UNIVERSITAT DE BARCELONA

Improving diagnostic strategies for latent tuberculosis infection in populations at risk for developing active disease

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UNIVERSIDAD DE BARCELONA

Facultad de Medicina

IMPROVING DIAGNOSTIC STRATEGIES FOR LATENT TUBERCULOSIS INFECTION IN POPULATIONS AT RISK FOR DEVELOPING ACTIVE DISEASE

Memoria presentada por

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Para optar al grado de Doctor en Medicina

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El Dr. Miguel Santín, profesor asociado de la Facultad de Medicina de la Universidad de Barcelona y Médico Adjunto del Servicio de Enfermedades Infecciosas del Hospital Universitario de Bellvitge, hace constar que la tesis titulada

"Improving diagnostic strategies for latent tuberculosis infection in populations at risk for developing active disease"

que presenta la licenciada Laura Muñoz, ha sido realizada bajo su dirección en el campus de Bellvitge de la Facultad de Medicina, la considera finalizada y autoriza su presentación para que sea defendida ante el tribunal que corresponda.

En Barcelona, marzo de 2017

Dr. Miguel Santín

A mis padres

A mi gran familia

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RESUMEN

INTRODUCCIÓN

La tuberculosis (TB) es una de las enfermedades más antiguas y devastadoras que ha acompañado al ser humano desde hace cientos de años. A pesar de ser una enfermedad tratable y prevenible desde el descubrimiento de los fármacos activos frente a su agente causal *Mycobacterium tuberculosis* (Mtb), la TB sigue siendo una de las diez causas de mortalidad más frecuentes en el mundo. En 2014 se declararon 1.5 millones de muertes por esta causa, concentrándose el 80% de casos en 22 países en vías de desarrollo. A pesar de esta distribución, la TB no es exclusiva de los países pobres: Mtb no conoce fronteras y ningún país ha logrado nunca eliminar esta enfermedad.

El reservorio de Mtb es casi exclusivamente humano, aunque antaño y todavía ahora en países del tercer mundo, ha existido transmisión zoonótica a través de la ingesta de leche no pasteurizada de ganado vacuno enfermo. El paciente enfermo de TB es fuente de contagio para el resto de personas: al toser o al hablar está dispersando secreciones respiratorias microscópicas que contienen Mtb. Se sabe que este bacilo puede mantener su viabilidad varios días expuesto al ambiente, lo que hace que el contagio sea extremadamente difícil de prevenir.

Al ser inhalado y alcanzar el espacio alveolar, Mtb se enfrenta a los macrófagos, la primera barrera que le presenta el sistema inmunitario. Tras un complejo sistema de citoquinas estimuladoras e inhibidoras de otros grupos celulares, se organizará el granuloma. Esta estructura histológica definitoria y característica de la reacción a Mtb, contendrá la infección en estado latente en la mayoría de los casos. El organismo

humano no puede aclarar la infección tuberculosa, pero es capaz de mantener un equilibrio a su favor. Sólo si se pierde dicho equilibrio, el paciente desarrollará TB. Clásicamente se ha diferenciado el desarrollo de TB tras el contagio (primoinfección) o tras un periodo de latencia que puede llegar a ser de varias décadas (reactivación).

Desde el descubrimiento de la estreptomicina en 1944 y la isoniazida en 1952, además de tratar a los individuos enfermos, el British Medical Council diseñó 5 ensayos clínicos a gran escala que permitieron demostrar la eficacia del tratamiento preventivo de infección latente para el desarrollo de TB. Los pacientes aleatorizados a la rama de tratamiento tenían una menor tasa de TB que los que habían recibido placebo tras un periodo de seguimiento de 5 años o más.

A pesar de que la principal arma para conseguir controlar la epidemia de TB es la precocidad en el diagnóstico y tratamiento adecuado de los individuos enfermos, la estrategia global de control de la enfermedad propuesta por la Organización Mundial de la salud (OMS) para después de 2015 incluye el tratamiento de las personas con infección tuberculosa latente (ITL), de donde surgirán los futuros enfermos de TB. Éste es el centro del presente proyecto de investigación.

Se ha estimado que un tercio de la población mundial está infectada por Mtb y que aproximadamente un 10% desarrollará TB a lo largo de su vida. En la mayoría de los casos existirá una condición inmunosupresora que favorecerá la pérdida del equilibrio entre Mtb y la inmunidad del huésped: son ejemplos las edades extremas de la vida, la malnutrición y enfermedades debilitantes, así como los tratamientos que alteran la inmunidad celular.

En cuanto al diagnóstico de infección tuberculosa en el paciente sano, no es posible confirmar el diagnóstico mediante cultivo. El bacilo está inmerso en la compleja arquitectura del granuloma y del organismo del huésped, lo que hace que sea inaccesible para la toma de muestras. La ITL sólo puede diagnosticarse de manera indirecta en un individuo asintomático, a través de la reacción inmunológica que desencadenen sus linfocitos al ser estimulados *in vivo* o *in vitro* con antígenos de Mtb.

Dado que no existe ninguna prueba diagnóstica "gold estándar" de ITL, la sensibilidad y especificidad de las pruebas diagnósticas utilizadas va a calcularse de manera imperfecta con los resultados de infección en los pacientes enfermos, ya que en éstos sí que se tiene certeza de la infección. Los individuos enfermos serán utilizados entonces como marcador subrogado de ITL, a pesar de la imperfección del comparador: de 100 individuos con TB confirmada por cultivo, sólo 70 tendrán una reacción positiva a la prueba de la tuberculina (PT).

Hasta la última década, únicamente se disponía de PT para el diagnóstico de infección tuberculosa. Esta prueba mide "*in vivo*" la respuesta de los linfocitos sensibilizados frente a determinados antígenos de Mtb contenidos en el derivado proteico purificado o PPD, que se inyecta en la dermis de la cara volar del antebrazo del paciente (técnica de Mantoux). Estos antígenos son comunes a la cepa de *M. bovis-BCG* utilizado para la vacunación infantil contra la TB, así como a algunas micobacterias no tuberculosas (MNT). Por este motivo, un resultado positivo de la PT no será exclusivo de la infección por Mtb, sobre todo en los individuos vacunados. Un metaanálisis de la última decada mostró que la proporción de resultados positivos de la PT en pacientes vacunados podría variar entre un 0-90%, reflejando la variablidad de los resultados en función del

reactante, la técnica, el punto de corte para considerar un resultado como positivo, el técnico que leía el resultado o la población donde se realizaban dichos estudios.

En los últimos quince años se han implementado las técnicas de detección de interferon-gamma *in vitro* (IGRAs), con capacidad de detectar inmunidad específica frente a Mtb en una muestra de sangre periférica. Los antígenos utilizados en los dos IGRAs disponibles: QuantiFERON-TB® Gold In-Tube (QFT-GIT) (Cellestis Limited, Carnegie, Victoria, Australia) y T-SPOT.TB® (Oxford Immunotec, Oxford, UK), no están presentes en la cepa de *M. bovis*-BCG contenida en la vacuna, por lo que no existirán "falsos IGRA positivos" de causa vacunal.

Ambas técnicas, PT e IGRAs ven disminuida su sensibilidad diagnóstica en los pacientes inmunosuprimidos, ya sea por enfermedades de base o por el tratamiento con corticoides o fármacos que alteran la funcionalidad de los linfocitos y les impiden generar una repuesta adecuada ante antígenos contra los que habían sido sensibilizados. Ninguna de las dos técnicas es capaz de distinguir si la infección es antigua o reciente, ni si está en forma activa o latente. Tampoco han demostrado ser capaces de predecir el desarrollo de enfermedad tuberculosa. El diseño ideal para determinar las diferencias en el valor predictor de tuberculosis entre IGRA y PT consistiría en dejar a todos los sujetos de ambos grupos sin tratar, y comprobar que la incidencia de tuberculosis en IGRA positivo es mayor que en PT positiva. Sin embargo, este diseño plantea un problema ético, ya que implicaría dejar sin tratar a personas en riesgo de desarrollar TB activa tras la primoinfección. La única manera, por tanto, de evaluar la eficacia de los IGRAs en las poblaciones de riesgo, es comprobar que las decisiones terapéuticas basadas en sus resultados disminuyen el riesgo de TB a

prácticamente cero (en un país de baja endemia y asegurando la toma del tratamiento).

El punto clave es dilucidar a qué individuos debe indicarse cribado y tratamiento de ITL, ya que a pesar de afectar a un tercio de la población mundial, el riesgo de reactivación y desarrollo de TB es sólo del 10%. No debe practicarse la prueba si no se tiene la intención de ser consecuente con el resultado de la misma. Deben someterse a una prueba diagnóstica aquellos individuos en riesgo de desarrollar enfermedad activa en caso de estar infectados. Son dos los grupos de riesgo identificados en la literatura: los pacientes inmunodeprimidos (fundamentalmente pacientes con infección por VIH, trasplantados y aquellos afectos de enfermedades inflamatorias inmunomediadas que precisan agentes biológicos) y los individuos con exposición reciente a un paciente enfermo de tuberculosis.

En estos grupos de riesgo y en la toma de decisiones en función del resultado de las pruebas diagnósticas de ITL se han centrado los trabajos recogidos en esta tesis doctoral.

CONTEXTO DE LA INVESTIGACIÓN

En países de baja endemia el control de TB se concentra en la prevención de tuberculosis a través del cribado de infección tuberculosa latente pacientes en riesgo de desarrollar enfermedad activa. La Unidad Clínica de TB del Hospital Universitari de Bellvitge dispone de personal a tiempo completo con amplia experiencia en educación sanitaria y protocolos asistenciales sistemáticos para los diferentes perfiles de pacientes. A raíz de la introducción de los IGRAs se realizaron tres estudios prospectivos que incluían estas técnicas para el cribado de ITL en poblaciones de riesgo para el desarrollo de TB, y que sirvieron de base para la realización de una tesis doctoral en 2012.

Desde entonces los IGRAs se han incluido en la práctica clínica. A pesar de que varias guías internacionales y recomendaciones de expertos los incluyen como parte del proceso diagnóstico de ITL, la realidad es que no existen directrices uniformes para su uso. La ausencia de "*gold standard*" para el diagnóstico de ITL dificulta la comparación de sus resultados con los obtenidos con la PT. En este sentido, la OMS definió en 2011 una escala jerárquica del grado de evidencia que podía generar el uso de los IGRAs. El primer paso consistía en valorar la concordancia de PT e IGRAs. El segundo, en valorar la sensibilidad y la especificidad de los IGRAs en pacientes con TB activa. En tercer lugar, estudiar la correlación de los resultados de los IGRAs con el grado de exposición a Mtb o en su defecto, a los factores de riesgo que se han asociado clásicamente a TB. El cuarto y penúltimo escalón estaba definido por el establecimiento de los valores predictivos positivo y negativo para el desarrollo de TB, y hasta este punto, la literatura científica ha generado resultados en esta progresión en la generación de

evidencia. En el momento de la realización de este estudio, y aún ahora al haberlo finalizado, no se han publicado estudios que satisfagan el objetivo del último escalón propuesto por la OMS: la valoración de la eficacia del tratamiento preventivo que se ha prescrito a tenor de los resultados de los IGRAs.

Con el objetivo de generar resultados con la evidencia necesaria para dar respuesta definitiva al valor de los IGRAs, se diseñó un ensayo clínico en el contexto del estudio de contactos de tuberculosis, comparando la estrategia habitual de PT a una estrategia secuencial que combina PT y su confirmación con QFT-GIT, y su eficacia en prevenir el desarrollo de TB a los dos años de seguimiento. Éste ha sido el principal proyecto de investigación de esta tesis. Durante la realización del mismo se ha llevado a cabo un meta-análisis que valora la capacidad de los IGRAs en reducir la proporción de pacientes candidatos a recibir tratamiento preventivo, sin que aumente el riesgo de TB ulterior. Asimismo se han llevado a cabo tres estudios longitudinales que describen la experiencia del uso de los IGRAs en la Unidad Clínica de TB de nuestro centro en tres poblaciones de riesgo, comparándolos con los resultados de las estrategias previas que no los incluían. Estas poblaciones de riesgo incluyen los contactos recientes de TB y dos grupos de inmunosupresión bien conocida: los pacientes sometidos a agentes anti-factor de necrosis tumoral (anti-TNF) y los candidatos a trasplante.

HIPÓTESIS DE TRABAJO

Premisas

1. A mayor proporción de diagnósticos de ITL entre los cribados como población en riesgo de desarrollar TB, más eficaz será la estrategia de prevención de enfermedad activa. Por este motivo se ha utilizado el booster y todavía se recomienda en algunas Guías Clínicas.
2. No existe evidencia sólida sobre cuál es la mejor técnica diagnóstica para ITL. Los IGRAs han demostrado superioridad en precisión diagnóstica (sensibilidad, especificidad y valores predictivos) y de hecho se han incorporado progresivamente a la práctica clínica ya sea sustituyendo o acompañando a PT, pero no existen estudios sólidos que favorezcan su uso. Esto se traduce en la heterogeneidad de las recomendaciones de expertos o en las directrices de sociedades científicas.
3. Los IGRAs pueden mejorar la estrategia diagnóstica de ITL en poblaciones en riesgo de desarrollar enfermedad activa.
    3.1. Pacientes inmunodeprimidos: los IGRAs pueden optimizar la sensibilidad de la estrategia diagnóstica si se añaden a la PT, ya que éstos han demostrado no estar tan influenciados por la inmunosupresión como la PT.
    3.2. Pacientes sanos con exposición reciente a Mtb: los IGRAs pueden mejorar la especificidad del diagnóstico. En diferentes series los IGRAs han demostrado un alto valor predictivo negativo, por lo que los pacientes con un resultado negativo no estarían en riesgo de desarrollar TB aún sin tratamiento preventivo.

4. Las pruebas diagnósticas constituyen una parte fundamental del manejo de la ITL. No obstante, el control de la adherencia y de los efectos secundarios son también fundamentales para conseguir la compleción del tratamiento, y por ende, su eficacia.

Hipótesis

El manejo de ITL es un componente crucial en la prevención de TB en poblaciones de riesgo . Una buena estrategia de cribado que incluya la compleción del tratamiento es garantía de eficacia. Aumentar la proporción de diagnósticos de ITL no es sinónimo de mejoría de los resultados. Al contrario, puede conducir a un aumento de tratamientos preventivos innecesarios.

1. En pacientes inmunosuprimidos: Partiendo de la mayor sensibilidad de los IGRAs respecto a PT en este grupo de pacientes, la implementación de los IGRAs en la estrategia de cribado de ITL podría aumentar el número de individuos diagnosticados y tratados de ITL, evitando los resultados falsos negativos de la PT y disminuyendo así el riesgo de reactivación de TB.

2. En pacientes sanos expuestos a Mtb (estudios de contactos): Asumiendo una sensibilidad equivalente y una mayor especificidad de los IGRAs respecto a PT, su utilización y la toma de decisiones en función de su resultado podrían optimizar la estrategia de selección de pacientes candidatos a recibir tratamiento de ITL en el estudio de contactos, reduciendo el número de pacientes tratados, sin aumentar el riesgo de TB.

