, including factors before and after entry into military service; 3) evaluate prediction models of targeted testing for LTBI among recruits; 4) identify factors associated with discordance between tuberculin skin test (TST) and the interferon-gamma release assays (IGRAs); and 5) determine the costs and effectiveness of current and alternative TB screening methods at time of accession. The study focused on two main areas. First, it estimated the potential impact of using a targeted testing approach as an alternative to the current policy of universal screening. Second, it compared the use of IGRAs with the TST to identify latent TB infection (LTBI) among military recruits. The central hypothesis was that targeted testing by use of the questionnaire followed by the TST or an IGRA, using either QuantiFERON® Gold In-Tube (QFT) or T-Spot®.TB (T-Spot), would reduce the amount of unnecessary testing among low-risk recruits while still detecting cases of LTBI among higher-risk recruits. This hypothesis was strongly supported by the evidence.

The current rate of active TB in the US military is very low, and it is much lower than the age-adjusted rate among the US population. This low rate meets the Healthy People

2010 goal of less than 1 per 100,000 person-years [1], and it approaches the defined goal of elimination of TB in the US of 1 per 1 million person-years [2-4]. Furthermore, active TB continues to decline in the US military despite large-scale operations in the TB-endemic countries of Afghanistan (beginning in 2001) and Iraq (beginning in 2003). This study had findings similar to those found in other studies describing the epidemiology of active tuberculosis in the military up to the mid-1990s, including low and declining incidence rates of active TB and associations with foreign birth and ethnic groups [5-7]. As expected, the risk factors for TB exhibited in the US military were also similar to those seen in the civilian US population, particularly foreign birth, race, and ethnic group [8-10].

The risk of active tuberculosis among the active component military is strongly associated with risk factors present prior to accession into military service. These factors include foreign birth, racial and ethnic group, and age. These findings are also consistent with those identified in three previous service-specific studies [5-7]. Stationing in Korea was the only military service-related risk factor found to have a strong and consistent association with active TB in this study. These associations were robust and were not altered in sensitivity analyses. The strong associations seen with risk factors existing prior to accession suggest a need for continuous TB surveillance during the service member's military career, beginning and focusing on the time of accession. The strong association with foreign birth suggests that these higher-risk individuals may need to be better targeted for interventions at accession, including LTBI diagnosis and treatment. Although all military services currently test for LTBI at entry into service, therapy is not

universally accepted or adhered to. The services should consider ways to improve both initiation and adherence to therapy, particularly among higher-risk recruits, such as those who are foreign-born.

Risk factors for LTBI at time of accession were similar whether measured by TST or one of the two commercially-available IGRAs. As in previous studies, birth in a country with a high prevalence of TB was an important risk factor for LTBI. Prediction models were constructed using this and additional variables such as close contact with an active TB case; living with a family member born outside the US; and history of a prior positive TB skin test. Use of these 4 variables resulted in 79% sensitivity, 92% specificity, and an area under the curve (AUC) of 0.871 in predicting a positive TB skin test. Targeted testing of only those with a positive response to one of these 4 questions would reduce testing by more than 90%. Prediction models for the IGRAs had similar specificities and reductions in testing but had lower sensitivities and AUCs. An important potential implication of this study is that targeted testing of a heterogeneous population is feasible with acceptable sensitivity and specificity using TST or either IGRA. Targeted testing would reduce testing by >90% and reduce costs of the TB screening program while still maintaining an acceptable level of effectiveness. Furthermore, more than 50% of all positives in this low-risk population are estimated to be false positives due to non-tuberculous mycobacteria (NTM) and other factors [11-13]. Targeted testing would be expected to reduce unnecessary treatment of false positives, with corresponding reductions in adverse events from unnecessary therapy.

