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REVIEW

Interferon-gamma release assays (IGRAs) in high-endemic settings: could they play a role in optimizing global TB diagnostics? Evaluating the possibilities of using IGRAs to diagnose active TB in a rural African setting

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Summary The number of patients suffering from tuberculosis (TB) globally is increasing. Due to the HIV epidemic, most patients suffering from TB reside in sub-Saharan Africa. In order to improve TB diagnostics, new tests — interferon-gamma release assays (IGRAs) — have been developed over the last decade. In this paper we evaluate the possible use of these tests in diagnosing or excluding active TB in high HIV-burden, resource-limited settings. The inability to differentiate between active and latent TB, limited data on IGRA performance in HIV-infected patients, observed false-negative results, high costs, and logistic problems limit the potential benefit of IGRAs. We also present two theoretical study designs in order to further assess IGRAs. Setting up a study on this subject is complicated by the frequent unavailability of mycobacterial cultures, the difficulty in acquiring prospective data, and the impossibility of denying treatment to a patient suspected of having active TB. We feel that current evidence does not support the implementing of IGRAs in clinical practice in settings with high endemic latent TB infection (LTBI) and high HIV prevalence. As these settings are the ones that suffer the most from the TB epidemic, we believe that the role of IGRAs in global TB control is questionable.

Introduction

Fuelled by the HIV epidemic, the number of tuberculosis (TB) patients in sub-Saharan Africa is increasing to almost unprecedented levels.[1,2] The majority of the HIV/TB co-infected patients reside in sub-Saharan Africa, resulting in

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high morbidity and mortality levels in that area.[3] The World Health Organization has called for ''urgent and extraordinary actions'' to control tuberculosis in Africa and launched the 'Global plan to stop tuberculosis', highlighting the need for accurate, simple, and low-cost diagnostic tests for the detection of TB infection.[4,5] In order to control the TB epidemic, the ability to make an adequate TB diagnosis in resource-limited settings is essential. However, diagnosing TB is challenging, especially in immunocompromised patients.

The gold standard test for the diagnosis of active TB is culture of *Mycobacterium tuberculosis* (MTB) in patients with signs and symptoms of active TB. In some patients it is not possible to isolate MTB from clinical specimens, or obtain clinical specimens. In HIV-positive patients with TB, an increased proportion of smear-negative and extrapulmonary disease is found.[6] Logistic reasons, such as time needed to culture MTB and costs, are additional reasons why culture is often omitted. Lacking a definite mycobacterial culture in patients suspected of having active TB, the decision to treat is often based on clinical signs and symptoms or typical findings on chest X-ray, whether or not combined with acid-fast bacilli (AFB) in sputum smear.

In 2006 the antenatal HIV seroprevalence in South Africa was 29% and the annual TB notification rate exceeded 700/100 000.[7] Because it is nearly impossible to convincingly exclude TB in primary care clinics in such a high-endemic TB country, and for fear of missing patients who are suffering from TB, the threshold to start anti-tuberculosis treatment is low. This might result in a considerable number of patients who are unnecessarily being exposed to a six-month course of tuberculostatics with the associated risks, side effects, and costs. On the other hand, a TB diagnosis may be missed in patients who are suffering from active TB, but who do not have clear symptoms (such as prolonged cough, fever, weight loss, night sweats, or lymphadenopathy) and have a normal chest X-ray and negative AFB on smears, thus running the risk of unnecessary morbidity and mortality and possibly infecting others.

The century-old tuberculin skin test (TST) has low specificity due to false-positive results in populations vaccinated with bacille Calmette—Guérin (BCG) and in patients infected with most non-tuberculous mycobacteria.[8] TST also has low sensitivity in immunocompromised patients and is therefore not recommended for this group by some of the current guidelines.[9]

In order to improve TB diagnostics and care worldwide, simple and reliable tests are needed to reduce false-positive and false-negative results (inherent in TST), equipping clinicians with more accurate tools for TB diagnosis, control, and elimination. However, the frequent inability to definitely confirm the presence of active TB by culture, hampers assessment of the accuracy of new TB tests.

Interferon-gamma release assays (IGRAs) for TB have been developed over the last decade.[10] Two IGRAs are currently commercially available, the QuantiFERON-TB Gold test (Cellestis, Carnegie, Australia) and the T-SPOT.TB assay (Oxford Immunotec, Oxford, UK). Both tests measure the interferon-gamma release by sensitized lymphocytes in response to specific MTB antigens, using methods such as ELISA (QuantiFERON) and enzyme-linked immunospot assay (T-SPOT.TB). IGRAs are considered positive when the amount of produced interferon is over a certain threshold. For reliable results, normal lymphocyte function is crucial. An indeterminate test result is usually due to reduced interferon-gamma production after stimulation with a non-specific antigen (phytohemagglutinin), resulting in a failed positive control. This often reflects underlying immunosuppression.[10]

As IGRAs are based on the cellular immune response, they are incapable of distinguishing between a latent and an active TB infection.