OBJETIVOS

Objetivo general: mejorar el manejo de ITL, especialmente a través de la implementación de los IGRAs, en poblaciones en riesgo de desarrollar enfermedad activa.

Objetivos específicos:

1. En el grupo de pacientes inmunosuprimidos

   1.1. Candidatos a recibir tratamiento con agentes anti-TNF

   - Determinar la eficacia de un protocolo sistemático de cribado y tratamiento de ITL en estos pacientes para prevenir el desarrollo de TB.
   - Determinar la eficacia y la seguridad de la estrategia que incluye PT en un tiempo y QFT-GIT comparándola con práctica previa de PT en dos tiempos (booster), en términos de reducción de tratamientos preventivos sin que exista un aumento de riesgo de desarrollo de TB.
   - Determinar de la rentabilidad de la repetición sistemática del cribado de ITL tras un resultado inicial negativo.

   1.2. Candidatos a trasplante

   - Valorar la utilidad de QFT-GIT y comparación con la utilidad de PT para la predicción del desarrollo de TB activa en pacientes candidatos a trasplante hepático y de precursores hematopoyéticos.

2. En pacientes sanos con exposición reciente a Mtb (estudio de contactos)
    - Determinar si la implementación de los IGRAs puede reducir el número de pacientes candidatos a recibir tratamiento preventivo sin aumentar el riesgo de TB activa ulterior.
    - Comprobar la no-inferioridad de una estrategia secuencial de PT confirmada con QFT-GIT comparada con la estrategia basada únicamente en PT para la prevención de TB.

METODOLOGÍA

Investigación Clínica

Todos los estudios se han realizado en la Unidad Clínica de TB del Hospital de Bellvitge, que desde el año 1988 funciona como una consulta monográfica, ocupándose de todos los pacientes con TB pulmonar y extrapulmonar, el estudio de contactos, programas de prevención de TB con protocolos de cribado de ITL para cada población de riesgo, y además es referente de infecciones por MNT y cepas MDR y XDR de Mtb.

El protocolo sistemático de cribado de ITL incluye

1. Evaluación clínica basal y descarte de enfermedad TB activa mediante radiografía de tórax y despistaje de síntomas, con recogida de datos demográficos, comorbilidades y tratamiento durante los últimos tres meses, antecedentes de tuberculosis o ITL, estado de vacunación BCG y factores de riesgo de tuberculosis.
2. Diagnóstico de infección tuberculosa latente: En los pacientes con antecedentes de TB o ITL documentadas, no se realizan pruebas diagnósticas. Si nunca han recibido tratamiento se les ofrece tratamiento preventivo en caso de que el riesgo sea superior a los inconvenientes del tratamiento. En pacientes que nunca han sido sometidos a pruebas de detección de tuberculosis, se administra la PT mediante el método de Mantoux utilizando 2 UT de PPD RT23 (Statens Serum Institute, Copenhague, Dinamarca) en la cara volar del antebrazo. Tanto la administración como su lectura a las 48-72 horas,

son evaluadas por dos enfermeras con experiencia en la Unidad de Tuberculosis. En caso de requerir un IGRA, QFT-GIT se realiza antes de la PT y de acuerdo con las instrucciones del fabricante.

3. Tratamiento de ITL: Todos los recién diagnosticados de ITL reciben seis meses de tratamiento con isoniazida. El régimen alternativo es de cuatro meses de rifampicina. Los candidatos a recibir tratamiento reciben un amplio asesoramiento sobre la infección tuberculosa, su tratamiento y posibles efectos secundarios. Durante el periodo de tratamiento, los pacientes tienen acceso telefónico directo a la Unidad de Tuberculosis 5 días a la semana. Las visitas de refuerzo y monitorización junto con las analíticas de control de la función hepática se realizan en los meses 1, 3 y 6. La adherencia a la isoniazida se evalúa mediante la detección de metabolitos del fármaco en orina con la prueba de Eidus-Hamilton. El control de lo pacientes que toman rifampicina se comprueba con el color de la orina.

Los trabajos observacionales que componen esta tesis han seguido la misma metodología. Los datos de los pacientes incluidos en los estudios se recogían de manera prospectiva como parte del trabajo asistencia de la Consulta. Para el desarrollo de TB se revisaron mediante un protocolo específico de recogida de datos las historias clínicas informatizadas de ámbito hospitalario y de la Atención Primaria. En caso de no poder obtener los datos necesarios, se realizaba una llamada telefónica al paciente. En caso de pérdida de seguimiento, los datos del paciente se contrastaban con el registro de declaraciones de TB en Cataluña.

Para el Ensayo Clínico se creó la RED OPTIMIST, compuesta de 12 centros hospitalarios repartidos por toda la geografía nacional. Algunos de estos centros trabajaban con un sistema parecido al del Hospital de Bellvitge antes del Ensayo (atención de los contactos en relación a cada caso índice, control sistemático de la adherencia y de los efectos secundarios), como por ejemplo el Complexo Hospitalario de Pontevedra, el Hospital de Mendaro; Son Llàtzer o el Hospital de Jerez de la Frontera. En otros centros se comenzó a trabajar con esta organización a raíz del inicio del Ensayo Clínico OPTIMIST (H. Gregorio Marañón, H.V. Macarena, Parc Taulí).

Aspectos Éticos

Los tres estudios observacionales fueron aprobados por el Comité Ético de Investigación Clínica (CEIC) del H. Universitario de Bellvitge. En cuanto al Ensayo Clínico OPTIMIST, el CEIC del H. Bellvitge actuó como Comité de referencia, y aprobó igual que en todos los centros participantes, la realización del estudio.

Financiación

La doctoranda recibió una Beca del Hospital de Bellvitge (julio 2009 – junio 2010). Además, en el año 2010 el ISCIII se le concedió una "Ayuda Predoctoral de Formación en Investigación de la Salud" (Expediente FI10/00443).

El ensayo clínico "Optimist", cuyo investigador principal es el Dr. Miguel Santín, director de la presente tesis, ha recibido financiación del ISCIII en su convocatoria 2009 del subprograma de proyectos de investigación clínica no comercial con medicamentos de uso humano (Expte EC 09/00113-TRA126).

RESULTADOS

1. En el grupo de pacientes inmunosuprimidos

1.1 Candidatos a recibir tratamiento con agentes anti-TNF.

Artículo 1. "Prevención de la tuberculosis asociada agentes anti-TNF: estudio de una cohorte longitudinal durante 10 años" (*Clin Infect Dis* 2015; 60: 349-356).

En este estudio se incluyeron 726 pacientes con enfermedades inflamatorias inmunomediadas candidatas a recibir agentes anti-TNF de los Servicios de Reumatología, Dermatología y Gastroenterología del Hospital de Bellvitge. Se compararon tres estrategias diagnósticas en tres periodos consecutivos: la primera incluía la PT en dos tiempos, la segunda añadía QFT-GIT a la PT en dos pasos, y la tercera simplificaba la estrategia a QFT-GIT y PT en un tiempo. Se evaluaron las diferencias en la incidencia de TB en dos años de seguimiento entre los pacientes expuestos a TNF y los no expuestos y entre los 3 períodos de estudio.

Cuatro pacientes desarrollaron TB en el primer año de seguimiento tras el cribado de ITL. La incidencia de TB fue de 2,85 casos por 1000 pacientes-año entre los expuestos (3/ 1052 pacientes-año) y de 1.77 por 1000 pacientes-año en los no expuestos (1/ 566 pacientes-año). No se produjeron casos más allá del primer año de tratamiento. En el tercer periodo del estudio (utilizando TST en un tiempo y QFT-GIT) la proporción de ITL fue de 26-5%, suponiendo una descenso significativo respecto al primer periodo (TST en dos tiempos) (42.5%; P <0.001) y al segundo (TST en dos tiempos y QFT-GIT) (38.5%; P = 0.02). La incidencia de tuberculosis entre los pacientes expuestos a

agentes anti-TNF no se modificó en los 3 períodos (2.63 , 3.91 y 2.4 por 1000 pacientes-año, respectivamente).

1.2 Candidatos a trasplante.

Artículo 2 “Valores predictivos de las pruebas inmunodiagnósticas de ITL para la progresión a tuberculosis en pacientes receptores de trasplante” (*Transplant Direct* 2015;1:e12).

En este estudio se incluyeron 50 candidatos a trasplante hepático (LT) y 26 candidatos a trasplante de células madre hematopoyéticas (HSCT) del Hospital de Bellvitge. Todos los pacientes fueron cribados mediante PT y QFT-GIT y ninguno recibió tratamiento preventivo por decisión En la cohorte de LT, un paciente (4,5%) entre los 22 sujetos con resultado positivo de QFT-GIT desarrolló TB activa. Ningún paciente con QFT-GIT negativo desarrolló TB. En la cohorte de HSCT, ninguno de los 7 pacientes con QFT-GIT positivo desarrolló TB, mientras que un caso (5,3%) progresó a TB activa entre los 19 pacientes con QFT-GIT negativo. Se obtuvieron resultados similares con la PT: en el grupo de LT, 1 de 23 pacientes con PT-positiva y ninguno de los 27 sujetos con PT-negativa desarrollaron TB; En el grupo de HSCT, ninguno de los 8 pacientes con PT-positiva y uno de los 18 pacientes con PT-negativa progresaron a TB activa.

Artículo 3. “IGRAs vs. prueba de la tuberculina para la selección de candidatos a tratamiento preventivo: Una revisión basada en la evidencia” (*J Infect* 2013; 66: 381-7)

En esta revisión sistemática se incluyeron los estudios longitudinales que incluían pacientes cribados para ITL con resultados de PT e IGRAs, entre enero de 2005 y mayo de 2012. Se evaluaron las reducciones en diagnósticos de ITL y el aumento de la incidencia de TB en personas consideradas no infectadas, utilizando IGRAs en lugar de PT o como prueba confirmatoria de PT (estrategia secuencial). En comparación con la estrategia basada únicamente en PT, la reducción de la proporción de diagnósticos de ITL al sustituir la PT por un IGRA fueron de 16.7% y 5.8% con los puntos de corte de PT en 5 y 10 mm respectivamente. Con la estrategia secuencial la reducción de diagnósticos de ITL fue de 24.5% y 12.4% con los puntos de corte de PT en 5 y 10 mm respectivamente. En comparación con la estrategia basada únicamente en PT, la incidencia de TB entre las personas consideradas no infectadas aumentó con la estrategia secuencial (0.94% con T-SPOT.TB y 1.1% con QFT-GIT) en uno de siete estudios realizados en países ricos. En los países con ingresos medios o bajos, dos de cuatro estudios presentaron aumentos (0.08 y 0.03 por 100 pacientes-año, respectivamente) con la estrategia secuencial.

2. En pacientes adultos sanos con exposición reciente a Mtb: estudio de contactos

Artículo 4: "Utilidad de QuantiFERON®-TB Gold In-Tube para el estudio de contactos de tuberculosis en pacientes vacunados con BCG". En revisión (*Plos One*).

En este estudio se compararon dos estrategias diagnósticas de ITL en el estudio de contactos de TB, correspondientes a dos periodos consecutivos en el Hospital de Bellvitge. En el primer periodo, el diagnóstico de ITL se basaba en la PT solamente (≥ 5 mm). En el segundo, se añadió QFT-GIT para el diagnóstico de ITL en los contactos vacunados con BCG.

Se incluyeron 671 contactos en el estudio de los cuales 290 estaban vacunados con BCG. En el segundo periodo se redujo la proporción de diagnósticos de ITL (77.4% vs. 51.2% p <0.01) y de tratamientos preventivos prescritos (62.1% vs. 48.2% p= 0.02) respecto al primer periodo, entre los contactos vacunados con BCG . Después de una mediana de seguimiento de 5 años, no hubo diferencias en las incidencia acumulada de TB (0.62 vs. 0.29%; p= 0.59) entre el primer y segundo periodo.

Artículo 5 : “Ensayo clínico comparativo de dos estrategias para la toma de decisiones terapéuticas en el estudio de contactos de tuberculosis: estrategia estándar, basada en la prueba de la tuberculina (PT) sola, frente a la combinación de PT y QuantiFERON®-TB Gold In-Tube; Estudio OPTIMIST” (Núm. EUDRACT: 2009-017430-49). En revisión (Annals of Internal Medicine)

Estudio prospectivo, multicéntrico y comparativo de dos estrategias de diagnóstico de infección tuberculosa en 12 hospitales de España con programas de estudio de contactos. Los sujetos del estudio fueron adultos sanos contactos convivientes de pacientes afectos de tuberculosis pulmonar y/o laríngea, confirmada por cultivo, los cuales fueron asignados de manera aleatoria a una de las dos estrategias: *Rama PT*, en la cual la decisión de tratamiento se llevaba a cabo en función del resultado de la PT; *Rama PT/QFT*, en la cual la decisión de tratamiento se llevaba a cabo en función del resultado del QFT-GIT. En la *Rama PT* se realizaba PT, y en la *Rama PT/QFT-GIT* se realizaba PT, y en caso de ser positiva, se seguía de QFT-GIT. Los sujetos con PT positiva (*Rama PT*) y QFT-GIT positivo (*Rama PT/QFT-GIT*) fueron diagnosticados de infección tuberculosa y se les indicó tratamiento con isoniazida durante seis meses. Todos los participantes fueron seguidos durante dos años. El punto final de evaluación fue desarrollo de tuberculosis a los dos años de seguimiento, y diferencia de la proporción de contactos a los que se les prescribía tratamiento de infección tuberculosa. El objetivo fue comprobar la no inferioridad de la estrategia secuencial de PT/QFT-GIT respecto al estándar de PT sola para la prevención de TB en el estudio de contactos con un margen de no inferioridad de 1.5 puntos porcentuales.

Un total de 871 contactos fueron asignados al azar. Cuatro contactos en la *Rama PT* y dos en la *Rama PT/QFT-GIT* desarrollaron tuberculosis. En el análisis por intención de tratar modificado, la incidencia de TB fue 0.99% en la *Rama PT* y 0.51% en la *Rama PT/QFT-GIT* (diferencia -0.48%, intervalo de confianza del 97.5% [IC], -0-90%–1.86%); En el análisis por protocolo, las tasas correspondientes fueron de 1.67% y 0.82% en las *Ramas PT* y *PT/QFT-GIT* respectivamente (diferencia -0.85%; 97.5% IC -1.43% –3.14%). De los 792 contactos analizados en el análisis por intención de tratar modificado, la proporción de diagnósticos de ITL fue de 65.3% en la *Rama PT* y de 42.2% en la *Rama PT/QFT-GIT* (diferencia 23.1%; 97.5% IC 16.4% –30.0%).

DISCUSIÓN

En los países de baja incidencia de TB, la principal contribución a la estrategia "*End TB*" propuesta por la OMS en 2015 consiste en el diagnóstico y tratamiento de los sujetos sanos con mayor riesgo de desarrollar tuberculosis activa.

