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Interferon Gamma Release Assay Result is Associated with Site of Disease and Death in Active Tuberculosis

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ABSTRACT

Interferon Gamma Release Assay Result is Associated with Site of Disease and Death in Active Tuberculosis

By Sara Cooper Auld

Introduction: The interferon gamma release assays (IGRAs) and tuberculin skin test (TST) are used to diagnose latent tuberculosis (TB) infection and support a diagnosis of active TB. We previously demonstrated that a negative TST in active TB is associated with disseminated disease and death. It is unknown whether the same associations exist for IGRAs and so we sought to determine the association between IGRA results and site of disease or death among persons with active TB disease.

Methods: We analyzed IGRA and TST results for all persons with culture-confirmed TB reported to the US National Tuberculosis Surveillance System from 2010–2014. We used logistic regression to calculate the association between IGRA or TST results and site of disease or death.

Results: Among 46,762 reported TB cases, 33,384 (71%) persons had a documented site of culture-confirmed disease and 24,803 (53%) had an IGRA or TST result documented. Compared to persons with a negative test result, those with a positive TST had lower odds of disseminated disease (adjusted odds ratio [aOR] and 95% confidence interval [CI] 0.33 [0.27, 0.39] for miliary; 0.57 [0.49, 0.65] for combined pulmonary/extrapulmonary). There was no difference in the odds of disseminated disease with a positive IGRA. However, persons with a positive TST or positive IGRA had lower odds of death after starting TB treatment (aOR [95% CI] 0.32 [0.27, 0.38] for TST; 0.66 [0.54, 0.80] for IGRA). An indeterminate IGRA was associated with greater odds of both disseminated disease and death as compared to a negative result (aOR [95% CI] 1.98 [1.36, 2.87] for miliary; 1.47 [1.11, 1.96] for combined pulmonary/extrapulmonary; 1.46 [1.09, 1.95] for death after starting TB treatment).

Conclusions: Despite perceived equivalence in clinical practice, IGRA and TST results have different associations with TB site of disease yet similar associations with risk of death. Furthermore, an indeterminate IGRA result in a person with active TB disease is not an unimportant result, and rather carries greater odds of disseminated disease and death. Prospective study may improve our understanding of the underlying mechanisms by which the TST and IGRA are associated with disease localization and death.

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INTRODUCTION

While the tuberculin skin test (TST) and interferon gamma release assays (IGRAs) can both be used to diagnose latent tuberculosis (TB) infection and to aid in the diagnosis of active TB disease, their significance as a prognostic indicator among patients with active TB disease has not been well explored. Current guidelines suggest that for the majority of patients these tests can be used interchangeably (1), yet there are important distinctions to keep in mind with these immunologic assays. A positive TST result represents an *in vivo* delayed-type hypersensitivity response of host memory T cells sensitized by prior mycobacterial exposure, whether to *Mycobacterium tuberculosis* (*Mtb*) complex (including bacillus Calmette-Guérin [BCG] vaccination) or nontuberculous mycobacteria. The TST reaction involves multiple cell types including lymphocytes, basophils, monocytes, and neutrophils, as well as local cytokine release, vasodilation, and edema (2-4). Meanwhile, a positive IGRA result, consisting of an *in vitro* interferon gamma (IFN-γ) release in response to specific antigens, detects prior sensitization to *Mtb* and will not cross react with BCG or most nontuberculous mycobacteria (5). IGRA testing can also yield an indeterminate result if there is failure of either the positive control (i.e., lack of response to mitogen) or negative control (i.e., high baseline levels of IFN-γ). An indeterminate IGRA result is felt to represent an uncertain likelihood of *Mtb* infection.

In order to investigate whether these reported differences in clinical disease by TST result were present for active TB disease, we previously analyzed the association between TST result and clinical characteristics of TB disease using US national surveillance data. We found that persons with a positive TST had lower odds of disseminated disease, even after adjusting for HIV status, birthplace, age, and sex (6). We also found that persons with a positive TST had lower odds of death after initiating TB treatment (7). Taken together, these findings further reinforce that a positive TST may be more than simply a marker of infection and may be directly relevant to the clinical presentation and prognosis of active TB disease.

Since the publication of our previous analysis, IGRA results were added to the US surveillance data and are now available for analysis. As there have been only limited reports to date on IGRA results among persons with active TB disease (8-10), we wanted to determine whether the significant associations that we previously observed between the TST result and active TB disease would hold true for the IGRAs. We also wanted to explore the significance of an indeterminate IGRA result in the setting of active TB, particularly as there is no analogous TST result category.

BACKGROUND

TST and IGRAs as markers of infection

In 1890 Robert Koch announced he had discovered a cure for tuberculosis: tuberculin. His theory of a cure was soon disproven yet the tuberculin skin test (TST) has become one of the most widely used diagnostic tests in the world (2). Since 1904, the TST has been used to aid in the diagnosis of tuberculosis disease and to identify individuals with latent tuberculosis infection who might benefit from treatment to prevent progression to TB disease (2, 11). A positive TST result, consisting of measurable skin induration at the site of tuberculin injection, is part of a delayed-type hypersensitivity response of T cells sensitized by prior TB infection (12). However, as a result of cross-reactivity between the tuberculin purified protein derivative (PPD), many nontuberculous mycobacteria, and the bacilli-Calmette Guérin (BCG) vaccine which continues to be administered to newborns in much of the developing world, the specificity of the TST is also quite limited in certain populations.