In our view, IGRAs are of little value in diagnosing or excluding active TB in high HIV-burden, resource-limited settings — areas where the TB epidemic rages most fiercely. We describe below the reasons why we believe this is the case, briefly summarize current evidence, and discuss theoretical study designs in order to further assess IGRAs in such settings.

Sensitivity and specificity of IGRAs

A number of papers on IGRA performance have been published. Sensitivity has been estimated by testing people with a confirmed pulmonary TB and specificity has been calculated in low-endemic countries with a high BCG vaccination rate. For immunocompetent patients, sensitivity is estimated to be between 83% and 97% for the T-SPOT.TB test and between 70% and 89% for the QuantiFERON-TB Gold test.[11—16] Specificity would be 96—98% for the QuantiFERON-TB Gold test and might be even higher for the T-SPOT.TB test.[11,12,17,18] Lacking a gold standard for the diagnosis of a latent TB infection (LTBI), most studies have compared IGRA results to the results of TSTs.[11—14,16,17] They have shown higher specificity (especially in BCG-vaccinated populations) and sensitivity rates for the IGRAs as compared to the TST. This is an important advantage of IGRAs over the TST, as BCG vaccine coverage is high in many sub-Saharan African countries. In South Africa, for example, coverage is over 95%.[19]

Diagnostic research on IGRAs

These studies, however, could all be seen as 'test research' as opposed to 'diagnostic research'. In test research, studies merely focus on the 'characteristics' of a test, such as sensitivity and specificity, instead of on the test's performance to confirm or exclude a diagnosis. Diagnostic research, on the other hand, refers to studies that aim to quantify a test's added contribution beyond test results readily available to the physician in determining the presence or absence of a particular disease, in this case TB.[20] A single test's sensitivity and specificity are of limited value in practice as they reflect the probability that a particular test result is positive or negative given the presence (sensitivity) or absence (specificity) of a disease. In practice, however, one is interested in the probability of having a particular disease given the test result. In order to determine whether or not a person is suffering from TB, a test has to truly increase, or decrease, the probability of disease presence as estimated from the previous data, such as clinical signs and symptoms, X-rays, and sputum tests. The 'post-test probability' should be greater or smaller than the 'pre-test probability'.[21] If such a test were available and affordable for TB, it would be of great value in high-endemic countries.

When setting up diagnostic research in order to calculate what added value implementing IGRAs in a sub-Saharan

African setting could have for the diagnosis of active TB, several issues have to be addressed. As already mentioned, IGRAs cannot differentiate between latent and active TB. In people infected with HIV, treating LTBI with isoniazid reduces the risk of developing active TB, but there is no evidence that such preventive therapy reduces all-cause mortality.[22] Although some do consider the use of mass isoniazid preventive therapy for HIV-infected individuals,[23] the implementation of LTBI treatment in resource-limited settings is limited due to difficulty in the identification of those at risk for developing active TB, uncertainty about effectiveness of preventive treatment in high-endemic areas, costs, and fear of enhancing the spread of resistant TB.[24–26] If diagnosing LTBI does not have therapeutic consequences, testing for it does not seem beneficial. Active TB on the other hand will be treated. So a benefit of implementing IGRAs in settings where there is a high LTBI prevalence, but where treating LTBI is not common practice, could only be expected if it were possible to reliably confirm or exclude active TB on the basis of IGRA results.

Prevalence of latent TB

Before being able to estimate the potential use of IGRAs in predicting active TB, the prevalence of latent TB should be determined. Lacking a gold standard test for LTBI, the exact prevalence cannot be calculated. Estimations have been made, however, using TSTs as well as IGRAs. In a recent case–control study in Cape Town, South Africa, TSTs and both IGRAs were each positive in over 70% of HIV-negative controls, indicating a very high community exposure to *M. tuberculosis*.[27] In another report from Khayelitsha, South Africa, an LTBI prevalence of 80% was estimated.[23] A person who does not show signs or symptoms indicating an active TB infection and who has a positive IGRA in such a setting would be considered to have latent and not active TB.