Desafortunadamente, hasta el momento actual, las pruebas diagnósticas de ITL no son lo suficientemente precisas para seleccionar qué individuos sanos son los que tienen mayor riesgo de desarrollar tuberculosis activa. Existen unos factores de riesgo claros para la reactivación de TB, pero no todos los individuos de esos grupos desarrollarán TB aunque no reciban tratamiento preventivo. Es más, incluso pautando tratamiento a todos los individuos en riesgo no se podría hablar de ausencia de riesgo para el desarrollo posterior de tuberculosis.

El manejo global de la ITL no incluye únicamente el diagnóstico. Son necesarios la educación sanitaria y el control de la adherencia y los efectos secundarios del tratamiento para asegurar el éxito de la estrategia preventiva. Los tratamientos actualmente disponibles son largos, no libres de efectos secundarios y requieren controles periódicos para la seguridad del paciente. Si bien se están investigando regímenes menos tóxicos, aprovechar al máximo las pruebas diagnósticas disponibles puede mejorar la estrategia de prevención de TB activa.

El objetivo de esta tesis es optimizar las estrategias de diagnóstico de ITL en dos grupos de riesgo: los individuos inmunosuprimidos en los que interesa maximizar la sensibilidad del proceso diagnóstico, y los sujetos sanos después de una exposición

reciente a Mtb, en los que se prefiere mejorar la especificidad de las pruebas hasta ahora disponibles.

En cuanto a los grupos con deterioro del sistema inmunitario, el estudio que recoge nuestra experiencia en la estrategia de prevención de la TB relacionada con el uso de agentes anti-TNF ha proporcionado tres resultados principales: la demostración de su eficacia sin poder anular por completo el riesgo de TB en el primer año de tratamiento y la evidencia que apoya la retirada del *booster* y la repetición sistemática del cribado de ITL tras un primer resultado negativo.

A pesar de que las guías españolas siguen recomendando el uso de la PT en dos tiempos buscando el "efecto *booster*", nuestros resultados han mostrado una asociación independiente entre la vacunación con BCG y un resultado positivo de la segunda PT, así como con los resultados discordantes (PT positiva/ QFT-GIT negativo), sugiriendo ambos datos que en los individuos vacunados con BCG, esta práctica aumenta el número de diagnósticos de ITL a costa de resultados falsos positivos. En nuestro estudio, la retirada de la PT en dos tiempos se asoció con una reducción significativa del número de diagnósticos de ITL en comparación con el periodo que la incluía, sin suponer un amento de riesgo de desarrollo de TB posterior.

En nuestra cohorte no se repitió de manera sistemática el cribado en pacientes con un resultado negativo inicial. Los dos años de seguimiento de la cohorte han demostrado la seguridad de este protocolo. Nuestra recomendación es la de repetir el cribado en caso de existir una exposición a un caso de TB, y educar a los pacientes en buscar atención médica en caso de presentar fiebre o cualquier otro síntoma sistémico. Los casos de TB activa que obtuvimos en nuestra cohorte, se presentaron antes del año de

la primera valoración, por lo tanto no se hubiesen podido detectar incluso con esta estrategia supuestamente más segura y que reiteradamente se plantea en los foros de facultativos tratantes de patologías que requieren agentes anti-TNF. Esta cuestión, compartida por clínicos de diferentes especialidades, no había tenido respuesta en la literatura hasta ahora.

En cuanto al segundo grupo de pacientes inmunosuprimidos, los candidatos a trasplante hepático y de progenitores hematopoyéticos, nuestra cohorte aporta los datos se seguimiento que permiten calcular los valores predictivos de PT y QFT-GIT para el desarrollo de TB. Nuestros resultados se suman a la evidencia de que los IGRA son pobres en predecir el desarrollo de la tuberculosis activa en los receptores de trasplante, de manera similar a los resultados reportados en cuatro cohortes de pacientes trasplantados en el momento de la publicación de este original.

Por lo que hace a las personas recientemente expuestas a un caso activo de tuberculosis, se ha confirmado la hipótesis de partida de este proyecto: la introducción de QFT-GIT en el cribado de pacientes en riesgo de desarrollar TB activa permite una reducción significativa de los tratamientos preventivos sin suponer un aumento de riesgo de TB ulterior. La hipótesis se ha confirmado en nuestra propia experiencia (estudio longitudinal) y con el máximo grado de evidencia mediante el Ensayo clínico “OPTIMIST”. Ambos estudios han mostrado además una alta tasa de compleción del tratamiento (>80%). Nuestra experiencia confirma que estas tasas son posibles cuando personal bien capacitado imparte educación sanitaria integral sobre el tratamiento y su toxicidad.

Por primera vez, un ensayo clínico controlado ha confirmado el beneficio de la inclusión de los IGRAs en el estudio de contactos adultos de pacientes con TB un entorno de baja incidencia. El beneficio estriba en evitar tratamientos preventivos innecesarios (PT positiva y QFT negativo) a pacientes que han demostrado muy bajo riesgo de desarrollo ulterior de TB. El ahorro de diagnósticos se ha ratificado no sólo en los contactos vacunados: en el grupo de los no vacunados y en los contactos valorados como de máximo riesgo (contactos íntimos de pacientes bacilíferos con cavitación en la radiografía de tórax) también se ha conseguido una reducción significativa de diagnósticos con la estrategia secuencial.

Aunque algunas sociedades e instituciones ya han implementado los IGRAs en su práctica cotidiana, las guías nacionales no los han incluido hasta el momento, en ausencia de evidencia firme de su beneficio y su mayor coste económico por unidad de test diagnóstico. El ahorro de prácticamente el 25% de los tratamientos, con sus controles, potenciales toxicidades y costes indirectos de su indicación supera con creces la diferencia económica de ambas pruebas.

Varias preguntas quedan pendientes de respuesta. El estudio coste-efectividad es necesario antes de implementar cualquier estrategia. Se necesitarán otros ensayos clínicos que comparen los resultados a largo plazo de decisiones basadas en el resultado de los IGRAs en otros grupos de riesgo. Los países con perfiles epidemiológicos diferentes necesitarán sus propios ensayos para determinar la mejor estrategia en términos de eficacia y rentabilidad. Por otra parte, la nueva generación de QFT-GIT (Plus) y de tuberculina específica (C-tb) suponen un aumento de las posibilidades diagnósticas respecto a los años del inicio de esta tesis doctoral. Estas

nuevas técnicas deberán ser probadas en las poblaciones de alto riesgo, no sólo para comparar sus resultados con los de PT e IGRAs “tradicionales”, sino para tomar decisiones basadas en sus resultados y calcular la incidencia de TB ulterior. Disminuir la posibilidad de desarrollar tuberculosis, reducirá el número de enfermos, y por tanto, de contagios. Con esta estrategia se pretende, además de la mejora individual de los sujetos, obtener beneficios en términos de salud pública.

CONCLUSIONES

Los resultados de estos estudios proporcionan evidencia firme sobre los beneficios de incluir los IGRA en el proceso de selección de los sujetos que se más se pueden beneficiar del tratamiento preventivo para el desarrollo de TB, dentro del conjunto de las poblaciones en riesgo de enfermar.

1. En cuanto a los pacientes candidatos a recibir agentes anti-TNF,
   1.1. La incidencia de tuberculosis asociada a estos tratamientos puede reducirse en gran medida a través de un protocolo sistemático de despistaje de infección tuberculosa latente, sin poder eliminar completamente el riesgo de TB, sobre todo en el primer año de anti-TNF.
   1.2. La estrategia de prueba de la tuberculina en dos pasos supone un aumento sustancial en la proporción de diagnósticos y tratamientos de infección tuberculosa, sin que ello modifique la incidencia de tuberculosis activa posterior. Esta práctica, por tanto no está justificada, a pesar de que conste todavía como parte de las recomendaciones actuales en nuestro país.
   1.3. No se requiere la repetición periódica y sistemática de las pruebas diagnósticas de infección tuberculosa latente si éstas han sido negativas al inicio.
2. En cuanto a los receptores de trasplante hepático y de progenitores hematopoyéticos, el valor predictivo positivo de QFT-GIT es bajo, y comparable al de la prueba de la tuberculina. Por tanto, la elección de una u otra técnica deberá fundamentarse en la especificidad esperada en cada entorno, factores operacionales y logísticos, económicos y en las preferencias de los pacientes.

3. Por último, en individuos adultos sanos con exposición reciente a un caso tuberculosis activa en un entorno de baja incidencia de TB, el uso de QuantiFERON®-TB Gold In-Tube para confirmar los resultados positivos de la prueba de la tuberculina, permite una reducción significativa de los diagnósticos y tratamientos de infección tuberculosa, sin aumentar el riesgo posterior de enfermedad activa.

INDEX

ABBREVIATIONS

ADA	Adenosine Deaminase
AFB	Acid-Fast Bacilli
AIDS	Acquired Immune Deficiency Syndrome
ALT	Alaninetransaminase
AST	Aspartatetransaminase
BCG	Bacillus Calmette-Guérin
CI	Confidence Interval
eCRF	Electronic Case Report Form
ESRD	End-Stage Renal Disease
HIV	Human Immunodeficiency virus
HSCT	Hematopoietic Stem Cell Transplant
IFN	Interferon
IGRAs	Interferon-gamma release Assays
INH	Isoniazid
IQR	Interquartile Range
LT	Liver Transplant
LTBI	Latent Tuberculosis Infection
MDR	Multi-drug Resistant
MHC	Major Histocompatibility Complex
Mtb	Mycobacterium tuberculosis
NPV	Negative Predictive value
NTM	Non-tuberculous Mycobacteria
PPD	Purified protein derivative

PPV	Positive Predictive Value
QFT-GIT	QuantiFERON-TB Gold In-Tube
RMP	Rifampicin
TB	Tuberculosis
TH	T-helper cells
TNF	Tumour Necrosis Factor
TST	Tuberculin Skin Test
TU	Tuberculin Unit
WHO	World Health Organisation
XDR	Extremely Drug Resistant

INTRODUCTION

1. TUBERCULOSIS: MAGNITUDE OF THE PROBLEM

Tuberculosis (TB) is one of the most ancient and devastating diseases, yet it still represents a global health challenge. The last World Health Organisation (WHO) Global tuberculosis report estimated that in 2014, 9.6 million people had fallen ill with tuberculosis, and 1.5 million people had died.[1] Alongside with HIV, tuberculosis ranks as a leading cause of death worldwide. The risk of contracting and dying of tuberculosis is not uniform around the globe, with maps of high-incidence countries showing the same tendencies of poverty and overcrowding.

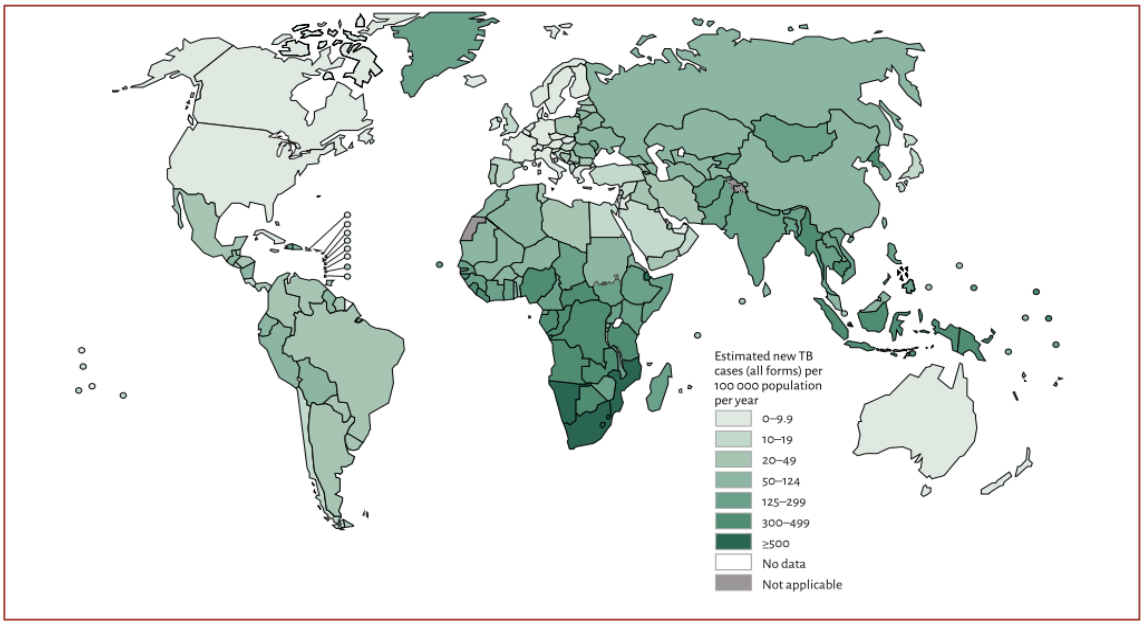

Figure 1. Estimated tuberculosis incidence rates in 2014.
Extracted from WHO Global tuberculosis report. Geneva, 2015.

Global efforts to control tuberculosis were reinvigorated in 1991, when a World Health Assembly resolution recognised TB as a major global public health problem in the light of the AIDS pandemic: "*Noting with concern that the current strategy for tuberculosis control has begun to lose its effectiveness in the industrialized countries, and that in these countries*

the declining trend of incidence has either slowed down or been reversed".[2] For the past decade, the *Stop TB Strategy,*[3] developed for the period 2006–2015, was WHO's recommended approach to achieving global targets for reductions in the occurrence of the tuberculosis disease. These targets aimed to halt and begin to reverse the incidence of tuberculosis by 2015, and to reduce prevalence and death due to tuberculosis by 50% compared to a baseline of 1990. Such targets were achieved on time globally and in 16 of the 22 high TB burden countries.