Given these limitations of the TST, there was great hope for an improved test with the release of the first interferon gamma release assay (IGRA) in 2001 (1). The IGRAs take advantage of the well characterized release of interferon-gamma (IFN-γ) by T cells in response to tuberculosis infection. These assays, of which there are currently four that are FDA-approved, QuantiFERON-TB, QuantiFERON-TB GOLD, QuantiFERON-TB Gold In-Tube, and T-SPOT.*TB*, measure *in vitro* IFN-γ release in response to a panel of tuberculosis antigens and will not cross react with BCG or most nontuberculous mycobacteria. When blood from persons exposed to tuberculosis are mixed with the tuberculosis antigens, their T cells produce IFN-γ and the test is interpreted as positive. IGRA testing can also yield an indeterminate result if there is failure of either the positive control (i.e., lack of response to mitogen) or negative control (i.e., high baseline levels of IFN-γ). Additional reasons for an indeterminate test include test performance issues such as under filling of tubes and processing delays. An indeterminate IGRA result, for which there is no analogous TST result, is felt to represent an uncertain likelihood of

M. tuberculosis infection. Additionally, it has been noted that IGRA responses can fluctuate over time with serial testing (13, 14). The clinical significance of these conversions and reversions remains unclear and could represent fluctuations in the test performance or in the actual biological response of individuals exposed to tuberculosis (15). Ultimately, as with the TST, the IGRAs are not directly measuring tuberculosis infection, but rather are measuring an indirect immune response to proteins found in tuberculosis.

TST and IGRAs and active TB disease

Despite its longstanding and widespread use, the TST remains an imperfect marker of TB infection. Previous studies have found that anywhere from 10–25% of patients with microbiologically-confirmed, active TB do not respond to tuberculin and have a negative TST result (2, 11, 16, 17). More recent studies of the IGRAs have had similar findings, with the test sensitivity ranging from 80-90% (18-22). Thus, in these individuals the negative TST or negative IGRA result does not correspond to microbiologically confirmed *M. tuberculosis* infection. Patients with immature or suppressed immune systems, including children, HIV-infected persons, and the elderly are among those who are more likely to have a negative TST or IGRA result in the setting of active TB disease (10, 23-25).

An indigenous population in the Brazilian Amazon provides yet another example of the complex relationship between our current tests for TB and the clinical presentation of disease. The Yanomani Indians have extraordinarily high rates of active tuberculosis but nearly half of those with active disease have a negative TST. These Indians also tend to have more severe disease and higher mortality from TB (26). In another cohort, Cambodian TB patients with a negative TST were found to have a decreased proliferative T cell response to PPD and poor clinical outcomes (27). Other studies have also documented functional differences in the cytokine profiles of TB patients who are TST-negative and TST-positive that are likely to reflect clinically meaningful differences in these groups of patients (27-30).

TST and IGRAs as windows into the host-pathogen interaction

There is increasing evidence that the immunologic processes underlying these assays may translate into biologically meaningful distinctions in the context of clinical disease. It has long been recognized that patients with an immature or suppressed immune system, the same patients more likely to have a negative TST or IGRA result, often have faster disease progression and more disseminated disease (23, 24, 31). A case series focused on the clinical manifestations of tuberculosis found that 69% of patients with meningitis or miliary TB had a negative TST (32). High rates of disseminated TB and poor clinical outcomes among patients with IFN-γ receptor mutations and other forms of inherited susceptibility to mycobacterial disease underscore the importance of cellular immune function, the basis of the TST and IGRA, in the successful treatment of TB (31, 33, 34).

A study of isoniazid preventive therapy (IPT) among people with HIV infection in Botswana found that extended prophylaxis was beneficial primarily for persons with a positive TST at baseline; IPT did not decrease the incidence of TB among those with a negative TST (35). Furthermore, among those given extended IPT TB incidence was higher for TST-negative persons than for TST-positive persons. The reason for this difference in the protective effect of IPT by TST result is not clear but suggests that the natural history of TB infection and response to therapy may be different for persons with a positive TST as compared to a negative TST.

Additionally, several recent studies suggest that *Mycobacterium tuberculosis* (*M. tuberculosis*) benefits from a more robust immune response and postulate that active engagement of *M. tuberculosis* with the human immune system favors cavity formation thereby increasing the likelihood of subsequent aerosol transmission (36-39). The TST was found to correlate with pulmonary cavitation in a rabbit model (40), but a previous study in humans did not find a relationship between TST and chest radiograph findings (41). These studies suggest a complex

interaction between *M. tuberculosis* and the host immune system that results in different disease manifestations and potential for transmission.

Previous analysis of TST and clinical characteristics of disease

While TST reactivity has long been recognized as an indicator of TB infection following exposure to persons with TB disease and had been widely studied in the context of latent TB infection,(42) we were not aware of any large studies that described the pattern of TST results among persons with different clinical presentations of active TB disease. Since the TST result and clinical manifestations of disease both depend on the host immune system, we previously analyzed the association between TST result and clinical characteristics of TB disease using US national surveillance data. In our initial analysis of the TST, we found that persons with a positive TST had lower odds of disseminated disease, i.e., both pulmonary and extrapulmonary disease or miliary disease, even after adjusting for the important clinical covariates of HIV status, birthplace, age, and sex (6). We also found that persons with a positive TST had lower odds of death after initiating tuberculosis treatment (7). Taken together, these findings further reinforce that a positive TST may be more than simply a marker of infection and may be directly relevant to the clinical presentation and prognosis of active tuberculosis

Since the publication of our previous analysis, IGRA results were added to the US surveillance data and are now available for analysis. Current guidelines suggest that for the majority of patients the TST and IGRA tests can be used interchangeably (1), yet it is unknown if the underlying differences in these indirect immune assays translate into meaningful differences in their relation to active disease . As there have been only limited reports to date on IGRA results among persons with active tuberculosis (8-10), we wanted to determine whether the significant associations that we previously observed between the TST result and active tuberculosis would hold true for the IGRAs. We also wanted to explore the significance of an indeterminate IGRA

result in the setting of active tuberculosis, particularly as there is no analogous TST result category.

METHODS

The main objective of this study was to determine whether there was an association between IGRA result and clinical manifestations of active TB disease, specifically the site of TB disease, while controlling for other risk factors. An additional goal was to determine whether there was an association between IGRA result and death in the setting of TB disease.