This study also provides the most complete, robust evaluation of current commercially-available TB diagnostics to date. It examines the performance of these diagnostic tests in a population representative of the underlying US source population, which is heterogeneous but generally at low risk for TB. The major findings of the study of discordance between TST and IGRA included similarities between all three tests in the proportions of positives, estimates of specificity, and associations of increased proportions of positive tests with increased TB exposure. Despite these areas of agreement, the three tests identified different people for the majority of positive test results. This suggests that in low-prevalence populations, the majority of positives resulting from any of the three commercially-available diagnostic tests are false positives. Factors associated with TST positive, IGRA negative discordance were very similar for both T-Spot and QFT. Strong associations were seen between discordance and BST reaction size as well as with BCG vaccination and TB prevalence in the country of birth. This suggests that a substantial proportion of discordance may be attributable to false-positive TST from cross-reactivity induced by NTM and BCG. The association of TST positive, IGRA negative discordance with TB prevalence in the country of birth or long-term residence, and the observed dose-response, suggests that a portion of the discordance may be attributable to false-negative IGRA results. No associations were found with TST negative / IGRA positive discordance.

This study suggests that current TB diagnostics have similar results in heterogeneous populations, although significant discordance still exists. However, as most positives by any test will be false positives, targeted testing should still be performed in this and other

heterogeneous populations. Although the pre-test probability in these populations is very low, the use of risk factors such as foreign birth and contact with a TB case can be used to increase this probability, reducing the proportion of false positives.

The robust comparison of the cost-effectiveness of TB screening strategies in the US military provides compelling evidence that targeted testing provided a superior value to universal testing among military recruits. This analysis not only included consideration of targeted, sequential, and universal testing strategies, but also comparisons between the different IGRAs (both T-Spot and QFT) and the TST. The current strategy of universal TST testing of military recruits was found to be extremely costly, at over 700,000 US Dollars (USD) incremental cost per case prevented. Universal testing with either IGRA or sequential testing with TST followed by an IGRA was also extremely costly. Targeted strategies using a risk factor questionnaire followed by a TST or either IGRA were found to prevent nearly the same number of cases as universal testing strategies at greatly reduced costs. The relative cost-effectiveness between the targeted testing strategies was sensitive to small to moderate changes in model assumptions. In sensitivity analysis, changes in the parameters of prevalence of LTBI and risk of progression to active TB resulted in the greatest changes in cost-effectiveness. However, at all but extreme ranges of the parameter estimates, the cost of preventing a case of active TB among US military recruits by any method was high, typically over 200,000 USD.

Limitations

There are several limitations to this study. The most important limitation in TB diagnostics is the lack of a gold standard to evaluate the presence of LTBI. Without such a standard, the significance of discordant TST and IGRA results will continue to have some uncertainty. This study addressed this limitation using a more complete and robust methodology than previously available, including assessment of prior risk factors, use of all three commercially available LTBI diagnostics, and use of the Battey Skin Test (BST) to assess for the presence of cross-reactive (nontuberculous) mycobacteria. Longitudinal studies will ultimately be required to assess the significance of this discordance by studying the outcome of active TB among thousands of persons over many years. However, the results from these studies will not be available for many years. In the absence of such longitudinal data, this study provides the most comprehensive understanding of LTBI diagnostic test performance in low-risk US populations currently available. The misclassification of other variables such as BCG vaccination and deployment history are also possible in this study, and this might have had an effect on study results. These differences should be non-differential, and thus would be expected to bias towards the null hypothesis of no association. Unmeasured confounding might also be present from adherence to LTBI therapy, other unmeasured TB exposures during military service, and other unmeasured TB exposures prior to accession. Finally, although this study is expected to be generalizable to the heterogeneous, low-risk general US population, it may not be generalizable to other higher risk groups. Furthermore, the US military is, on average, healthier than the general US population due to the "healthy

worker effect," leading to lower risk for TB due to the lack of risk factors such as HIV and other immunosuppressive conditions.