As for incidence, the rate has fallen at an average rate of 1.5% per year since 2000. Globally, the mortality rate in 2015 was 47% lower than in 1990: the target of a 50% reduction was almost met. Globally, the tuberculosis prevalence rate in 2015 was 42% lower than in 1990. Particularly in the European region, the absolute number of incident cases is slowly falling, at an average rate of 3% per year (2009-2015).[1] Spain notified 5,018 tuberculosis cases in 2014, equivalent to 10.8 cases per 100,000 population. This rate is 10% lower than the previous year -12.04 cases per 100,000-.[4]

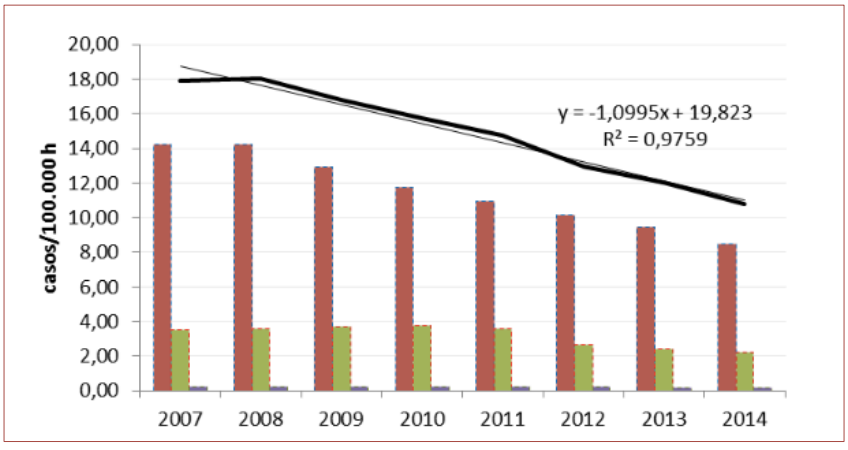

Figure 2. Tuberculosis Incidence rates expressed in cases per 100,000 population. Extracted from the Epidemiologic report of tuberculosis in Spain, 2014.[4]

The year 2015 was a watershed moment in the battle against tuberculosis. It marked the deadline for global targets set in 2006, and the transition from the *Stop TB Strategy* to the *End TB Strategy*. From 2016 onward, the goal is to end the global tuberculosis epidemic by implementing the *End TB Strategy* by means of reducing the number of tuberculosis deaths by 90% by 2030 (compared to 2015 levels) and reduce the number of new cases by 80%.[5]

2. TUBERCULOSIS: THE FIGHT AGAINST THE EPIDEMIC

2.1. From Koch's fluid to modern chemotherapy

Soon after the discovery of *Mycobacterium tuberculosis* (Mtb) as the causative agent of tuberculosis in 1882, Robert Koch created Koch's fluid ("*a glycerin extract of pure cultivations of tubercle bacilli*",[6] for use as a live vaccine; it turned out to be an ineffective preventive agent. Koch´s fluid, which was given the name of tuberculin, later proved to be a valuable diagnostic tool for active tuberculosis in cattle. Koch fluid experiments on cattle showed that some cows developed a febrile reaction 14-18 hours after tuberculin administration.[7,8] Although apparently healthy, reactive cattle were slaughtered; some of the necropsies revealed active tubercles, while others showed no active disease. Positive reactions in cattle without tubercles were attributed to dormant Mtb bacilli, as quiescent tuberculous foci were identified in some of the necropsies.[8]

The first attempts to use tuberculin in humans caused highly symptomatic reactions that hampered further investigation.[9] However, description of a new technique to administer tuberculin (the "tuberculin skin test" (TST)) by Charles Mantoux in 1908, together with its improved composition in 1934 (purified protein derivative (PPD)), made its use as a human diagnostic method possible, as systemic reactions were not observed in tested subjects; positive responders showed only an induration of the skin where the intradermal injection

had been administered.[10] The first trials, which tested tuberculin on tuberculosis patients, also showed positive results in healthy controls. It was at this time that the notion of latent bacteria or bacillary allergy emerged.[11]

In the pre-antibiotic era of sanatoriums, bed rest and physical interventions were used to treat tuberculosis.[12] The most common surgical procedure consisted of therapeutic pneumothorax: collapsing an infected lung to "rest" and favour the healing process.[13]

In 1944 Albert Schatz, Elizabeth Bugie, and Selman Waksman isolated *Streptomyces griseus* or streptomycin, the first antibiotic and first bacterial agent effective against Mtb.[14] This discovery is generally considered the beginning of the modern era of TB, although the true revolution began some years later, in 1952, with the development of isoniazid, the first oral mycobactericidal drug.[15] The advent of rifampicin in the 1970s shortened recovery times,[16] and significantly reduced the number of tuberculosis cases until the 1980s. Since 1976 there have been no changes in the short-course chemotherapy for pan-susceptible organisms, comprising two months of a quadruple regimen (isoniazid, rifampicin, pyrazinamide and ethambutol), followed by four months of isoniazid and rifampicin.[17] During the last decade, the emergence of resistant TB encouraged the development of new drugs, new dosages, and new indications and combinations of drugs.[18] Several bactericidal drugs were found to have anti-mycobacterial effects, such as quinolones and linezolid, which are now included as part of regimens that cannot rely upon isoniazid and rifampicin.[19] Current trials are looking at shortening therapy for pan-susceptible strains from six to four months by substituting rifapentine for rifampicin,[20] using high-dose rifapentine[21] and switching ethambutol for moxifloxacin,[22] or isoniazid for moxifloxacin.[23] As for multi-drug resistant (MDR) and extremely resistant (XDR) strains, bedaquilin[24] and delamanid[25] have been recently

introduced as highly effective anti-tuberculous drugs. Currently several molecules are in the pre-clinical development phase, e.g. Pretomanid (PA-824), Sutezolid (PNU-100480), SQ109, and benzothiazinones (BTZ043).[26]

As for preventive purposes, the twentieth century saw the introduction of several successful vaccines, and great expectations for an effective preparation against TB arose. In 1902 Behring proposed to utilize the bacillus isolated from humans for the vaccination of cows.[27] Calmette and Guérin passaged *Mycobacterium bovis*, which has similar antigenic properties to the virulent *M. tuberculosis* bacilli and the ability to provoke antibody formation in man, to obtain the attenuated Bacillus Calmette-Guérin (BCG) strain.[28] The immunological responses to *M. bovis* and *M. tuberculosis* are very similar, allowing the effective use of BCG as a vaccine and tuberculin as a diagnostic reagent. The significance of BCG vaccination for the evaluation of TST reactions lies in this relation.[29] BCG was successfully tested in infants exposed to family cases of tuberculosis, and although not wholly protective, BCG proved to reduce the incidence of disseminated TB disease in infants and subsequently reduced mortality.[10]

To accomplish the goals of the *End TB Strategy* there is a need to either develop a new vaccine for TB or to enhance the efficacy of BCG, the only licensed TB vaccine product in the world. There are 15 TB vaccines in clinical trials by now[10] and these employ one of three strategies: pre-exposure vaccines similar to BCG, a prime-boost strategy that seeks to augment BCG-vaccinated infants and children, and a therapeutic vaccine that enhances medical therapy.[30]

2.2. Treatment of latent tuberculosis infection as a measure of tuberculosis control

Soon after the discovery of streptomycin and isoniazid for active TB, the first experiments using isoniazid as a preventive treatment in an animal model were undertaken.[31] Initial clinical trials in humans began in 1957, with results reported in the mid-sixties, showing the benefits of isoniazid in preventing active TB.[32–36]

The management of latent tuberculosis infection (LTBI) is viewed as a critical component of the new post-2015 *End TB Strategy*, and is one of the interventions that can help countries to achieve the ambitious targets of a 90% reduction in the tuberculosis incidence rate and a 95% reduction in deaths by 2035, compared to 2015 levels.[1] It is estimated that one third of the world's population is latently infected with Mtb[37], meaning that LTBI individuals as a group constitute a huge reservoir for future cases of tuberculosis. However, given the 10% progression rate, clinical and public health services must primarily focus their energies on groups at a higher risk of disease. To this effect, the WHO issued guidelines on the management of LTBI for upper-middle and high-income countries with an estimated incidence rate of less than 100 per 100,000 population.[38] Preventive treatment of persons at high risk (people living with HIV, adult as well as child contacts of pulmonary TB cases, patients initiating anti-tumour necrosis factor (TNF) treatment and transplant recipients) has been included, for the first time, as one of the four integrated, patient-centred care and prevention components of the *End TB Strategy* for the years 2016-2035.

2.2.1. Natural history of tuberculosis. Latent tuberculosis infection

The most important reservoir of Mtb is humans, while cattle are the equivalent host for *M. bovis*. Zoonotic transmission of *M. bovis* was frequent in ancient times;[39] however, pasteurization of milk and the testing of herds decreased the public health impact of *M.*

bovis in humans in wealthy countries.[40] There are many developing countries in which zoonotic transmission is still observed.[41] Humans with active pulmonary disease currently play the dominant role in the transmission and maintenance of tuberculosis. Studies by Flügge in 1899 showed that both dried tuberculous sputum dust and the minute drops coughed out by pulmonary tuberculosis patients were capable of surviving for several days while remaining infectious.[42] These tiny particles containing tubercle bacilli -known as droplet nuclei-, are easily inhaled, and constitute the route of entry and first contact with the healthy subject. Upon inhalation, Mtb reaches the main airway and alveoli and faces the first cell-mediated barrier of the innate immune system. Adaptive immunity, however, can take as long as 42 days after Mtb exposure and infection to be established.[43–45] Alveolar macrophages phagocytose the pathogen and isolate it through a process of membrane invaginations that finally culminates in phagosome formation. From this point, infected macrophages and dendritic cells display Mtb antigens via major histocompatibility complex (MHC) class II molecules. These professional antigen-presenting cells expressing Mtb molecules travel to the lymph system and then into local mediastinal lymph nodes, where they will activate $CD4^+$ T-helper cells (TH).[46] Reaching the lymph system allows the dispersal of infection, particularly to well-oxygenated areas such as lung apices, suprarenal glands, the brain cortex, and bone and growing joints. From two to four weeks after the first encounter between bacteria of the *Mycobacterium tuberculosis* complex and macrophages, the human host prepares two types of response.[47] The first is a delayed-type hypersensitivity to tubercle bacilli, which is tissue damaging as it promotes destruction of non-activated macrophages containing proliferating Mycobacterium. The second is a cell-mediated response, which activates macrophages to kill bacteria. There is a dynamic

equilibrium between these two responses, which can lead to either TB progression or containment of Mtb.[48]

Lymphocytes TH response regulate the secretion of cytokines via endocrine and paracrine signalling, inducing a localized pro-inflammatory response that attracts mononuclear cells and T lymphocytes to build up a granuloma "the hallmark tissue reaction of TB".[49] At this point a large number of activated macrophages are recruited to the site of infection and construct the granuloma, to which lymphocytes, epithelioid and giant cells are also attracted. By depriving the centre of the granuloma of oxygen and lowering the pH, necrosis occurs internally, preventing bacterial replication, but enabling dormancy.[50]

Asymptomatic people considered to have latent TB might be better considered as part of a spectrum of infection states where at one end infection may have been eliminated, while at the other end dis-ease may be active but in a subclinical form, and between these two extremes infection is controlled in a quiescent state.[51] Of all asymptomatic subjects, only about 10% of infected individuals develop active disease;[52] most of them will progress in the first two years after exposure.[37,53] The rest will reactivate from a latent stage, usually due to a failure of the host immune response. There are two main factors in the maintenance of health or development of active disease, those depending on the Mtb and those depending on the host.[54] *M. tuberculosis* has a series of tools to enable survival inside the human host. First, a unique cell *wall* structure with high *lipid* content protects the bacterium from the highly acidic pH of the phagosome. Second, it is able to arrest phagosome-lysosome fusion. Third, it interferes in antigen presentation as well as in the normal function of $CD8^+$ T-cells, natural killer cells and the complement attack complex. Fourth, Mtb can resist host-derived antimicrobial substances (reactive nitrogen and oxygen intermediates).[47,49] As described

above, the human immune response against Mtb includes both the innate defence system (macrophage and natural killer cells, complement activation, ciliary clearance and secretions of the main airways) and the adaptive response, comprising both cellular and humoral immunity. T-cells are the main component of the cellular response that provides protection against tuberculosis and promotes memory response and granuloma formation.[49]

The main benefit of the adaptive response is the presence of immunological memory, so that a copy of each antigen found is stored in memory T-cells and will lead to a rapid and enhanced response to subsequent encounters with that same antigen.[10] This is the basis for the TST; a delayed-type hypersensitivity reaction caused by cytokines released from previously sensitized T-cells when they are exposed to PPD antigens. Although hypersensitivity is linked to protective immunity, protection is not complete. Different studies have shown that TST-positive reactors are less likely to be re-infected than non-responders,[53,55] but there is no way to prevent reactivation without specific treatment.

LTBI is defined by the detection of a specific immune response to Mtb antigens in a healthy subject (i.e. with no symptoms or signs of active disease).[56] Although LTBI represents a heterogeneous condition, with a complex spectrum of metabolic and physiologic states for both the host and Mtb,[57] the dichotomous distinction between the active and latent stages of tuberculosis allows for a more pragmatic and approachable understanding of clinical decision-making.[51]

As Mtb can only be isolated from humans when it is causing illness, i.e. in an active phase, the detection of the latent form of tuberculosis is wholly reliant on indirect measurements of immune reactivity to antigenic challenge. No culture is achievable in the latent phase; thus, there is no gold standard for its diagnosis. Since the first trials on isoniazid

effectiveness, positive reactors to TST were considered at a high risk of progression to active TB, as all active tuberculosis cases emerged in the follow-up period of positive TST individuals.[58] It was not the deep understanding of immune reactions that mattered; it was the division between two categories of risk (positive TST reactors) and low risk (negative TST responders) for the development of active tuberculosis. Those trials showed that positive reactors who received preventive therapy suffered an equal risk of subsequent development of tuberculosis to individuals who were TST negative at baseline.[58]

Progression from latent to active tuberculosis can be prevented with antibiotic treatment;[59–61] the duration of global standard regimens is six to nine months with a single drug, or at least three months with two antibiotics.[60] Given the small proportion of LTBI positive individuals who develop the active disease, the intrinsic difficulties in LTBI diagnosis, the length of the treatment, and its associated side effects, the critical question is therefore: *who should be targeted for treatment?* This question is pertinent because none of the tests currently available for diagnosing latent infection are able to accurately predict future progression to active tuberculosis.[62–65] It is widely recognised that the risk of progression is highest in young children,[52,66,67] the immunosuppressed,[68] and shortly after infection.[69] Pragmatically, therefore, these individuals should be targeted for LTBI screening and treatment.

2.2.2. Diagnosis of latent tuberculosis infection: From TST to the Interferon-gamma Release Assays

As stated above, there is no way to directly diagnose LTBI, as Mtb cannot be recovered from the host unless active tuberculosis is present. An indirect immunological assessment of exposure is made instead, by ascertaining the reactivity of host lymphocytes to mycobacterial antigens, either by testing the *in vivo* response with the TST or *in vitro* with interferon-γ Release Assays (IGRAs).

The TST consists of an intradermal injection of tuberculin. Although a variety of approaches have been used, the Mantoux technique, which is administered on the inner surface of the forearm, is one of the most widely used globally. The more common tuberculin products are PPD-S2 (Sanofi Pasteur Limited, Swiftwater, PA, USA) and PPD-RT23-SSI (Statens Serum Institute, Copenhagen, Denmark). The standard doses are 5 PPD-S units (5 Tuberculin Units (TU); 0.1 ml) or 2 TU PPD-RT-23, which are equivalent to each other. Both types of tuberculin contain a complex mixture of antigens, including those of *M. bovis*-BCG strains and several antigens of non-tuberculous mycobacteria, as well as antigens of Mtb.[10] Tuberculin will stimulate a delayed-type hypersensitivity response via T-lymphocytes. A positive TST can be detected by an induration on the site of the injection after 48-72 hours. The result is the transverse diameter of the induration, which is usually recorded in millimetres. The interpretation of TST results should take into account immunosuppression as well as the prevalence of tuberculosis in each particular setting: 5 mm is considered positive if the patient is immunosuppressed, but will be considered negative in healthy patients living in high-prevalence areas, for whom a 15 mm cut-off will apply. In some settings a uniform cut-off of 10 mm is used. These different and subjective considerations

make it difficult to compare TST properties between studies in different countries and populations.