Null Hypothesis

A positive or indeterminate IGRA result is not associated with increased odds of disseminated TB disease as compared to a negative IGRA result.

Alternative hypothesis

A positive or indeterminate IGRA result is associated with increased odds of disseminated TB disease as compared to a negative IGRA result.

Study Design and Population

This analysis was a retrospective cohort study including persons with culture-confirmed TB reported to the US National Tuberculosis Surveillance System (NTSS) during January 1, 2010 through December 31, 2014. The NTSS collects annual TB case information from 60 reporting areas including all 50 states and the District of Columbia. NTSS data include demographic, clinical, and risk factor information for all reported cases of TB. These data are collected by state and local TB programs and submitted electronically to the Centers for Disease Control and Prevention (CDC). The IGRA variable was added to the national TB case report forms in January 2009 and all states were required to report using the revised forms by 2010. Of note, reports from California from 2010 were excluded from this analysis because HIV status was not routinely reported from that jurisdiction in that year (43).

In our main analysis, we included all persons with culture-confirmed TB who also had (1) either a documented IGRA or TST result and (2) a documented site of disease.

Measurements and Definitions

For the primary analysis, IGRA and TST results were the independent predictor variables and site of disease was the dependent outcome variable. IGRA results were classified as negative, positive, or indeterminate as documented in the NTSS case report forms. The TST result is documented in millimeters of induration. However, for our analysis, we classified a TST result of 0–4 mm as negative and a result ≥ 5 mm was classified as positive. (Although CDC guidelines for diagnosing latent TB infection stipulate differential interpretation of the TST result based on patient risk factors (42), we previously determined that any result ≥ 5 mm could be classified as positive when analyzing cases of active TB disease (6).)

Site of TB disease was defined as one of the following mutually exclusive categories: miliary disease, combined pulmonary and extrapulmonary disease, extrapulmonary only disease, and pulmonary only disease. Pulmonary only disease was further divided into noncavitary pulmonary disease and cavitary pulmonary disease on the basis of the initial chest radiograph or CT scan. Of note, a designation of miliary disease was based on either clinical impression or a miliary radiographic pattern on either chest radiograph or CT scan. Disease that was either miliary or combined pulmonary and extrapulmonary was considered to be disseminated.

For our secondary analysis of the association between IGRA and TST results and death, we looked at two different times of death: (1) death at the time of TB diagnosis, which is designated as vital status at the time of diagnosis, and; (2) death after initiating TB treatment, which is included as a reason for which therapy was stopped on the standard report forms. The dates on which therapy was started and stopped are also documented and were used to calculate the interval between treatment initiation and death.

Data Analysis

We first compared the sociodemographic and case characteristics for persons with and without documented IGRA or TST results using Pearson's chi-square statistic. A p-value < 0.05 was considered significant for this and subsequent comparisons. This comparison allowed us to determine whether there were case characteristics associated with having had a particular test, i.e., IGRA, TST, or both IGRA and TST, performed and documented, and whether the cohort of patients with a documented test result were representative of all TB cases.

Descriptive statistics were then used to compare case characteristics by IGRA or TST results (i.e., positive, negative, indeterminate). Pearson's chi-square statistic was used to assess differences in the distribution of IGRA or TST results by these characteristics.

We next examined the association between IGRA or TST result and site of TB disease using multinomial logistic regression to calculate odds ratios and 95% confidence intervals. For the multinomial analysis, noncavitary pulmonary disease was selected as the referent outcome category both because it was the largest site of disease category and because it was felt to represent a clinically useful comparator group as a middle ground between disseminated disease and cavitary pulmonary disease. A negative IGRA (or TST) was used as the referent predictor category. Thus, persons with noncavitary pulmonary disease with a negative IGRA (or TST) served as the comparison group to calculate odds ratios and 95% confidence intervals for each of the respective site of disease/IGRA (or TST) result category combinations (e.g., miliary disease with an indeterminate IGRA was compared to noncavitary pulmonary disease with a negative IGRA, and cavitary pulmonary disease with a positive IGRA was also compared to noncavitary pulmonary disease with a negative IGRA). We also conducted a sensitivity analysis with TST cutoffs of 10 and 15 mm and found no appreciable change in the results (data not shown).

As the NTSS data set is well established and rigorously maintained, there is not a significant amount of missing data. However, for model covariates that did have a missing value,

we chose to classify those values as unknown since persons with undocumented values might differ in some meaningful way from those with documented values.

With regards to model building and variable selection, we considered the following covariates for inclusion: sex, age, race and ethnicity (self-designated), HIV status, birthplace, incarceration at the time of diagnosis, homelessness in the 12 months prior to diagnosis, and excessive alcohol or illicit drug use in the 12 months prior to diagnosis. Based on our previous analyses, clinical relevance, and scientific judgment, we had an *a priori* interest in HIV status, birthplace, age, and sex and so these variables were forced into the model. Subsequent addition of the remaining covariates did not appreciably impact the point estimates (i.e., by $\geq 20\%$) and so no further covariates were included in the preliminary model. We then evaluated for interaction between each of the model terms and TB site of disease by sequentially adding interaction terms to the model one by one; no significant interaction was found. We also analyzed IGRA result by the test type, i.e., any QuantiFERON (QFT®) assay versus T-SPOT®.TB, and did not find evidence of confounding or interaction and so test type was not included in the models.

We next determined the association between death and IGRA or TST results. We used multivariate logistic regression to determine the association between IGRA or TST results and (1) death at the time of TB diagnosis and (2) death after starting TB treatment. As before, IGRA or TST results were the independent predictor variables, and death was the dependent outcome variable. In order to account for severity of TB disease, we included site of disease as an additional covariate in both models. Additionally, for the model looking at death after starting TB treatment, in the interest of comparing groups with similar drug susceptibility patterns and treatment histories we also limited the analysis to persons with (1) drug-susceptible TB (i.e., no documented resistance to any of the standard first line drugs [isoniazid, rifampin, pyrazinamide, and ethambutol]) with no prior history of TB who were started on standard four-drug first line therapy; (2) isoniazid monoresistant TB (i.e., documented resistance to isoniazid but rifampin-susceptible); or (3) multidrug-resistant (MDR) TB (resistance to at least rifampin and isoniazid).