Predictive values of IGRAs

Could IGRAs then play a role in reducing the number of patients who are needlessly being exposed to tuberculostatics? If so, this would decrease morbidity and costs for both patients and healthcare facilities and would therefore be valuable. Before receiving tuberculostatics, patients have to present with signs or symptoms indicating TB. Within this group of patients that are eligible for TB treatment, three groups of patients can be distinguished. The first and probably largest group consists of patients who have been diagnosed with TB correctly. The second group of patients consists of those who have an LTBI, but whose actual signs and symptoms are caused by a different ailment, and the last group of patients will have neither active, nor latent TB. Ideally it should be possible to determine which patients are in the second and third groups and withhold TB treatment from them. A positive IGRA cannot differentiate between the first two groups, but is it correct to assign a patient to the third group if the IGRA result is negative? Or, in other words, what is the negative predictive value of IGRAs?

As prospective data on IGRAs are limited, predictive values of these tests are not known. An ideal study design to determine the predictive values would be a prospective cohort study where patients, clinically suspected of having active TB, but with negative IGRA and sputum-test results, would be denied TB treatment and would be followed up to see if those who tested IGRA-positive actually develop active TB and those who tested negative stay well. Such a study is not feasible, however, for obvious ethical reasons.

A prospective study that followed up persons who had tested IGRA-negative in an LTBI screening program showed that no patient with a negative test result subsequently developed active TB.[28] This study was set in an area where TB prevalence is low, and none of the studied persons had signs or symptoms indicative of TB. The pretest probability of developing a new TB infection in such a setting is much lower, and therefore one could state that the generalizability of these results is limited. Another prospective study concluded that negative QuantiFERON test results should not be used to exclude the diagnosis of TB in persons with suggestive signs or symptoms, as 14 out of 69 patients with culture-confirmed TB had a negative QuantiFERON test result.[29] The US Centers for Disease Control and Prevention came to the same conclusion and state in their guidelines that a negative QuantiFERON test cannot be used alone to exclude the diagnosis of active tuberculosis.[30]

Test validity in immunocompromised patients

Another issue when determining whether or not it is justifiable to withhold TB treatment from people who test IGRA-negative, is the test's validity in immunocompromised patients. Impaired immune functionality can possibly reduce interferon-gamma responses, and in the severely immunocompromised the test may be impaired by T-cell anergy.[31] Data on the performance of these tests in HIV-positive patients, especially in patients with low CD4 T-cell counts, are still limited though. Most studies are case–control studies on diagnosing LTBI,[31,32] often comparing IGRA results to TST outcomes.[27,33,34] Studies on the performance of IGRAs in HIV-infected patients suspected of having active TB are even scarcer, and in most only a subgroup of patients is HIV-positive.[13,23,29,35,36] Moreover, a high frequency of indeterminate IGRA results in immunocompromised patients has consistently been shown, especially where the QuantiFERON test is used.[13,29,36,37] The number of indeterminate results seems to be somewhat lower with the T-SPOT.TB assay.[32,34,38]

Two theoretical study designs

In 2006, a group of experts gave directions for future research on IGRAs.[39] Test performance in high-risk populations, such as those with HIV infection, was considered an important research question. In order to study if IGRAs would be useful in diagnosing active TB in HIV-positive patients, we considered two study designs. One possible design would be to include all patients eligible for tuberculostatics, i.e., those suspected of having active TB. By splitting the group according to HIV status and after performing an IGRA for each patient, the 2 × 2 table shown in Table 1 can be constructed.

Even though the negative predictive value of the IGRAs is unknown, some will regard a negative IGRA result in an immunocompetent patient sufficient to withhold TB

Table 1 2 × 2 Contingency table IGRA results depending on HIV status

All patients suspected of active TB	IGRA result (QuantiFERON-TB/T-SPOT.TB)	
HIV status	Positive	Negative
Negative	A	B
Positive	C	D

IGRA, interferon-gamma release assay; TB, tuberculosis.

treatment and actively search for an alternative diagnosis.[40–42] Assuming that the proportion of patients who actually have a TB infection (latent or active) and those who have not is the same for HIV-positive and HIV-negative patients, one could decide to withhold TB treatment from all patients with a negative IGRA result if the proportion of IGRA-negative patients in HIV-positive patients (D/C) does not differ too much from that in HIV-negative patients (B/A). If the prevalence of LTBI is high, for example 70%, the number of patients with suspected TB and a negative IGRA result would be low.

Let us say that at least 80% of HIV-negative patients suspected of having active TB will test IGRA-positive. If a 10% difference in test results between HIV-positive and HIV-negative patients is still considered acceptable, and one wants to test the null-hypothesis that this is the case, a study population of 6609 patients is needed. A more realistic scenario, for example with the assumption of only 10% of HIV-negative patients having a negative IGRA result, will already call for a study population of 14 950 patients. Accepting a difference in test results between HIV-positive and HIV-negative patients of only 5%, increases this number to 25 782. Power calculations for various assumptions on the proportion of HIV-negative patients testing IGRA-positive and for various differences in test results between HIV-positive and HIV-negative patients are shown in Table 2.