The main limitation of TST is its low specificity due to previous BCG vaccination[70] and non-tuberculous mycobacteria infections.[71] Tuberculin contains several antigens shared by the *Mycobacterium tuberculosis* complex, *M. bovis*-BCG and non-tuberculous mycobacteria, thus a positive TST does not necessarily equate to TB infection, especially in BCG-vaccinated subjects, among whom a higher proportion of false positive results can be found. There are also other drawbacks in this apparently simple test: in subjects with chronic debilitating conditions or immunosuppression, TST may have a lower sensitivity than in healthy people. It must be administrated and read by trained professionals so as to avoid variability of the results. It also requires two clinical visits, and a positive reaction may interfere with confidentiality issues. Finally, there are very few countries (the United States, Spain and Portugal) where the two-step TST or "booster" is heeded.[72,73] This strategy aims to obtain a LTBI diagnosis in older and immunosuppressed patients, where an initial TST may boost the immune system against an older latent infection that would be evident after the second dose of tuberculin. However, the effectiveness of this second test is usually low, and several studies have reported a non-negligible proportion of false positive results, mainly in BCG-vaccinated individuals.[74,75]

Based on the quantification of the cellular immune response, *in vitro* immunodiagnostic methods have been developed by detecting interferon-gamma (IFN-γ) released by sensitized T-cells stimulated with specific Mtb antigens. These tests use a blood sample taken from the patient to measure T-cell release of IFN-γ *in vitro* after stimulation by specific *Mycobacterium tuberculosis* complex antigens. Two IGRAs have been developed:

the QuantiFERON-TB® Gold In-Tube (QFT-GIT) (Cellestis Limited, Carnegie, Victoria, Australia) and the T-SPOT.TB® (Oxford Immunotec, Oxford, UK).[76] QFT-GIT is an ELISA-based test performed on whole blood. It measures levels of IFN-γ in the supernatant of a cell suspension. The result is easily processed by software and is reported in international units per ml (IU/ml). T-SPOT.TB is an enzyme-linked immunospot assay (ELISPOT) performed on separated T-lymphocytes. The result is reported as number of IFN-γ producing T-cells. Both tests use the early-secreted antigenic target 6 (ESAT-6) and culture filtrate protein 10 (CFP10), which are encoded by genes located in the region of difference 1 (RD-1) segment of *Mycobacterium tuberculosis* complex genome. QFT-GIT also includes a third antigen, TB7.7 (RD-11). A new generation of QuantiFERON, QuantiFERON-TB Plus (Qiagen, Hilden, Germany), which includes an additional antigen tube for the stimulation of both $CD4^+$ and $CD8^+$, has recently been commercialized.[77]

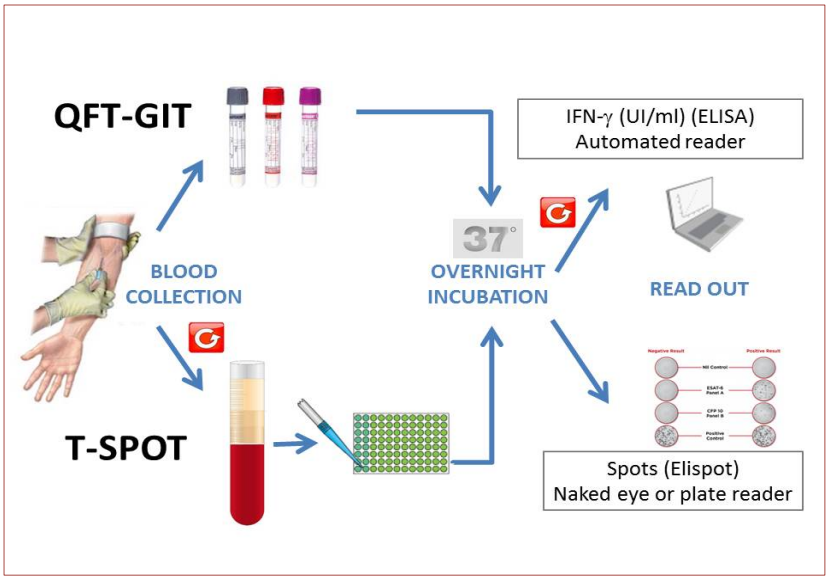

Figure 3. Diagrammatic representation of elispot and ELISA for diagnosing M. tuberculosis infection.

IGRAs detect interferon-gamma (IFN-γ) released by sensitized T-cells stimulated with specific M. tuberculosis antigens.

Table 1. Differences between both IGRAs, extracted from Lalvani, 2009.[78]

Variables	**ELISpot (T-SPOT. *TB*)**	**ELISA (QuantiFERON Gold In -Tube)**
Antigens	ESAT-6 and CFP10	ESAT-6, CFP10 and TB7.7
Positive internal control	Yes	Yes
Potential for boosting effect in repeated test	No	No
Need for return visit	No	No
Time required for results, h	16-20	16-24
Readout units	IFN-γ spot-forming cells	International units of IFN-γ
Technology platform	ELISpot	ELISA
Test substrate	Peripheral blood mononuclear cells	Whole blood
Outcome measure	Number of IFN-γ-producing T cells	Serum concentration of IFN-γ produced by T cells
Readout system	Enumeration of spots by naked eye, magnifying lens, or automated reader	Measurement of optical density values using an automated reader

Neither TST nor IGRAs are able to detect tuberculosis infection in its first stages. As they measure the adaptive immune response to Mtb, an approximately eight-week period after infection is required for a reliable result.[79] As there is no "gold standard" for LTBI diagnosis, it is not possible to properly measure the sensitivity and specificity of the available diagnostic tests. The only individuals with proven tuberculosis infection are patients with active disease, who act as surrogate markers of tuberculosis infection.

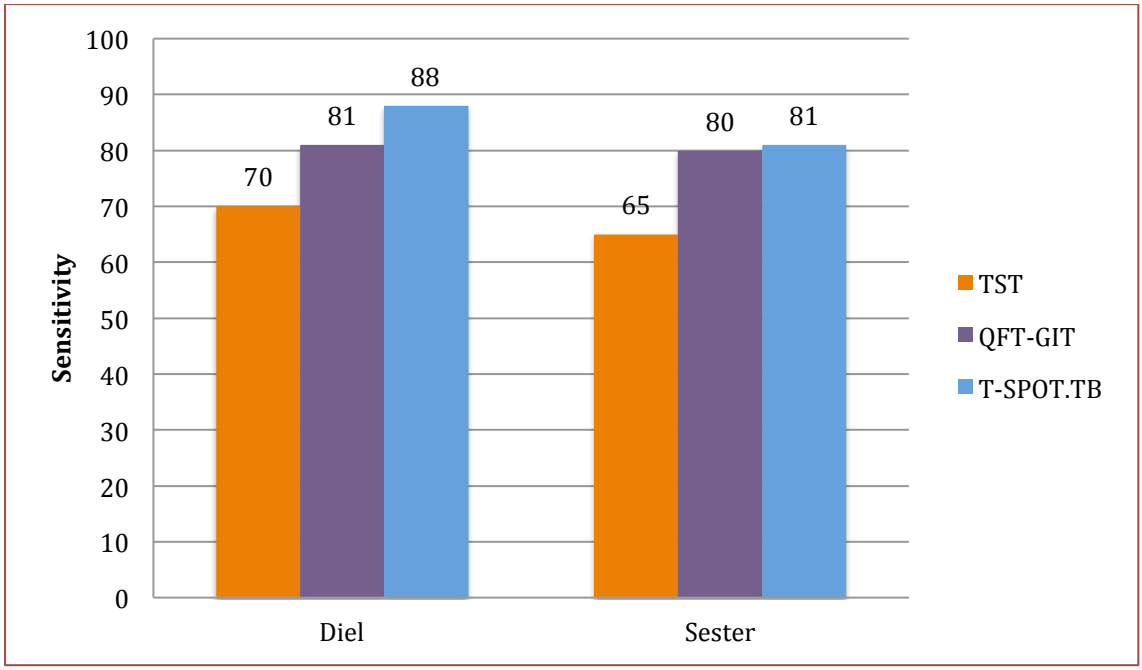

Figure 4. Sensitivity results for TST, QFT-GIT and T-SPOT.TB when tested on active disease patients. Data extracted from Diel and Sester's metaanlyses.[123,124]

The immune responses of latently infected and active tuberculosis patients are not likely to be identical, as tuberculosis itself is a well-known cause of anergy.[80] Thus, sensitivity of both TST and IGRAs may be even higher in healthy people. In such individuals a negative TST or IGRA rules out TB infection and has an almost 100% negative predictive value (NPV) for progression to active tuberculosis. A negative result, however, may not be reliable in other clinical scenarios, such as clinical suspicion of active TB or immunosuppressed patients. Several studies have tested how IGRAs perform in these specific circumstances; overall, IGRAs seem to be less affected by immunosuppression than the TST.[81–84]

The main advantage of IGRAs over TSTs is their specificity, as ESAT-6, CFP10 and TB7.7 are found in the *Mycobacterium tuberculosis* complex genome, but not in *M. bovis*-BCG, nor in the majority of non-tuberculous mycobacteria.[71] Moreover, IGRAs can differentiate a

negative result from anergy by means of a positive control, which uses phytohaemagglutinin to stimulate the production of IFN-γ.[85–87]

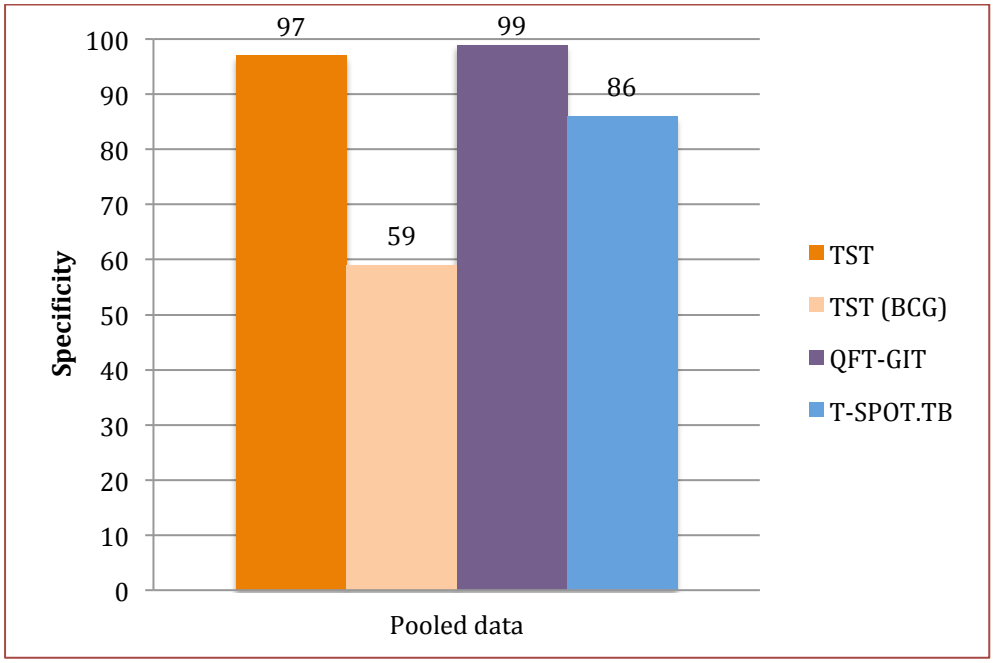

Figure 5. Specificity results for TST in general and in BCG-vaccinated subjects[117] *and for both QFT-GIT and T-SPOT.TB*[123] *in healthy, native residents of low- incidence countries without any previously known exposure to TB. Data extracted from Pai, 2008 and Diel, 2010.*

Several studies have tried to correlate the risk of progression to the response of both *in vivo* tests (TST measurement) and *in vitro* tests. This is the real and valuable patient-centred outcome. The first published meta-analysis on these data showed a slightly better positive predictive value (PPV) with IGRAs as compared to TSTs for the development of active disease. As for NPV, IGRAs presented significantly higher NPV than TSTs.[88,89]

Table 2. Positive (PPV) and negative predictive values (NPV) for developing active TB of both TST and IGRAs in overall and high-risk populations. Data extracted from Diel, 2012.[88]

Overall	**PPV (95% CI)**	**NPV (95% CI)**
TST	1.5% (1.2-1.7)	99.4% (99.2-99.5)
IGRAs	2.7% (2.3-3.2)	99.7% (99.5-99.8)
High risk groups		
TST	2.4% (1.9-2.9)	--
IGRAs	6.8% (5.6-8.3)	--

There are three notable limitations shared by both TST and IGRAs. The first is the previously mentioned low PPV; hence, neither test is useful for predicting active tuberculosis or to select who is at highest risk for developing it. The second is the inability of TSTs and IGRAs to distinguish between LTBI and active disease.[90,91] The third limitation is that the tests are unable to distinguish between recent and past infections; the response profiles for past and recent infection are identical according to the current diagnostic tools. Each result must be carefully examined and tailored, as peculiarities of the host may modify the sensitivity and specificity of both TSTs and IGRAs.

A novel specific skin test (C-tb) based on ESAT-6 and CFP10 antigens has been recently released.[92] Data on safety, concordance with QFT-GIT, and association with risk of infection were published in February 2017.[93]

2.2.3. Screening for latent tuberculosis infection: "intention to test is intention to treat"

Individuals should not be tested for LTBI unless the responsible clinician has a management plan; "intention to test is intention to treat". The low risk of progression, difficulties in treatment completion, as well as the adverse effects of preventive therapy should be taken into account when testing is offered.

The most important issue for LTBI management is to properly target candidates for screening and giving preventive therapy. There are three main populations at risk for developing tuberculosis: newly infected people –specially children-, immunocompromised patients, and newly arrived migrants from high-incidence settings.[38] The first two years after infection carry the highest risk for developing active disease.[37] For this reason, individuals at risk for Mtb infection found to have converted from a negative to a positive result for a LTBI diagnostic test, as well as individuals testing positive after a contact tracing study will be the most likely to benefit from preventive therapy. After diagnosing an individual with pulmonary TB it is mandatory to get information regarding transmission settings in order to assign priorities for the investigation.[94] The purpose of a contact examination of a TB case is to evaluate individuals for active TB disease (either to find the source or secondary cases) to administer treatment, stop further transmission, and enable diagnosis and treatment of recently infected contacts.[95]

Priorities for contact investigation depend upon:

- Characteristics of the index case, i.e. patients with cavitation on chest X-ray and positive acid-fast bacilli (AFB) smear sputum are the most infectious[96]

- Susceptibility and vulnerability of contacts: children under five years of age and immunocompromised patients are assigned the highest priority[97] and
- Circumstances of the exposure: household contacts, congregate settings.[98]

All referred contacts should be specifically asked for respiratory symptoms and current general health. Then, a TST or an IGRA (either alone or in a two-step strategy) should be undertaken. Patients with negative results obtained prior to eight weeks after the end of exposure to the index case should have the test repeated so as to avoid false negative results in the window period.[79] Subjects reporting any suggestive symptom of active tuberculosis and all individuals with positive results on the LTBI diagnostic tests should have a chest X-ray to rule out active disease. After tuberculosis disease is excluded, all LTBI patients should be offered treatment with one of the approved regimens.[60] Preventive treatment implies adherence control and screening of adverse events related to drugs, specifically liver toxicity. Liver function tests should be obtained before the beginning of treatment and checked in three to four weeks.