Drug susceptibility class was then also included in the model, in addition to HIV status, birthplace, age, sex, and site of disease.

Finally, Kaplan-Meier survival curves for the probability of survival following treatment initiation were constructed and compared using the Wilcoxon test. All persons who initiated TB treatment were included. Further, all observations were censored at 180 days as the majority of deaths occurred in the first several months of treatment and deaths occurring later would be more likely to reflect heterogeneity in treatment and clinical care. All analyses were conducted using R statistical software (44).

Ethics

Approval by an institutional review board was not required because data were collected and analyzed for this project as part of routine TB surveillance, and the project is therefore not considered research involving human subjects.

RESULTS

Of 46,762 TB cases reported in the United States during 2010 through 2014, 35,447 (76%) were culture-confirmed, 33,384 (94%) had a documented site of TB disease, and 24,811 (74%) had either an IGRA or TST result reported and were eligible for inclusion in the study (Figure 1). There were 11,393 persons with an IGRA result and 15,019 persons with a TST result reported; 3,397 persons had both an IGRA and a TST result reported, and these individuals were analyzed independently for each test result.

Case characteristics by availability of IGRA or TST result

Among persons with culture-confirmed tuberculosis and a documented site of disease, there were significant differences in sociodemographic and clinical characteristics according to which test was documented, i.e., IGRA, TST, or neither (Table 1). Notably, a greater proportion of US-born persons had only a TST result reported, and a greater proportion of foreign-born persons had only an IGRA result reported. A greater proportion of children (i.e., < 15 years) had a TST result or both an IGRA and a TST result documented. Persons with an unknown or positive HIV status had the greatest proportion of not having had either test result documented. Death before or during treatment was also more common among those without a test result.

Case characteristics by IGRA result

Among persons with a documented IGRA result, 9,232 (81%) had a positive IGRA, 1,520 (13%) had a negative IGRA, and 641 (6%) had an indeterminate IGRA (Table 2). Negative and indeterminate IGRAs were more common among those older than 65 years, infected with HIV or with unknown HIV status, and with either miliary or combined pulmonary and extrapulmonary disease. A positive IGRA was more common among those younger than 45 years, and a lower proportion of persons between the ages of 5 and 44 had an indeterminate IGRA. Non-Hispanic whites and US-born persons had lower proportions of a positive IGRA.

There were no noteworthy differences in the distribution of IGRA results according to sex or baseline sputum smear result. A greater proportion of those with a negative or indeterminate result died before or during treatment.

Case characteristics by TST result

Among persons with a documented TST result, 11,678 (78%) had a positive TST and 3,341 (22%) had a negative TST (Table 3). As with the IGRA results, a negative TST was more common among those older than 65 years, infected with HIV or with unknown HIV status, and with either miliary or combined pulmonary and extrapulmonary disease. A positive TST was more common among those younger than 45 years, females, Asians, and foreign-born.

Association between IGRA result, TST result, and site of TB disease

For the analysis of IGRA or TST and site of TB disease, we first confirmed the associations between TST and disseminated disease (i.e., miliary or combined pulmonary and extrapulmonary disease) identified in our previous analysis (6). We found that persons with a positive TST had 67% lower odds of miliary disease (adjusted odds ratio [aOR] 0.33 [95% confidence interval [CI] 0.27, 0.39]) and 43% lower odds of combined pulmonary and extrapulmonary disease (aOR 0.57 [95% CI 0.49, 0.65]) than those with a negative TST (Table 2 and Figure 2a). There was no association between a positive TST and either extrapulmonary only disease or cavitary pulmonary disease. Next, in examining IGRA result and site of TB disease, we found no association between a positive IGRA and site of disease when compared to a negative IGRA. However, an indeterminate IGRA was significantly associated with site of disease such that persons with an indeterminate IGRA had nearly two-fold greater odds of miliary disease (aOR 1.98 [95% CI 1.36, 2.87]) and nearly 50% greater odds of combined pulmonary and extrapulmonary disease (aOR 1.47 [95% CI 1.11, 1.96]) than those with a negative IGRA.

Association between IGRA result, TST result, and death

Among 11,393 persons with an IGRA result, 113 (1%) were dead at the time of TB diagnosis and 732 (8%) died after starting treatment (Figure 1). Similarly, among 15,010 persons with a TST result, 139 (1%) were dead at the time of diagnosis and 702 (6%) died after starting treatment. Persons with a positive TST had 75% lower odds of death at the time of diagnosis (aOR 0.25 [95% CI 0.17, 0.36]) (Table 3, Figure 2b) and 68% lower odds of death after initiating treatment than those with a negative TST (aOR 0.32 [95% CI 0.27, 0.38]). A similar pattern was observed with IGRA where persons with a positive IGRA had 66% lower odds of death at diagnosis than those with a negative IGRA (aOR 0.34 [95% CI 0.17, 0.73]) and 34% lower odds of death than those with a negative IGRA (aOR 0.66 [95% CI 0.54, 0.80]). Persons with an indeterminate IGRA had an adjusted odds of death at diagnosis that was 3.85 (95% CI 1.78, 8.62) times greater than those with a negative IGRA and 11.18 (95% CI 5.76, 21.53) times greater than those with a positive IGRA. An indeterminate IGRA was also associated with a 46% increase in the odds of death after starting treatment as compared to a negative IGRA (aOR 1.46 [95% CI 1.09, 1.95]) (Table 3, Figure 2c).

Kaplan-Meier survival curves depict the worse survival for persons with a negative TST, negative IGRA, or indeterminate IGRA as compared to persons with a positive test (p-value < 0.001 for all comparisons) (Figure 3).