Public Health Relevance

Tuberculosis is a disease of public health importance in the United States and throughout the world because of its impact on human health and because of its transmissibility. Although the incidence of TB in the US military continues to decline, TB still has the potential to cause disease during military service as well as future morbidity and mortality after the termination of service. Similar to the general US population [14, 15], the majority of TB seen in the US military is from reactivation of latent TB acquired prior to accession rather than transmission during military service. Individual military personnel may also have exposures that put them at risk for *Mycobacterium tuberculosis* infection while in congregate settings or during deployment to areas where TB is endemic. However, these exposures were not found to be important risk factors for active TB in this study.

All three services are currently reviewing TB screening policy in light of the recent licensure of the IGRAs, including both the QFT and the T-Spot. However, it is uncertain whether the current literature supporting the use of the IGRAs are generalizable to a low-prevalence military population. The current US military policy of universal testing is not the standard of care for LTBI diagnosis, as evidenced by recommendations from the CDC and IOM, which state that "targeted tuberculin testing programs should be conducted only among groups at high risk and discouraged in those at low risk" [16]. The challenge

for the US military is to mitigate the risk of TB in those few recruits in our population with these risk factors without exposing low-risk recruits to unnecessary therapy. This study provides evidence that targeted testing through use of a short questionnaire can greatly reduce the amount of testing while still allowing the capture of higher-risk recruits. Although some recruits with positive tests were missed by this strategy, all of these recruits lacked risk factors for the development of TB, so would not be high priority candidates for therapy according to CDC guidelines. As seen in the cost-effectiveness analysis, targeted testing would dramatically reduce the costs and logistic burden of universal testing. It would also result in reductions in false positives, with concomitant reductions in adverse drug events from unnecessary therapy, pseudoepidemics of skin test conversions, unnecessary worry, and diversion of resources from more effective interventions.

Finally, this study is the most comprehensive evaluation of TB diagnostics performed to date, including the use of the risk factor questionnaire (RFQ), TST, both commercially-available IGRAs, and the Battey Skin Test (BST) to assess the potential contribution of NTM as a source of discordance between the TST and the IGRAs. There is uncertainty whether or not the IGRAs are more accurate in determining whether an individual has LTBI. Although these tests were developed to have better specificity than the TST through the use of region of difference 1 (RD1) and other antigens, considerable discordance has been seen between the TST and IGRAs in this and other studies [17-20]. Understanding this discordance is critical in the choice of TB diagnostics, since these discordant pairs may or may not be at higher risk for activation of LTBI. This study

showed a strong association of TST positive, IGRA negative discordance with sensitization to NTM as measured by the BST. A strong association was also seen with BCG vaccination, another common cause of false positive TSTs. This suggests that the IGRAs may indeed be more specific tests for LTBI, resulting in less false positives due to NTM. However, the similar overall specificities suggest that switching from the TST to an IGRA will not solve the problem of false positives in a low-prevalence population. Instead, the emphasis of military TB control programs should be on targeted testing using scientifically defensible risk factors for TB exposure. Furthermore, birth or residence in a TB endemic country was also associated with discordance even after adjusting for BCG status, suggesting that the IGRAs might also be less sensitive than the TST.

Recommendations

General Recommendations

This study population was heterogeneous and generally at low risk for infection with TB, making it very similar to the underlying US source population. Therefore, the results from this study should be generalizable to the US population, although it may not be generalizable to higher risk subpopulations such as hospital workers, prison guards, long-term civilian travelers, or deploying military personnel. This study suggests that targeted testing can be accomplished in low-risk populations using the TST or either IGRA with comparable effectiveness, including sensitivity, specificity, and cost-effectiveness. Targeted testing also results in a better value than universal or sequential testing strategies. The low pre-test prevalence seen in this and most other US populations leads to poor positive predictive value with any of these three tests. If universal testing is done

with any of these tests, the majority of positives will be false positives. In contrast, with targeted testing, the pre-test probability is increased, leading to a higher positive predictive value and much lower likelihood of identifying and treating a patient with a false positive result.