Household children should be started on preventive treatment even though a negative result may be found in the first LTBI test (TST or IGRA).[67] Children, especially those under five years of age, have an immature immune system that both favours false negative results and has a higher risk of developing active tuberculosis. If active disease has been excluded and a second LTBI test remains negative after the window period, treatment can be stopped. Although small children diagnosed with tuberculosis are rarely infectious, a contact investigation among their close contacts is also mandatory, as infant TB is usually the clue for finding an adult with active TB.

An IGRA or TST may be used for persons with occupational exposure (i.e. health care workers) to Mtb. If tuberculosis infection is systematically tested for and ruled out when the individuals commence their new jobs, and then every two years as long as they remain negative responders, a conversion from negative to positive result may be detected and thus treatment for a recent infection may be offered to prevent TB development. Prospective studies have shown a low risk of TB development in this classic risk group; recent recommendations do not encourage serial testing nor treatment of these individuals in a systematic programme.[99,100]

There is a vast array of immunocompromised patients who are at risk of developing active TB: people living with HIV,[101] transplantation candidates,[102,103] those exposed to biological agents,[104] patients on corticosteroids, and patients suffering from other chronic debilitating conditions such as malignancies, end-stage renal disease or diabetes.

Screening and prescription of preventive therapy in these groups have shown to reduce their tuberculosis incidence rate. As a consequence, systematic screening for TB infection in individuals has been recommended.[68]

Migrants from endemic areas arriving in high-income countries have a higher risk of TB than the autochthon population, especially in the first five years.[105] As the majority of TB in migrants arises through the reactivation of infections acquired overseas and developed in the first few years post entry, screening for LTBI in this population upon arrival may an essential element of any TB elimination strategy.[105] This group may be a hard-to-reach population, with low rates of returns to have TSTs read, thus an IGRA test might be preferred. The use of IGRAs can increase test completion rates, so control efforts should focus on those most likely to benefit from further evaluation and treatment.

2.2.4. Management of latent tuberculosis infection

The treatment of LTBI has a long history, starting from the early studies of the 1950s through to modern trials of novel combination regimens in high-risk populations.[38] Drugs for treating latent infection should possess sterilizing properties against dormant mycobacteria,[106] as such is the status of bacteria in these patients. As previously described, the very fact that isoniazid is active against LTBI suggests that at least some replication is occurring, as this drug inhibits the synthesis of mycolic acid, required by the mycobacterial cell wall during replication. Numerous randomised controlled trials and a handful of systematic reviews have demonstrated the safety and efficacy of isoniazid in the general population,[60] HIV infected,[101] anti-TNF recipients[107] and post-transplant patients[108] for preventing TB reactivation.

Rifampicin, the second proven active drug for LTBI, is a well-known antibiotic with intracellular activity that inhibits the bacterial DNA-dependent RNA polymerase and has been used against other bacterial infections in their latency period. Globally, it is most common to use six months of daily isoniazid monotherapy (dose 300 mg) although recent data suggest that four months of daily rifampicin (dose 600 mg), or three months of combined therapy with both antibiotics (isoniazid 300 mg plus rifampicin 600 mg). A novel regimen consisting of 12 doses of a weekly regimen of rifapentin (900 mg) and high-dose isoniazid (900 mg) has recently been described as effective,[109] and is currently used in the USA under directly observed therapy.

The three common treatment regimens are generally well tolerated. Patients must always be told about symptoms that may indicate the development of adverse effects so that they seek care promptly. The most common life-threatening adverse event resulting from

isoniazid is liver toxicity. Not only is a clinical assessment needed, but liver function tests are also essential in the third or fourth week of treatment, and then in the second and third month, as well as in the instance of patients reporting hepatitis signs and symptoms. Biochemistry variations on alaninetransaminase (ALT) and aspartatetransaminase (AST) precede symptoms as indicators of liver damage, which is the reason for testing apparently healthy patients. Another frequent adverse event of isoniazid is peripheral neuropathy, which can easily be prevented with daily pyridoxine.[110]

Patients taking rifamycins can present flu-like symptoms in their first week of treatment. This adverse event is more common in intermittent regimens (i.e. weekly doses). Hypersensitivity reactions are more frequently related to rifamycins, although they might also be caused by isoniazid. These reactions are mostly seen in the second to fourth week of treatment and consist of a rash and fever, ranging from harmless itching of the skin to severe clinical pictures including Stevens-Johnson syndrome.

Although the prevention of drug resistant TB cases is of great concern for clinicians and health authorities, there are no recommended regimens for LTBI treatment of those exposed to MDR-TB cases, as there are no sterilizing drugs for dormant bacilli apart from isoniazid and the rifamycins. Guidelines advise only “watchful waiting” of these individuals to assure an early diagnosis in case of the development of active TB.[38] Some studies have reported the experience of prophylactic treatment given to young children, who are household contacts of MDR-TB cases.[111] This should always be supervised by a physician with knowledge and experience of MDR-TB prophylactic treatment, based on drug susceptibility results of the index case.

For all LTBI patients diagnosed within a contact study, it is mandatory for the clinician to check the drug susceptibility of the index case and to ensure that the transmitted bacteria are likely to be sensitive to the chosen LTBI treatment regimen.

The first and major reason for treatment failure, and thus for the development of active TB, is poor medication adherence.[59] Unfortunately, direct observation of each patient is unaffordable for most countries. LTBI treatments are lengthy and a close monitoring of subjects is required to ensure high treatment compliance. Several methods for checking treatment compliance have been validated: they include the Eidus-Hamilton test[112] and commercially available reactive stripes that can easily detect urine metabolites of isoniazid. Checking the orange-coloured urine of subjects taking rifampicin may also be useful for adherence control.

CONTEXT OF THE INVESTIGATION;

JUSTIFICATION

In low-incidence TB settings, screening and prescription of preventive therapy in populations at risk of developing active tuberculosis represent the main workload in TB units.[113] Healthy individuals with recent exposure to an active tuberculosis case, whether in their household or their work environment (i.e. healthcare professionals), as well as the growing group of patients about to receive immunosuppressive drugs constitute the largest part of the collaboration in the *End TB Strategy*. Each diagnosis and decision for treatment is part of a tailored preventive strategy. With the same test results one individual might receive treatment and another might not. Age, comorbidities, type of exposure and type of immunosuppression need to be balanced, and benefits must outweigh the risks of preventive treatment. The prescription, the control of adherence, and the rapid detection of adverse events are all equally important for the strategy to yield results, i.e. to achieve high rates of treatment completion.

The Bellvitge TB Clinical Unit benefits from full-time staff, comprehensive protocols for different patients' profiles, and prospective gathering of epidemiological, diagnostic and outcome data from all evaluated individuals. The staff prioritizes contact investigation in all tuberculosis cases, and receives every patient prior to immunosuppression with anti-TNF agents from Dermatology, Rheumatology and Gastroenterology Departments, as well as kidney transplant recipients as a part of their pre-transplant evaluation. Other kinds of immunosuppressed patients are also examined. Preventive therapy is prescribed, if needed, and checked in the outpatient clinic.

The current project was conceived in this fertile ground, seeded by a previous doctoral project in the management of LTBI in immunosuppressed patients soon after IGRAs were implemented in our centre in 2006.[114–116]

TST has been the only method for diagnostic approach to tuberculosis infection for a hundred years, although sensitivity is less than 70% in culture-proven active cases.[117] As there is no gold standard for LTBI diagnosis, studies aiming to assess the accuracy of new diagnostic tests for LTBI, are inexact. For this reason, the WHO developed a hierarchy of reference standards to assess the performance of IGRAs.[118] Direct comparison and agreement tests between IGRAs and tuberculin have been explored in several settings for the last decade, as have studies assessing sensitivity and specificity and correlation of IGRA results and exposure gradient.[119] In the last years several cohorts have been followed and data on predictive values have been provided.[120]

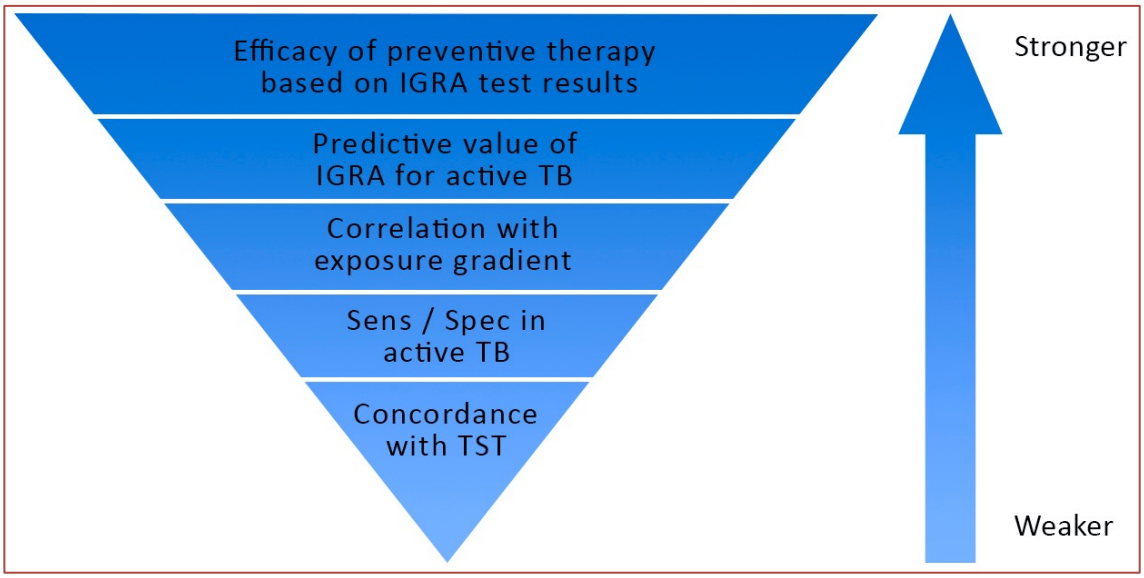

Figure 6. Hierarchy of reference standards used to assess the evidence base. Modified from WHO, 2011[118].

At the time this project began, there was little information on longitudinal and prospective studies assessing IGRA results and patients' outcomes. Until then there had been no conclusive evidence-based recommendations, as no studies had used IGRAs in their

decision-making process to provide preventive therapy. The fact that IGRAs are currently recommended by international guidelines and societies as part of several diagnostic approaches for latent TB infection diagnosis under low quality evidence, contributes to non-standardized practices around epidemiologically similar countries.

In immunosuppressed populations, sensitivity is the most desirable property for a diagnostic test, so as not to leave any subject unprotected from tuberculosis reactivation. As mentioned previously, a former fellow published the assessment of QFT-GIT in different cohorts of immunosuppressed populations.[114–116] The analysis of the anti-TNF cohort in our centre before the implementation of QFT-GIT led to a modification in the clinical protocol of LTBI screening, since a higher than expected prevalence of LTBI was found. It was almost 50%, higher than the estimated "third of the global population".[37] The diagnostic strategy was then modified by means of adding QFT-GIT and simplifying the two-step TST to a single-step practice.

A cohort of liver transplant candidates and hematopoietic stem cell transplant recipients was also evaluated for QFT-GIT performance in an observational study.[114] None of these patients received preventive therapy, as treatment decisions relied on the transplant group, which decided that the risk of side effects were too great. The follow-up period allowed the calculation of predictive values of both TST and QFT-GIT for the development of active disease in theses populations.

In healthy populations evaluated after recent exposure to an active case of tuberculosis, IGRAs had shown a high NPV.[62] However, they were only slightly better than TSTs in terms of predicting development of subsequent active TB. This fact led to a shared disenchantment after the initial expectations of IGRA tests. In this project, we relied on the

true benefit that IGRAs could add to the screening of latent TB infection by better selecting healthy patients at risk for developing TB. This solid conviction led to three actions. First, IGRA were included as part of the diagnostic process of LTBI in BCG-vaccinated contacts of TB at the Clinical TB Unit. Second, the doctoral candidate elaborated a literature study on the ability of IGRAs to reduce the number of people considered for preventive treatment. Third, we decided to test our hypothesis through a clinical trial.

HYPOTHESIS

Assumptions

1. The higher the proportion of LTBI diagnosis among tested individuals, the better the strategy for preventing development of active tuberculosis. For this reason, a two-step TST practice has been used for years, and is still recommended in several guidelines.[121] However, this practice poses a high rate of false positive results, especially in BCG-vaccinated patients.

2. There is no solid evidence on the best strategy for diagnosing LTBI. IGRAs exceed TSTs in diagnostic accuracy, and have been progressively assimilated into clinical practice, either replacing or accompanying TSTs. The lack of well-evidenced practices impedes the homogeneity of recommendations and guidelines.

3. IGRAs could improve LTBI diagnosis strategy in both populations at risk for developing active tuberculosis.

3.1 Immunosuppressed individuals: IGRAs can improve TST sensitivity in this high-risk population. Maximum sensitivity in these populations is foreseen, as they pose the highest risk for development of active tuberculosis. IGRAs have shown better diagnostic performance than TSTs in such settings.[81]

3.2. Healthy subjects who have recently been exposed to an active case of pulmonary or laryngeal tuberculosis: IGRAs can improve TST specificity in this setting. IGRAs have shown an almost 100% NPV for developing tuberculosis.[62] Leaving negative-IGRA responders without preventive therapy could reduce the number of individuals receiving treatment, without increasing the risk of subsequent active tuberculosis.

4. Diagnostic tests are of great importance for LTBI management. However, control of treatment adherence as well as adverse events, which are the clue for treatment completion, and thus for efficacy, are usually overlooked.

Hypothesis

LTBI management is a crucial component of tuberculosis prevention in high-risk individuals. A global approach including diagnosis and treatment completion ensures good outcomes. Increasing the proportion of LTBI diagnosis does not imply better results; on the contrary, it could lead to an increase in unnecessary treatments.

1. In immunosuppressed individuals: The addition of an IGRA test might benefit the diagnostic strategy in order to maximize TST sensitivity.

2. In recently infected individuals (contact tracing studies): Implementing the use of IGRAs could narrow the proportion of preventive therapy in individuals at risk of developing active tuberculosis, without increasing its risk.