DISCUSSION

In this comprehensive cohort of all persons with culture-confirmed active TB disease reported in the US between 2010 and 2014, we were surprised to find different associations for TST and IGRA results and TB disease. While a positive TST was significantly protective against disseminated TB disease, there was no association between a positive IGRA and site of disease. However, both a positive TST and a positive IGRA were associated with decreased odds of death. These findings contrasted with our initial hypothesis that the TST and IGRA would have similar patterns of association for persons with active TB disease. Additionally, an indeterminate IGRA result was significantly associated with both site of disease and with an increased odds of death as compared to a negative IGRA.

It is not clear why there would be inconsistent associations between these two assays and site of disease. Given that IGRA positivity is not associated with site of disease, our findings suggest that the narrow, antigen-specific T-cell response represented by a positive IGRA is not important for disease localization. This finding should not be wholly unexpected since the antigens included in the IGRAs, CFP-10, ESAT-6 (and TB7.7 in the newest QuantiFERON-TB Gold In-Tube assay), were selected for their sensitivity and specificity for *Mtb* and not because a T-cell response to these antigens is necessarily relevant to disease pathogenesis (45). In contrast, the TST response includes many cell types responding to a broader mix of antigens that may represent a more physiologic and coordinated response comparable to an *in vivo* infection. A study of household contacts of persons with pulmonary TB found different regulatory T cell subsets for contacts with a positive TST and IGRA as opposed to persons with a positive TST and a negative IGRA (46). Such reports, in combination with our data, reinforce that the TST and IGRA measure distinct immunological processes, despite their relative equivalence in routine clinical practice (1).

We found that both a positive TST and a positive IGRA were associated with an approximately 70% lower odds of death at the time of diagnosis and a 40-70% lower odds of

death among persons who initiated TB treatment. An indeterminate IGRA was associated with the greatest odds of death, with more than a three-fold increase in the odds of death at the time of diagnosis and a nearly 50% increase in the odds of death after starting treatment as compared to persons with a negative IGRA. The reason for these increased odds of death seen with a negative TST or a negative or indeterminate IGRA cannot be discerned from these surveillance data.

One possibility is that the inability to generate either an *in vitro* IFN-γ response or an *in vivo* TST response is associated with death. There are several smaller studies of children, persons with HIV, and immunocompetent adults where a negative TST in the setting of active disease has been associated with an increased risk of death (30, 47, 48). The risk of death associated with a negative TST at the time of disease is also evident in a native Brazilian population with high rates of anergy (46%), severe clinical manifestations, and high mortality from TB disease (26).

The benefit of having a positive TST at the time of disease may also have parallels with the effects of BCG vaccination in early childhood. In general, children who have been vaccinated show a transient positive TST response and are less likely to develop disseminated forms of disease, and are less likely to die if they do develop TB disease (49, 50). Our results suggest that the host immune response in the setting of active TB disease that is measured by a TST might be similar to the immune response triggered by BCG vaccination in children and potentially mitigates against death.

Another possibility for the association between negative or indeterminate tests and death is that negative or indeterminate results could contribute to diagnostic delays if they are mistakenly interpreted as definitive evidence against a TB diagnosis. Previous studies have found that atypical features of TB disease, such as smear-negative or extrapulmonary disease, are indeed associated with diagnostic delays and death prior to treatment (51, 52). It is plausible that a negative (or indeterminate) TST or IGRA result could have a similar effect.

In considering our findings about the associations for indeterminate IGRA results, it is important to keep in mind that an indeterminate IGRA result can come either from a failure of the

positive control with lack of response to the mitogen (positive control) or from a failure of the negative control due to high circulating levels of IFN-γ (1, 53). Unfortunately, the IGRA results are reported in the NTSS with only the qualitative categories of positive, negative, and indeterminate and the quantitative results for the negative control, mitogen, and TB response are not collected. Thus, we were unable to determine what proportion of indeterminate results in this study population are due to failure of the positive versus negative controls. Failure of the positive control can be considered analogous to TST anergy seen among immunosuppressed patients and has been reported among persons infected with HIV, lymphopenic patients, and elderly patients (9, 10, 54-59). Meanwhile, failure of the negative control attributable to elevated IFN-γ levels might be hypothesized to arise in the setting of overwhelming systemic inflammation that can be seen with disseminated disease. Failure of the negative control has been previously reported among patients with miliary disease, even in the presence of lymphopenia (60). Prospective studies with quantitative IGRA results could confirm whether failure of the positive control, negative control, or both, are driving the associations with disseminated disease and death that we observed in our study.

There are several limitations to our study. Although there were 49,949 cases of TB reported during the study period, an IGRA result was only documented for 34% of those cases. However, we were still able to analyze a very large study population of nearly 25,000 persons, including more than 11,000 persons with an IGRA result, which we believe is the largest cohort to date for IGRA results among persons with active TB disease. Additionally, while there were differences between the sociodemographic and clinical characteristics of those who had a TST or an IGRA test performed, most notably that US-born persons were more likely to have a TST test and foreign-born persons were more likely to have an IGRA test, we attempted to address these differences in our statistical models, but the possibility of uncontrolled confounding remains.

We also do not have data on CD4 count or antiretroviral therapy and so we are unable to account for differences in the extent of immunosuppression among persons with HIV-infection.