Testing can be accomplished through the use of the TST or either IGRA, but none of these tests can currently be preferred to the others in all situations. Comparisons of testing using the IGRAs with testing using the TST resulted in findings of similar sensitivities and specificities. This was true using universal or targeted testing approaches. Costs and effectiveness are also very similar between the three diagnostic tests, and the small differences seen in cost-effectiveness were sensitive to small changes in baseline parameter assumptions.

Finally, future studies should consider use of the methodology in this study to allow for a more robust analysis of the causes of discordance. This study provides the most complete, comprehensive methodology in the understanding and interpretation of commercially-available LTBI diagnostics. Future studies of TB diagnostics in other populations should also include these methods, including evaluation of effectiveness of the three diagnostic tools, risk factor analysis, assessment of NTM as a source of cross-reactivity, and the analysis of TST and IGRA discordance.

Military Recommendations

Targeted Testing

The current service-specific policies for LTBI testing at accession are shown in Table 33, along with the recommended policy. Since 2000, targeted testing has been the recommended approach to diagnosing and treating LTBI in the US, with the CDC stating that "…targeted tuberculin testing programs should be conducted only among groups at high risk and discouraged in those at low risk [16]." The TST has an estimated sensitivity of 77% and specificity of 97% [21], although the estimate of specificity among Army recruits obtained in this study was higher (99%). False positive TSTs may result from exposure to cross-reactive or non-tuberculous mycobacteria (NTM), BCG vaccination, poor TST administration reading and documentation, and other factors [22]. Because the use of the TST results in a high proportion of false positives in the low prevalence setting of the US military (>50%), targeted testing is indicated. The CDC recommends targeted testing using a risk stratified interpretation of the TST using the 5, 10, and 15 mm criteria [16]. This adjusts the sensitivity and specificity of the test according to the pre-test probability in order to maximize predictive accuracy, thus reducing false positives. The military should continue to use these criteria as well, but should only be testing groups that would fall into the 5 and 10 mm criteria groups which have definable risks for infection.

Estimates of sensitivity for the IGRAs range from 70-90%, and estimates of specificity range from 93 to 99% [21], similar to the TST. Again, in this study, the estimates of IGRA specificity among Army recruits were at the higher end of this range (99%). The

IGRAs are generally thought to have better specificity in BCG-vaccinated populations and populations sensitized to NTM [21, 23], as also suggested by this study. However, as their net specificity was similar to the TST, they also suffer from the same problems with poor PPV in low prevalence populations as does the TST. ***Therefore, only targeted testing should be performed in US military populations using the IGRAs, as with the TST.*** Also similar to the TST, IGRA results should be reported quantitatively rather than simply as positive or negative [24], as all three tests should be interpreted more cautiously near the cutoff points.

The military should generally be considered a low risk population. There may, however, be special subpopulations within the military at higher risk for TB, including health care personnel, prison guards, and those born in TB endemic countries. These recommendations for targeted testing should not affect or change testing in these groups. Military policies for these and other specific high-risk groups should conform to CDC recommendations for testing and treatment.

The Importance of Risk Factors at Accession

Deployment risks should be continuously evaluated, but they were not found to be a risk factor for active TB in this analysis. However, a substantial amount of heterogeneity in TB exposure occurs, and individual patients may have substantial exposures to TB from deployment which place them at risk for latent and active TB. Therefore, assessment of TB risk from exposure should continue to be individualized to mitigate these risks, however rare.

In contrast, birth in a TB-endemic country and other risk factors existing prior to accession were much stronger risk factors for developing active TB during military service, resulting in a much larger burden of disease. In the US military, activation of LTBI present at accession leads to the greatest burden of disease. Therefore, increased emphasis and focus is warranted on the screening of recruits as an opportunity for prevention, including quality of testing, adherence to therapy, and other factors. Therapy for LTBI should be given and well-documented during basic training for all those found to be infected in the targeted TB testing program.

Which test to use: TST or IGRA?