AIMS

Global aim:

Improve the management of LTBI—particularly by the implementation of IGRAs—in populations at risk of developing active tuberculosis

Specific aims:

1. In immunosuppressed populations

1.1 Candidates to anti-TNF agents

First, to assess whether a comprehensive latent tuberculosis screening and treatment program can prevent anti-TNF–associated tuberculosis.

Second, to determine whether the currently recommended two-step TST approach can be safely replaced by a single-step TST plus QFT-GIT screening strategy by reducing the number of patients treated without increasing the risk of subsequent active tuberculosis.

Third, to evaluate the need for systematic retesting in patients with negative latent tuberculosis screening at baseline.

1.2. Transplant candidates

To assess the usefulness of QFT-GIT in predicting the development of active tuberculosis in comparison to the TST in patients undergoing liver transplant and hematopoietic stem cell transplant.

2. In recently tuberculosis-infected individuals (contact tracing)

First, to ascertain whether using IGRA, either in place of TST or to confirm a positive TST, might reduce the number of people considered for preventive treatment, without leading to a significant increase in the risk of subsequent active tuberculosis.

Second, to determine whether the use of QFT-GIT as a confirmatory test of the TST to target preventive therapy in tuberculosis contacts might reduce the number of individuals receiving treatment without an increased risk of subsequent active tuberculosis, as compared to a TST-based strategy.

MATERIALS AND METHODS

1. SETTING

Since the foundation of the Infectious Diseases (ID) Department at Bellvitge University Hospital in 1977, it has traditionally been admitting tuberculosis patients for isolation purposes. On discharge, patients were systematically followed up by ID staff and a clinical nurse at a single-tuberculosis outpatient clinic. In 1988 the Tuberculosis Unit, as it is called today, took its first steps, aiming to avoid unnecessary admissions of tuberculosis patients and to provide simple and efficient care to both patients and their contacts. The health district where the unit is located established a protocol to ensure the prevention, control and treatment of tuberculosis. In summary, these were the objectives to develop this New Plan of Tuberculosis Control:

1. Ensure treatment completion of TB patients.
2. Ensure contact investigation and preventive therapy indications.
3. Standardize of the TST and microbiological procedures.
4. Enhance declaration of each tuberculosis case to the Health Department.
5. Improve adherence to tuberculosis treatment in those groups at risk of withdrawal.
6. Screen latent and active tuberculosis in difficult to reach populations (homeless and those living in poverty belts).
7. Train General Practitioners and Clinical Nurses in order to ensure rapid diagnoses of active TB cases.
8. Care for patients in special situations: people living with HIV, disease with resistant strains or patients infected with non-tuberculous mycobacteria.

The clinical activity at the TB Unit has expanded since the first anti-TNF treatments were prescribed at the hospital. In 2002 we began the screening of LTBI in candidates to anti-TNF, in collaboration with the Rheumatology, Dermatology and Gastroenterology Departments.

In 2006, the QuantiFERON®-TB Gold In-Tube was implemented in our clinic, initially as a part of three research projects with liver and stem cell transplantation candidates, patients considered for anti-TNF agents and HIV patients. After the study projects, we began to use QFT-GIT in BCG-vaccinated contacts of TB, and after two years of positive experience in reducing the proportion of diagnoses and treatments prescribed, the test became available to the rest of the hospital. In the last four years a new screening program for LTBI in candidates for kidney transplantation is being carried out. Also, our unit acts as a reference centre for MDR-TB and difficult-to-treat mycobacterial infections.

2. CLINICAL PROTOCOL FOR LATENT TUBERCULOSIS INFECTION AT THE TB UNIT

The clinical protocol includes:

2.1. Baseline assessment

- Data gathering: demographics, comorbidities and treatment during the previous three months, previous history of tuberculosis or LTBI, BCG vaccination status, and past risk factors for tuberculosis, description of the current risk factor for developing active TB.
- Exclusion of active tuberculosis through suggestive symptoms (fever, cough, weight loss, and drenching sweats) and chest X-ray.

2.2. Diagnosis of latent infection

- Patients who have already been diagnosed or have radiographic findings of healed tuberculosis are assumed to have “past” LTBI and no immunodiagnostic tests are carried out. If previously treated, they do not undergo further evaluation. If not, preventive therapy is offered.
- In patients who have never been tested for tuberculosis infection, the TST is performed by the Mantoux method using 2 TU of PPD RT23 (Statens Serum Institute, Copenhagen, Denmark) in the volar aspect of the forearm. TST is administered and assessed by two experienced nurses at the Tuberculosis Unit. When appropriate, QFT-GIT is performed before TST and in accordance with the manufacturer’s instructions.

2.3. Preventive therapy

All newly LTBI diagnosed patients receive six months of isoniazid treatment. The alternative regimen is four months of rifampicin. Patients about to receive preventive treatment receive extensive counselling about tuberculosis infection, its treatment, and possible side effects. During treatment, patients have access to their treatment team at the Tuberculosis Unit. Reinforcement visits and blood test monitoring are performed at months 1, 3 and 6. Adherence to isoniazid is assessed by detecting isoniazid metabolites in the urine with the Eidus-Hamilton test,[112] and adherence to rifampicin is assessed by checking the urine colour.

3. SCIENTIFIC PRODUCTION

The clinical studies included in this thesis comprise three longitudinal cohort studies, a systematic review and a clinical trial. The three observational studies are retrospective analyses of prospectively gathered data, recorded as part of the clinical assistance in the Tuberculosis Unit.

3.1. Study design

Article 1. Prevention of Anti–Tumour Necrosis Factor– Associated Tuberculosis: A 10-Year Longitudinal Cohort Study (Clin Infect Dis 2015;60:349-356).

A prospective observational cohort study, originally designed in 2005 aiming to evaluate the efficacy of the comprehensive clinical program for tuberculosis prevention in patients receiving anti-TNF therapy. We included consecutive patients between January 2003 and December 2013. Three periods with different LTBI diagnostic strategies and their outcomes during a two-year follow-up were evaluated. The Ethics Committee of Bellvitge University Hospital approved the study (approval number PR235/11).

Article 2. Immunodiagnostic Tests' Predictive Values for Progression to Tuberculosis in Transplant Recipients. A Prospective Cohort Study (Transplant Direct 2015;1:e12).

A prospective cohort study was conducted to assess predictive values of TST and QFT-GIT for developing active TB in consecutive transplant candidates between July 2008 and July 2010; it was originally designed in 2006 to assess the usefulness of the QFT-GIT for predicting the development of active TB in comparison to the TST in patients undergoing liver transplant (LT) and hematopoietic stem cell transplant (HSCT). These patients did not

receive preventive therapy as their treating physicians considered that risks outweighed the benefits of such prescription. The ethics committee of Bellvitge University Hospital approved the study (approval number PR248/06).

Article 3. Interferon-gamma release assays versus tuberculin skin test for targeting people for tuberculosis preventive treatment: An evidence-based review (J Infection 2013;66:381-387)

We aimed to ascertain whether using an IGRA either in place of TST or to confirm a positive TST might reduce the number of people considered for preventive treatment, without leading to a significant increase in the risk of subsequent active tuberculosis.

This literature review ensured the hypothesis of the next two studies in contact investigation, taking advantage of the better specificity and NPV of IGRAs for targeting preventive treatment.

Article 4. QuantiFERON®-TB Gold In-Tube for contact screening in BCG-vaccinated adults: A longitudinal cohort study (PloS One; under review).

A retrospective comparative study of two screening strategies for tuberculosis contact tracing (before and after the implementation of QFT-GIT in BCG-vaccinated contacts) was performed between January 2006 and December 2010. The study was designed in 2008, when QFT-GIT was added to the LTBI screening strategy in BCG-vaccinated contacts. We hypothesized that using the QFT-GIT to target TB contacts would reduce the number of individuals diagnosed with, and treated for, TB infection compared to the previous TST-only

strategy, without an increased risk of active TB in the follow-up period. The Ethics Committee of Bellvitge University Hospital approved the study (PR269/11).

Article 5. QuantiFERON®-TB Gold In-Tube as a confirmatory test for tuberculin skin test in tuberculosis contact tracing: A non-inferiority clinical trial (Annals of Internal Medicine; under review)

We conducted an open-label, multicentre, randomised trial, to test the non-inferiority of a two-step strategy with the tuberculin skin test, followed by QuantiFERON®-TB Gold In-Tube as a confirmatory test (the TST/QFT arm) to the standard TST-alone strategy (TST arm), for targeting preventive therapy in household contacts of tuberculosis. Eight hundred and seventy-one participants from 12 Spanish hospitals were randomized. The follow-up period was 24 months. The primary endpoint was the development of tuberculosis, with a non-inferiority margin of 1.5%. The Research Ethics Committee at each participating site approved the study protocol, with Bellvitge University Hospital acting as Research Ethics Coordinator (ref AC111/09).

3.2. Participants and follow-up

As mentioned, consecutively evaluated patients for LTBI screening at the TB Unit were included in the cohort groups of anti-TNF recipients, transplant candidates or contact investigation, if they met the inclusion criteria for the respective analysis.

The three observational studies had different periods of follow-up: 24 months for the anti-TNF cohort, 48 months for the transplant candidates and 5 years for the contacts of active TB. We checked and updated the final outcomes annually until the pre-established period was completed. This update consisted of reviewing the medical charts and contacting patients to assess their vital status and the development of tuberculosis. We recorded the type and length of anti-TNF therapy, date and drugs after transplantation and further contacts with TB cases for each study respectively. Patients lost to follow-up were matched with the registry of tuberculosis patients at the Health Department of Catalonia to ensure that no incident cases were missed.

As for the clinical trial, all adult household contacts of pulmonary tuberculosis patients who were evaluated at any of the participating sites were invited to take part in the trial. Inclusion and exclusion criteria as well as the schedule of appointments were specified in the protocol (Annex).

3.3. Statistical analyses

Each paper describes the ad-hoc statistical analysis in great detail. In general terms, the statistical analyses were made with the SPSS (Statistical Package for the Social Sciences) software (version 17 and 22). Differences between groups were assessed using the $\chi 2$ or the analysis of variance tests if ≥2 groups were compared. Student t test and Mann–Whitney U nonparametric test compared continuous variables, as appropriate. The level of significance was fixed at $\alpha = 5\%$, and confidence intervals (CIs) for differences in proportions were estimated using OpenEpi software version 2.3.1.[122] The incidence of active tuberculosis was expressed as the incidence density rate with 95% CIs. The risk of tuberculosis was assessed by Cox proportional hazard regression analysis to assess the effect of covariates and the

differences between groups.

As for the clinical trial, an electronic case report form (eCRF) was designed in Access and built with an online database in which the information was recorded. Randomisation was stratified by centre in a 1:1 allocation ratio, using a computer-generated randomisation list integrated into the eCRF. Details on the statistical analysis and the included variables can be found in the trial protocol. The Clinical Trials and Statistical Unit at Bellvitge University Hospital-IDIBELL downloaded and analysed the data contained in the electronic case report forms (eCRF) following the statistical analysis plan.

4. ETHICS

In all clinical studies approval was obtained from the Ethical Committee, and written informed consent was obtained from all subjects.

5. FUNDING AND GRANTS

The doctoral candidate received the following grants and funding during her research process:

-Post-Residence grant from Bellvitge University Hospital (July 2009)

-Grant P-FIS from the Insituto de Salud Carlos III [PI10/00443]

In addition, the clinical trial "Comparison of two strategies for therapeutic decision-making in tuberculosis contact tracing: a standard strategy based on TST alone vs. TST combined with QuantiFERON®-TB Gold In-Tube (QFT)", was partially supported by *Insitituto de Salud Carlos III – Convocatoria 2009 de Ayudas para el fomento de la traslación de la aplicación de medicamentos de uso humano, huérfanos y terapias avanzadas (Exp TRA-126)*.

RESULTS

1. Immunosuppressed population

1.1 Patients about to receive anti-TNF agents

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MAJOR ARTICLE

Prevention of Anti–Tumor Necrosis Factor–Associated Tuberculosis: A 10-Year Longitudinal Cohort Study

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Background. The extent to which anti–tumor necrosis factor (TNF)–associated tuberculosis can be prevented is unclear, and there is no established guidance on the optimal screening strategy for latent tuberculosis (LTBI) in patients about to start anti-TNF therapy. We aimed to determine the effectiveness of a comprehensive program for the prevention of anti-TNF–associated tuberculosis, and to evaluate 3 LTBI screening strategies and the need for retesting patients with negative results at baseline.

Methods. In total, 726 patients were screened prior to anti-TNF therapy using 1 of 3 diagnostic strategies over 3 consecutive periods: first, a 2-step tuberculin skin test (TST); second, a 2-step TST plus QuantiFERON-TB Gold In-Tube test (QFT-GIT) (2-step TST/QFT); and third, a single-step TST plus QFT-GIT (TST/QFT). Infected patients were offered preventive therapy. We assessed differences in the incidence of tuberculosis between anti-TNF exposed and nonexposed patients, and between the 3 study periods.

Results. Tuberculosis developed during the first year in 2.85 per 1000 exposed patient-years (3/1052 patient-years) and 1.77 per 1000 nonexposed patient-years (1/566 patient-years). No cases occurred beyond the first year of treatment. LTBI diagnoses decreased with the single-step TST/QFT (26.5%) compared with the 2-step TST (42.5%; $P < .001$) and 2-step TST/QFT (38.5%; $P = .02$); the incidence of tuberculosis among exposed patients did not change significantly across the 3 periods (2.63/1000, 3.91/1000, and 2.4/1000 patient-years, respectively).

Conclusions. Although anti-TNF–associated tuberculosis can be reduced, some risk remains during the first year of therapy. Neither the 2-step TST nor systematic retesting after negative baseline testing is justified.

Keywords. tuberculosis; latent tuberculosis infection; anti-TNF; QuantiFERON-TB Gold In-Tube; IGRAs.

Biological agents, particularly tumor necrosis factor–α antagonists (anti-TNF), have dramatically improved the clinical course of patients with immune-mediated inflammatory diseases (IMIDs). However, their introduction has been associated with an increase in the risk of tuberculosis. The reported incidence of tuberculosis ranges from 24.4 cases per 100 000 in the United States to 522 cases per 100 000 in Spain, reflecting the different tuberculosis burdens of each region [1, 2]. Differences have also been related to the type of anti-TNF, with the monoclonal antibodies infliximab and adalimumab posing the greatest risk [3, 4].

Anti-TNF–associated tuberculosis mainly occurs due to the reactivation of a latent tuberculosis infection (LTBI) [5]. Consequently, screening and treatment for LTBI before starting anti-TNF therapy are essential for preventing tuberculosis reactivation [6], and its effectiveness has been proved in the BIOBADASER (Spanish Society of Rheumatology Database on Biologic Products) group [7]. Nonetheless, tuberculosis still occurs, leading to questions regarding the effectiveness

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of current clinical practice. Although incomplete protection has been attributed to poor compliance with the guidelines [8], the suboptimal diagnostic tests, poor adherence with preventive therapy, and incidental new infection may also be contributing factors [8–10].