Nor were we able to account for the role of host and mycobacterial genetics in our analysis, both of which have been shown to influence TST result and clinical presentation (61-66). One recent report identified a single gene locus that corresponds to the size of a TST response, and it would be interesting to explore whether this locus also impacts localization of disease. (67)

In summary, although the ability to mount a positive TST response or generate a positive IGRA result are both protective against death among persons with active TB disease, a positive TST is also protective against disseminated TB disease but a positive IGRA is not. Thus, while the TST and IGRA are considered largely interchangeable for screening purposes, these indirect immune assays are not equivalent in their associations with site of disease. Additionally, although an indeterminate IGRA does not help to confirm a diagnosis of either latent TB infection or active TB disease, our findings indicate that an indeterminate IGRA can be a very meaningful result. In the context of active TB disease, an indeterminate IGRA can identify persons with an increased risk of disseminated disease and death. It is important for clinicians to be aware of the negative prognostic significance of a negative TST, negative IGRA, or indeterminate IGRA even if the underlying immune responses driving these different associations remain unclear. The differences in site of disease by IGRA and TST result that we have described may indicate that these result can be a useful adjunct for identifying patients with different underlying immune system engagement with TB. These differences may predict different response to vaccine candidates, different biomarker profiles, or even differential response to TB treatment. Future prospective studies of the TST and IGRA among patients with active TB could help to better define the immune response and biomarkers associated with these assays. Further understanding of the underlying biology represented by these different assays may lead to greater insight into the complex relationship between host and pathogen that results in TB infection, disease, localization, and either cure or death.

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TABLES/FIGURES

Table 1. Comparison of culture-confirmed TB cases reported in the United States from 2010 through 2014 by availability of IGRA and/or TST results.

	Total	Only IGRA result reported (n, %)	Only TST result reported (n, %)	Both IGRA and TST results reported (n, %)	Neither IGRA nor TST result reported (n, %)
Total	33,384	7,996 (24)	13,418 (40)	3,397 (10)	8,573 (26)
Age					
0–4	391 (0)	30 (0)	249 (2)	69 (2)	43 (1)
5–14	325 (1)	43 (1)	182 (1)	71 (2)	29 (0)
15–24	3,633 (10)	785 (10)	1,788 (13)	498 (15)	562 (7)
25–44	10,670 (32)	2,539 (32)	4,709 (35)	1,123 (33)	2,299 (27)
45–64	10,466 (31)	2,516 (31)	4,083 (30)	998 (29)	2,869 (33)
65+	7,898 (26)	2,083 (26)	2,406 (18)	638 (19)	2,771 (32)
Sex					
Male	20,833 (61)	4,896 (61)	8,478 (63)	2,046 (60)	5,413 (63)
Female	12,543 (39)	3,100 (39)	4,933 (37)	1,351 (40)	3,159 (37)
Race/ethnicity[†]					
Hispanic	9,365 (27)	2,186 (27)	3,995 (30)	964 (28)	2,220 (26)
American Indian	470 (1)	49 (1)	208 (2)	40 (1)	173 (2)
Asian	9,655 (34)	2,723 (34)	3,334 (25)	1,019 (30)	2,579 (30)
Black	7,298 (21)	1,662 (21)	2,990 (22)	804 (24)	1,842 (21)
Native Hawaiian	966 (1)	59 (1)	750 (6)	28 (1)	129 (2)
White	5,124 (15)	1,175 (15)	1,967 (15)	486 (14)	1,496 (17)
Birthplace					
US-born	12,035 (30)	2,390 (30)	5,225 (39)	1,230 (36)	3,190 (37)
Foreign-born	21,301 (70)	5,600 (70)	8,173 (61)	2,167 (64)	5,361 (63)
HIV status					
Negative	27,020 (81)	6,681 (84)	11,265 (84)	2,983 (88)	6,091 (71)
Positive	2,023 (7)	520 (7)	672 (5)	154 (5)	677 (8)
Unknown	4,341 (13)	795 (10)	1,481 (11)	260 (8)	1,805 (21)
Clinical category of disease					
Miliary	1,480 (5)	396 (5)	527 (4)	153 (5)	404 (5)
Pulmonary/extrapulm	3,054 (11)	909 (11)	1,005 (7)	424 (12)	716 (8)
Extrapulmonary	5,949 (20)	1,582 (20)	1,967 (15)	608 (18)	1,792 (21)
Noncavitary pulmonary	14,809 (42)	3,360 (42)	6,223 (46)	1,432 (42)	3,794 (44)
Cavitary pulmonary	8,092 (22)	1,749 (22)	3,696 (28)	780 (23)	1,867 (22)
Sputum smear					
Negative	12,965 (44)	3,515 (44)	5,015 (37)	1,534 (45)	2,901 (34)
Positive	15,833 (44)	3,504 (44)	6,944 (52)	1,461 (43)	3,924 (46)
Death before treatment					
Survived	32,564 (99)	7,896 (99)	13,275 (99)	3,381 (100)	8,012 (93)
Died	814 (1)	97 (1)	141 (1)	16 (0)	560 (7)
Death during treatment					
Survived	31,060 (93)	7,412 (93)	12,735 (95)	3,249 (96)	7,664 (89)

Died	2,324 (7)	584 (7)	683 (5)	148 (4)	909 (11)

*Pearson's chi-square test p-value < 0.001 for all groups. †All races are non-Hispanic.