The sensitivity and specificity of the TST and IGRAs are considered to be similar [21], although different individuals are often identified as positive by each test [18, 27], as was also seen in this study. Although the IGRAs are commonly thought to be better than TST in BCG vaccinated populations and populations sensitized to NTM, in practice very little difference is seen between the TST and IGRAs [17]. The pros and cons of other characteristics of the TST and IGRAs are compared in Table 34. Although the cost of the IGRAs is higher from the health care perspective, typically about $50 as compared to $2, there are indirect cost advantages to the IGRA. The most important indirect cost savings are from time saved by the patient not requiring a second visit, as is required to read the TST. There are also theoretical savings possible from having a more specific test in reducing the amount of unnecessary follow-up and treatment of false positives. However, since the overall specificities of the TST and IGRAs are similar in military

populations, these savings may not be realized in practice. Similarly, the IGRAs have advantages in logistics and quality assurance from the perspective of the clinical staff by reducing the workload and need for proper training and oversight required for a good quality TST screening program. However, the IGRAs also have disadvantages in both of these areas from the laboratory perspective from increasing workload and complexity, largely transferring the burden of screening to the laboratory. After considering all these aspects, it is difficult to recommend one of these tests or the other in all situations. All three commercially-available tests are approved by the Food and Drug Administration (FDA) and are considered acceptable for use in diagnosing LTBI by the CDC [24]. Therefore, the decision to use a particular test should be based on the testing circumstances and capabilities at each location.

Finally, other countries such as the United Kingdom and other countries use a sequential testing method, testing first with TST and then confirming positives with an IGRA [28, 29]. This approach is advocated in order to reduce false positives thought to be likely among BCG vaccinated populations in low-risk European countries. However, this approach is discouraged by the CDC [24] and not recommended by the author. As the US has never used BCG vaccination as a national policy, those with BCG are largely from high-risk, TB endemic countries that are by definition at higher risk for LTBI. Use of sequential testing in a US population results in increased cost, logistical burden, and results in lower sensitivity than either test alone.

The TST, T-Spot, and QFT all had similar estimates of costs and effectiveness in this study. It is reasonable to use any of these tests in a low-risk population where the prevalence of LTBI is low, as most positives from any of the diagnostic tests are likely to be false positives. Similar conclusions were found in recently updated CDC guidelines on the use of IGRAs [24]. The convenience of the IGRAs may make them most desirable for patients and providers, as well as the potential for reduction in false positives from cross-reactivity due to BCG and NTM. However, the high proportion of discordance seen between the IGRAs and the TST among higher risk recruits tempers the enthusiasm for the IGRAs, as this may also be due to poor IGRA sensitivity. Furthermore, the low concordance between the three tests suggests that using any of the tests in a low prevalence population will result in the majority of tests (>50%) of positives identified being false positives, as has been previously demonstrated [12, 13]. Thus, ultimately the choice of which test to use will depend on local laboratory, clinical, and logistic considerations, as well as patient population characteristics. Therefore, testing with any of these tests should be permitted and none of these tests preferred over the others. Continuous evaluation and re-evaluation of the data supporting the effectiveness and costs of these and other new TB diagnostics should be undertaken in order to ensure the best protection of our military service members.

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Table 33. Current service-specific and recommended military practices for tuberculosis control

Accession Testing	***Army***	***Navy***	***Air Force***	***Recommended***
Who tested	Universal	Universal	Universal	***Targeted***
Test used	TST	TST*	TST	***TST or IGRA***
Treated	No	Yes	No	***Yes***

* 10-14 mm TST reactions are confirmed by IGRA

IGRA=interferon-gamma release assay; TST= tuberculin skin test

Table 34. Pros and Cons of TST and IGRA Use in Military Populations

Attribute	*TST*	*IGRA*
Costs		
Direct	+	
Indirect		+
Test Characteristic		
Sensitivity	+	
Specificity		+
Logistics		
Patient/Soldier		+
Clinical requirements		+
Laboratory requirements	+	
Quality assurance and reliability		
Clinical requirements		+
Laboratory requirements	+	

TST=tuberculin skin test

IGRA=interferon-gamma release assay

+ = test advantage