Even if it is possible to set up and finance a study with enough participants in order to test such a hypothesis, we still do not know the exact significance of a negative test result. Prospective follow-up would be needed to show if these patients indeed stay free of tuberculosis, and in many resource-limited settings such follow-up is not feasible.

Another possible study design would be to include patients eligible for tuberculostatic treatment, but split the group according to the sputum smear results instead of their HIV status. By doing this, the 2 × 2 table shown in Table 3 can be constructed.

Although patients who are not suffering from TB, but who have AFB in their sputum have been described previously,[43] pulmonary disease due to environmental mycobacteria is very rare.[44] The positive sputum smear could thus serve as an alternative 'gold standard' for the diagnosis of active TB, and patients with a positive sputum test could be used as a reference for the other groups.

If an immunocompetent patient is AFB- and IGRA-negative (D), it might be justified to withhold TB treatment from him

Table 2 Power calculations for a theoretical study design on the use of IGRAs to exclude active TB in HIV-positive patients. Various assumptions on the proportion of HIV-negative patients testing IGRA-positive and various differences in test results between HIV-positive and HIV-negative patients are used to calculate the number of patients needed for a study

Assumed percentage of HIV-neg patients with a positive IGRA test result ('A' in Table 1)	Difference in IGRA test results between HIV-pos and HIV-neg patients deemed acceptable ('D/C' versus 'B/A' in Table 1)	*N* Group[a]	*N* Total[b]
60%	25%	407	814
	20%	628	1256
	15%	1101	2202
	10%	2439	4878
	5%	9592	19 184
70%	25%	649	1298
	20%	996	1992
	15%	1737	3474
	10%	3829	7658
	5%	14 988	29 976
80%	25%	1133	2266
	20%	1732	3464
	15%	3009	6018
	10%	6609	13 218
	5%	25 782	51 564
90%	25%	2586	5172
	20%	3940	7880
	15%	6825	13 650
	10%	14 950	29 900
	5%	>25 000	>50 000

IGRA, interferon-gamma release assay; TB, tuberculosis.
[a] *N* Group: number of patients needed per group.
[b] *N* Total: total number of patients needed for the study.

Table 3 2 × 2 Contingency table IGRA results depending on sputum smear (AFB) results

All patients suspected of active TB	IGRA result QuantiFERON-TB/T-SPOT.TB	
Sputum smear (AFB)	Positive	Negative
Positive	A	B
Negative	C	D

IGRA, interferon-gamma release assay; AFB, acid-fast bacilli; TB, tuberculosis.

or her. However, many patients are co-infected with HIV and TB. As mentioned earlier, HIV co-infection increases the probability of having a negative sputum test as well as testing IGRA-negative. Being associated with both the exposure as well as the outcome, HIV co-infection is an important confounder and will decrease the internal validity of the study. If all HIV-positive patients are excluded in order to avoid this problem, the internal validity will increase, but the external validity and thus generalizability, of the study will decrease. A possible solution to this problem would be to analyze data from HIV-negative and HIV-positive patients separately, enabling a comparison of the results later on.

Costs and logistics

Apart from methodological problems in setting up studies on the use of IGRAs in diagnosing active TB and in interpreting IGRA test results, there are other hurdles to be overcome when implementing IGRAs in resource-limited settings. Using IGRAs in clinical practice will result in a substantial financial and logistic burden on healthcare institutions and laboratories. Therefore, future research on cost-effectiveness will also be needed.

Conclusions

In summary, the inability to differentiate between active and latent TB, the limited data on IGRA performance in HIV-infected patients, the observed false-negative results, high costs, and logistic problems limit the potential benefit of IGRAs in the diagnosis of active TB. Setting up a study on this subject is complicated further by the frequent unavailability of mycobacterial cultures, difficulty in acquiring prospective data, and the impossibility of denying treatment to a patient suspected of having active TB. We therefore feel that current evidence does not support the implementing of IGRAs in clinical practice in settings with high-endemic LTBI and high HIV prevalence. As these settings are the ones that suffer the most from the TB epidemic, we believe that the role of IGRAs in global TB control is questionable. If the results of future research make it possible to differentiate between latent and active TB (possibly by defining separate interferon-gamma cut-off values), or if more evidence is published on the performance of IGRAs in HIV-infected patients, the area in which IGRAs are useful might expand.

Conflict of interest: No conflict of interest to declare.

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