Table 2. IGRA result and characteristics of culture-confirmed TB cases.*

	Total	IGRA positive (n, %)	IGRA negative (n, %)	IGRA indeterminate (n, %)
Total	11,393	9,232 (81)	1,520 (13)	641 (6)
Age				
0–4	99	85 (86)	8 (8)	6 (6)
5–14	114	102 (89)	9 (8)	3 (3)
15–24	1,283	1,137 (89)	110 (9)	36 (3)
25–44	3,662	3,100 (85)	398 (11)	164 (4)
45–64	3,514	2,803 (80)	491 (14)	220 (6)
65+	2,721	2,005 (74)	504 (19)	212 (8)
Sex				
Male	6,942	3,693 (83)	528 (12)	230 (5)
Female	4,451	5,539 (80)	992 (14)	411 (6)
Race/ethnicity[†]				
Hispanic	3,150	2,627 (83)	366 (12)	157 (5)
American Indian	89	72 (81)	11 (12)	6 (7)
Asian	3,742	3,019 (81)	515 (14)	208 (6)
Black	2,466	2,062 (84)	260 (11)	144 (6)
Native Hawaiian	87	77 (89)	6 (7)	4 (5)
White	1,661	1,224 (74)	329 (20)	108 (7)
Birthplace				
US-born	3,620	2,825 (78)	554 (15)	241 (7)
Foreign-born	7,767	6,404 (82)	964 (12)	399 (5)
HIV status				
Negative	9,664	7,955 (82)	1,219 (13)	490 (5)
Positive	674	456 (68)	148 (22)	70 (10)
Unknown	1,055	821 (78)	153 (15)	81 (8)
Site of disease				
Miliary	549	413 (75)	78 (14)	58 (11)
Pulmonary/extrapulmonary	1,333	1,035 (78)	192 (14)	106 (8)
Extrapulmonary	2,190	1,791 (82)	293 (13)	106 (5)
Noncavitary pulmonary	4,792	3,902 (81)	647 (14)	243 (5)
Cavitary pulmonary	2,529	2,091 (83)	310 (12)	128 (5)
Sputum smear				
Negative	5,049	4,127 (82)	687 (14)	235 (5)
Positive	4,965	4,008 (81)	634 (13)	323 (7)
Death before treatment				
Survived	11,280	9,180 (81)	1,490 (13)	610 (5)
Died	113	52 (46)	30 (27)	31 (27)
Death during treatment				
Survived	10,661	8,754 (82)	1,365 (13)	542 (5)
Died	732	478 (65)	155 (21)	99 (14)

*Pearson's chi-square test p-value < 0.001 for all groups. [†]All races are non-Hispanic.

Table 3. TST result and characteristics of culture-confirmed TB cases.*

	Total	TST negative† (n, %)	TST positive (n, %)
Total	15,019	3,341 (22)	11,678 (78)
Age			
0–4	300	42 (14)	258 (86)
5–14	238	24 (10)	214 (90)
15–24	2,110	252 (12)	1,858 (88)
25–44	5,228	924 (18)	4,304 (82)
45–64	4,488	1,105 (25)	3,383 (75)
65+	2,654	994 (37)	1,660 (63)
Sex			
Male	9,461	2,349 (25)	7,112 (75)
Female	5,551	992 (18)	4,559 (82)
Race/ethnicity‡			
Hispanic	4,495	1,002 (22)	3,493 (78)
American Indian	223	42 (19)	181 (81)
Asian	3,839	625 (16)	3,214 (84)
Black	3,308	689 (21)	2,619 (79)
Native Hawaiian	772	148 (19)	624 (81)
White	2,176	788 (36)	1,388 (64)
Birthplace			
US-born	5,787	1,645 (28)	4,142 (72)
Foreign-born	9,213	1,693 (18)	7,520 (82)
HIV status			
Negative	12,801	2,602 (20)	10,199 (80)
Positive	712	311 (44)	401 (56)
Unknown	1,506	428 (28)	1,078 (72)
Site of disease			
Miliary	605	267 (44)	338 (56)
Pulmonary/extrapulmonary	1,250	374 (30)	876 (70)
Extrapulmonary	2,172	440 (20)	1,732 (80)
Non-cavitary pulmonary	6,909	1,415 (20)	5,494 (80)
Cavitary pulmonary	4,083	845 (21)	3,238 (79)
Sputum smear			
Negative	5,873	1,169 (20)	4,704 (80)
Positive	7,583	1,771 (23)	5,812 (77)
Death before treatment			
Survived	14,879	3,259 (22)	11, 620 (78)
Died	139	82 (59)	57 (41)
Death during treatment			
Survived	14,317	2,930 (20)	11,387 (80)
Died	702	411 (59)	291 (41)

*Pearson's chi-square test p-value < 0.001 for all groups. †TST negative = 0-4mm, TST positive = 5+ mm. ‡All races are non-Hispanic.

Table 4. Multinomial associations between site of disease and TST result and IGRA result.*

	Miliary disease	**Pulmonary & Extrapulmonary disease**	**Extrapulmonary only disease**	**Cavitary pulmonary disease**
	aOR (95% CI)	aOR (95% CI)	aOR (95% CI)	aOR (95% CI)
TST†				
Negative	Ref	Ref	Ref	Ref
Positive	**0.33 (0.27, 0.39)**	**0.57 (0.49, 0.65)**	0.92 (0.81, 1.04)	0.92 (0.83, 1.02)
IGRA				
Negative	Ref	Ref	Ref	Ref
Positive	0.95 (0.73, 1.23)	0.89 (0.75, 1.07)	0.90 (0.78, 1.05)	1.03 (0.89, 1.19)
Indeterminate	**1.98 (1.36, 2.87)**	**1.47 (1.11, 1.96)**	0.96 (0.73, 1.25)	1.11 (0.86, 1.43)

*Models are adjusted for age, sex, HIV status, and birthplace. Non-cavitary pulmonary disease, with the largest number of cases, is the referent clinical category.

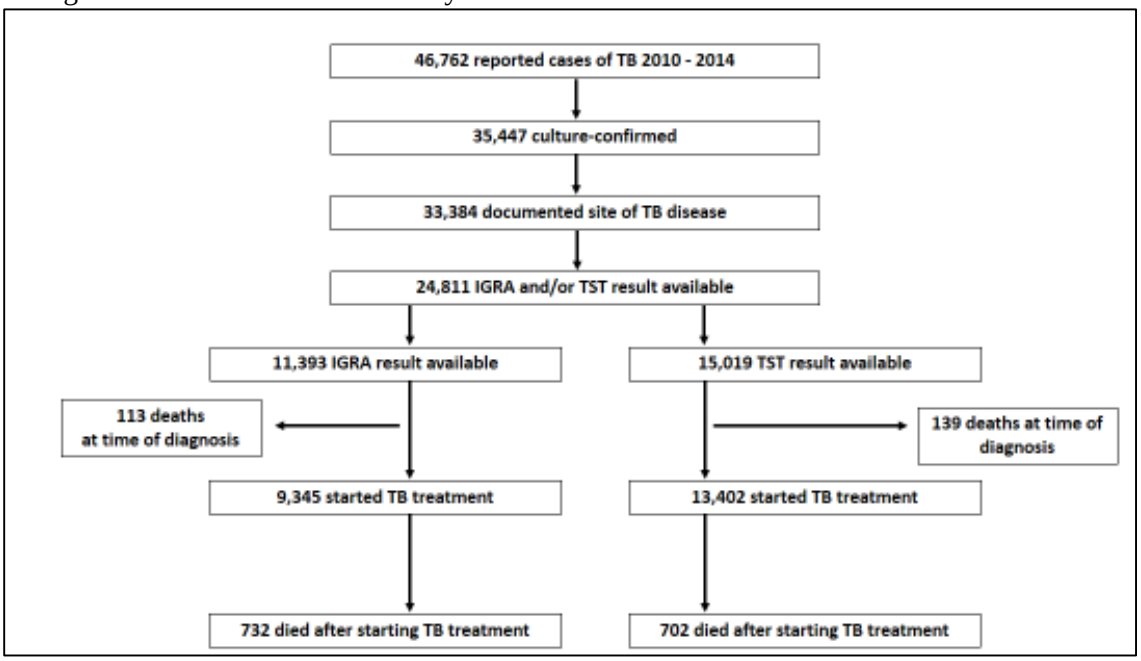
†TST negative = 0-4 mm, TST positive = 5+ mm

Table 5. Association between TST result, IGRA result, and death.*

	Death at time of diagnosis aOR (95% CI)	**Death after starting TB treatment**‡ aOR (95% CI)
TST†		
Negative	Ref	Ref
Positive	**0.25 (0.17, 0.36)**	**0.32 (0.27, 0.38)**
IGRA		
Negative	Ref	Ref
Positive	**0.34 (0.17, 0.73)**	**0.66 (0.54, 0.80)**
Indeterminate	**3.85 (1.78, 8.62)**	**1.46 (1.09, 1.95)**

*Models are adjusted for age, sex, HIV status, birthplace, and site of disease. Probability modeled is of death.
†TST negative = 0-4mm, TST positive = 5+ mm.
‡Model is also adjusted for baseline drug susceptibility status.

Figure 1. Flow diagram for selection of United States TB cases reported to the CDC during 2010 through 2014 for inclusion in the analysis**.***



* Note: Cases from California in 2010 were not included because of lack of HIV reporting in that year.

Figure 2. Association between IGRA result, TST result, and (A) site of disease, (B) death at the time of diagnosis, and (C) death after initiation of TB treatment. In panel A, black circles represent adjusted odds ratio (aOR) for miliary disease, dark grey diamonds for combined pulmonary and extrapulmonary disease, grey downward triangles for extrapulmonary only disease, light grey upward triangles for cavitary pulmonary disease. Odds modeled are those of each disease outcome category (the referent disease category is noncavitary pulmonary disease) for a positive (vs. negative) TST result or a positive or indeterminate (vs. negative) IGRA result. Lines represent the 95% confidence intervals for the point estimates. In panels B and C, odds modeled are those of death and arrowheads represent a 95% confidence interval extending beyond the vertical axis.

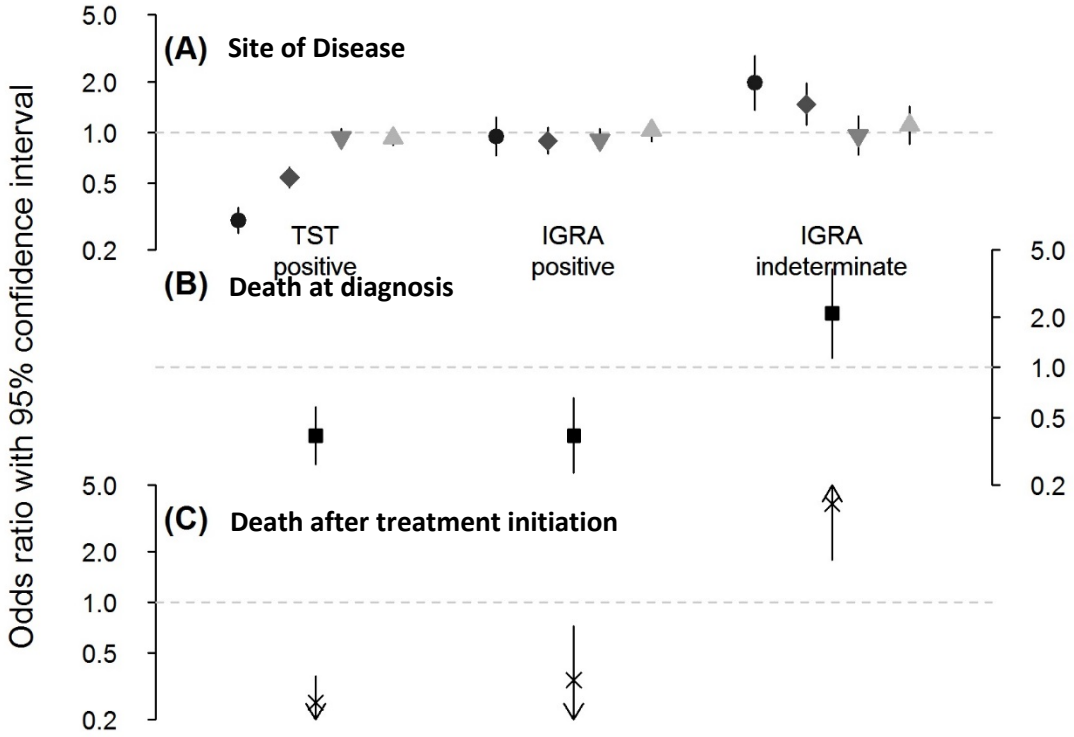


Figure 3. Kaplan-Meier survival curves for patients starting TB treatment by (A) TST or (B) IGRA result.