The best diagnostic strategy for LTBI prior to anti-TNF therapy remains a matter of debate. The only evidence on the effectiveness of preventive measures so far is based on screening with a 2-step tuberculin skin test (TST) [7]. However, although the 2-step TST approach increases the detection of remote infection, it may also increase the false-positive rate, leading to a greater number of unnecessary preventive treatments. The ex vivo interferon-γ release assays (IGRAs) may overcome the limitations of the TST [11, 12]. However, it remains unclear whether or not they should be used and how best to implement them. This is reflected in differences in national guidelines [6, 13–20].

It is also unclear if patients who tested negative for LTBI before starting anti-TNF therapy should undergo systematic reassessment. Despite being advocated [21–23], there is no supporting evidence for this practice.

In this study we aimed to answer 3 main questions. First, to what extent can anti-TNF–associated tuberculosis be prevented by a comprehensive LTBI screening and treatment program? Second, can the 2-step TST approach be replaced by a single-step TST plus QuantiFERON-TB Gold In-Tube (QFT-GIT) screening strategy? Third, is systematic retesting necessary for patients with negative baseline LTBI screening?

METHODS

Study Design, Setting, and Participants

A prospective observational cohort study was conducted between January 2003 and December 2013, at the Tuberculosis Unit of Bellvitge University Hospital, Barcelona, Spain.

Consecutive patients with IMIDs needing anti-TNF agents and referred for LTBI assessment between January 2003 and December 2011 were eligible for the study. Follow-up data were collected up to 31 December 2013. Diagnostic results from the first 214 patients were published in a previous study on the performance of QFT-GIT in anti-TNF candidates [24].

Clinical Program and Interventions

Screening for LTBI followed a preestablished clinical protocol, as follows: Baseline assessment included demographics, the type and duration of IMID, and treatment during the previous 3 months. Comorbidities, previous history of tuberculosis or LTBI, BCG vaccination status, and risk factors for tuberculosis were also collected. To rule out active tuberculosis, all patients had a chest radiograph done and were asked for specific symptoms of active tuberculosis (fever, cough, weight loss, and drenching sweats). If there was evidence of radiographic signs or suggestive symptoms of active tuberculosis, respiratory specimens were collected for smear and culture. The decision on whether to start anti-TNF was postponed until a firm diagnosis of the current respiratory process was obtained.

As for diagnosis of LTBI, patients who had already been diagnosed or had radiographic findings of healed tuberculosis were assumed to have "past" LTBI and no immunodiagnostic tests were carried out. If previously treated, they did not undergo further evaluation. If not, preventive therapy was offered. The whole diagnostic assessment of LTBI, including immunodiagnostic tests, was carried out among asymptomatic patients with normal chest radiograph and no previous testing for tuberculosis infection. Those with a positive result in any immunodiagnostic test were diagnosed as being "new" LTBI. We changed the diagnostic strategy twice over the study period as new evidence on the field became available.

We defined 3 consecutive periods in which 3 different diagnostic strategies were applied. In the first period (January 2003–October 2006), a 2-step TST strategy was followed: either an induration of ≥5 mm in the first test or an increase of ≥5 mm in the second test was considered positive [25]. In the second period (November 2006–May 2008), QFT-GIT was added to the previous 2-step TST, and a LTBI diagnosis was established based on positive TST or QFT-GIT results. In the third period (June 2008–December 2010), the strategy was simplified to a single-step TST and QFT-GIT.

The TST was performed by the Mantoux method using 2 U of purified protein derivative RT23/0.1 mL (Statens Serum Institut, Copenhagen, Denmark) in the volar aspect of the forearm. TST was administered and assessed by 2 experienced nurses at the Tuberculosis Unit. QFT-GIT was performed before TST and in accordance with the manufacturer's instructions [24]. LTBI screening was not repeated in patients with negative results at baseline.

In all newly LTBI diagnosed patients, a 9-month isoniazid treatment schedule was recommended. Rifampin for 4 months was used as the alternative regimen. Patients about to receive preventive treatment received extensive counseling about tuberculosis infection, its treatment, and the possible adverse events. During treatment, patients had access to their treating team at the Tuberculosis Unit. Reinforcement visits and blood test monitoring were performed at months 1, 3, 6, and 9. Adherence to isoniazid was assessed by detecting isoniazid metabolites in the urine with the Eidus–Hamilton test [26], and adherence to rifampin was assessed by checking the color of the urine. Anti-TNF treatment was postponed until completion of at least 4 weeks of therapy for LTBI.

Main Outcome Measures

We aimed to achieve at least 24 months of follow-up, and checked and updated the final outcomes annually until 31

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December 2013. This update consisted of reviewing the medical charts and contacting patients to assess their vital status, the development of tuberculosis, and the type and length of anti-TNF therapy. Patients lost to follow-up were matched with the registry of tuberculosis patients at the Health Department of Catalonia to ensure that no incident cases were missed [27].

Data Analysis

We defined 2 cohorts according to anti-TNF therapy exposure: patients who received anti-TNF agents at any time since screening (exposed) and patients who did not (nonexposed). Candidates who were not prescribed any anti-TNF agents contributed person-time to the nonexposed cohort. Patients who received at least 1 dose of anti-TNF therapy were included in both groups: the time from the date of screening (date S) to the first dose of anti-TNF (date D) represented the person-time attributable to the nonexposed cohort (date S–D); from date D onward, they contributed person-time to the exposed cohort (Figure 1). The effectiveness of the program was assessed by comparing the tuberculosis incidence rates of both the exposed and nonexposed cohorts. For risk estimation purposes, patients were censored after 2 years from date D (exposed cohort) or date S (nonexposed cohort), as previous studies had defined the highest risk of tuberculosis reactivation during the first 2 years of exposure [1, 4]. Early censoring causes included death, development of tuberculosis, or loss to follow-up, whichever came first.

Differences between groups were assessed using the χ^2 or the analysis of variance tests if ≥2 groups were compared. Student *t* test and Mann–Whitney *U* nonparametric test compared continuous variables, as appropriate. The level of significance was fixed at $\alpha = 5\%$, and confidence intervals (CIs) for differences in proportions were estimated using OpenEpi software version 2.3.1 [28]. The incidence of active tuberculosis was expressed as the incidence density rate with 95% CIs. The risk of tuberculosis was assessed by Cox proportional hazard regression analysis to assess the effect of covariates and the differences between groups (exposed, nonexposed, and exposed in different periods). Agreement between TST and QFT-GIT was calculated using the Cohen kappa coefficient (κ). Statistical analyses were performed with SPSS statistical software (version 17.0, SPSS Institute Inc, Chicago, Illinois) and the VassarStats website [29].

Ethical Considerations

The ethics committee of Bellvitge University Hospital approved the study (approval number PR235/11).

RESULTS

Characteristics of the Cohort

During the study period, 726 patients were referred for evaluation. Changes in the characteristics of the cohort over the 3 periods are shown in Supplementary Table 1. Five hundred forty-two patients (74.7%) started at least 1 course of anti-TNF treatment at some point during the study period. No cases of active tuberculosis were diagnosed at baseline.

Diagnosis of LTBI and Preventive Therapy According to the Study Period

Overall, among 726 screened patients, 34 (12.7%) were excluded from the final analysis of differences in LTBI prevalence. Reason

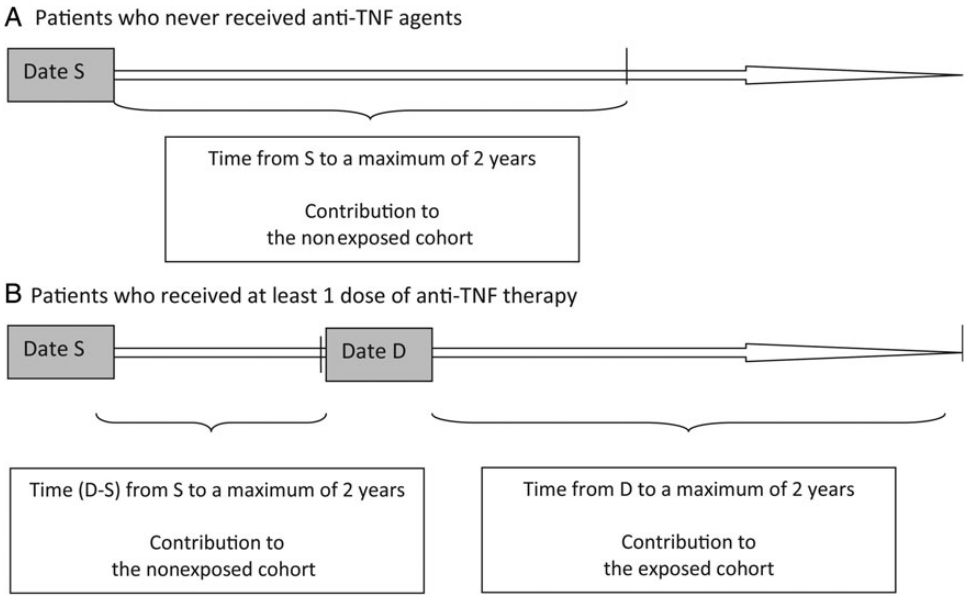

Figure 1. Models for calculating time at risk in the exposed and nonexposed cohort. Abbreviation: TNF, tumor necrosis factor.

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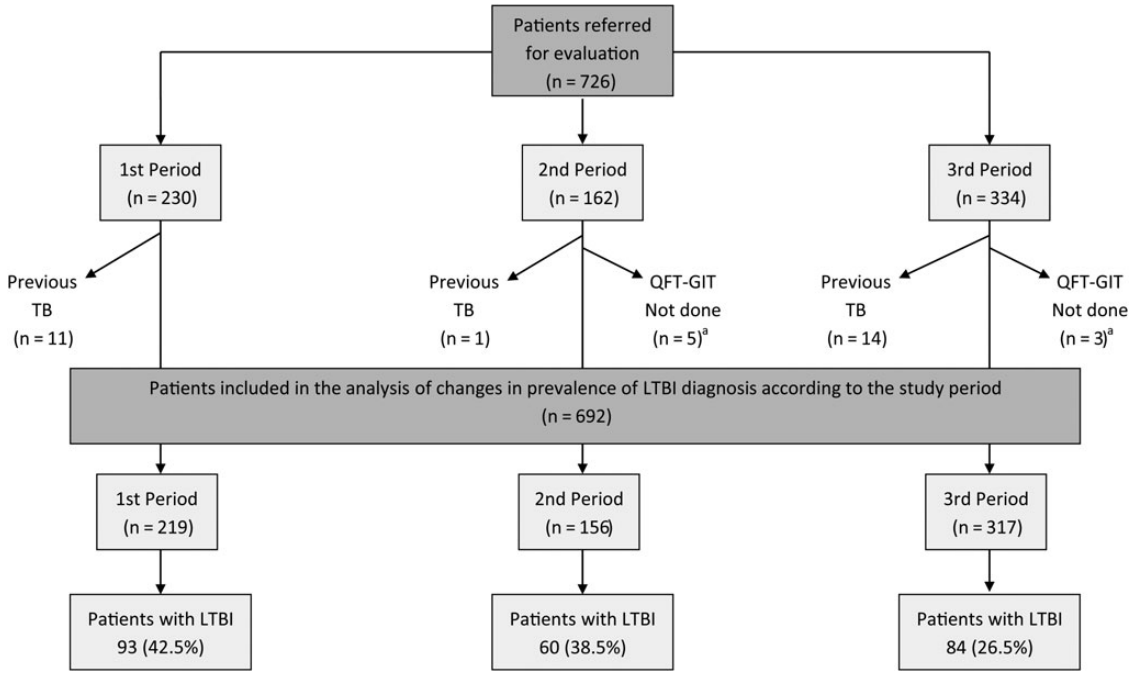

Figure 2. Flowchart of patients referred for evaluation and included in the final analysis. [a]Diagnosis out of protocol: 8 patients were only evaluated with tuberculin skin test (TST): 1 of 5 patients in the second period and 3 patients in the third period were diagnosed with LTBI based only on a positive TST. Abbreviations: LTBI, latent tuberculosis; QFT-GIT, QuantiFERON-TB Gold In-Tube; TB, tuberculosis.

for exclusion included past LTBI (n = 26) and incomplete diagnostic strategy (n = 8). Comparison between periods was finally restricted to 692 patients who had undergone complete evaluation according to the study protocol (Figure 2).

The prevalence of new LTBI diagnoses over the study period was 34.2% (237/692). There were no significant differences between the first and second periods (42.5% and 38.5%, respectively; $P = .37$), but a notable decrease was observed in the third period (26.5%; $P < .02$) (Figure 3). Five of 473 (1.1%) QFT-GIT tests yielded indeterminate results.

Of the 237 newly diagnosed patients, 226 (95.4%) were treated for LTBI. The proportions of patients on preventive therapy were 41%, 38.5%, and 24% in first, second, and third period, respectively ($P < .01$). Specifically, 212 patients (93.8%) were treated with isoniazid for 9 months, and 14 (6.2%) were treated with rifampin for 4 months. During treatment, 11 (5.2%) patients on isoniazid developed liver toxicity necessitating treatment termination. Of these, 6 completed LTBI therapy with rifampin, but 5 did not because they no longer required anti-TNF therapy. Overall, 221 (97.8%) patients completed a full course of treatment.

Impact of BCG Vaccination on the Diagnosis of LTBI

We conducted a stratified analysis by the BCG vaccination status for differences in prevalence of LTBI, and obtained 3 main findings. First, the prevalence of positive TST results was higher in BCG-vaccinated than in non-BCG-vaccinated patients (41.8% and 27.8%, respectively; $P < .01$), whereas there were nonsignificant differences in the prevalence of positive QFT-GIT results between the 2 groups (20.1% and 16.9% for BCG-vaccinated and non-BCG-vaccinated patients, respectively; $P = .39$) (Table 1).

Second, the concordance between TST and QFT-GIT was lower in BCG-vaccinated than in nonvaccinated patients ($\kappa = 0.36$ and 0.67, respectively; Table 2). BCG vaccination was predictive of discordant results (positive TST/negative QFT-GIT) after making adjustments for sex, age, type of IMID, and previous immunosuppressive treatment (odds ratio [OR], 4.0 [95% CI, 2.24–7.26]). Third, BCG vaccination was found to be independently associated with the booster effect (OR, 2.54 [95% CI, 1.25–5.16]), as was age <65 years (OR, 7.92 [95% CI, 1.05–59.9]).

Development of Active Tuberculosis

After a median observation of 5.47 years (interquartile range, 3.67–7.91), representing 3985 patient-years, 54 (7.4%) patients were lost to follow-up and 41 (5.6%) died. Causes of death were as follows: respiratory infection and failure having excluded tuberculosis by culture results or necropsy (n = 8), cancer (n = 7), cardiovascular events (n = 14), sepsis (n = 9), end-stage liver disease (n = 1), and renal failure (n = 1).

During the entire follow-up period, 4 patients (0.6%) born in Spain and without specific risk factors for tuberculosis developed active disease. Three were receiving anti-TNF treatment: 2 had

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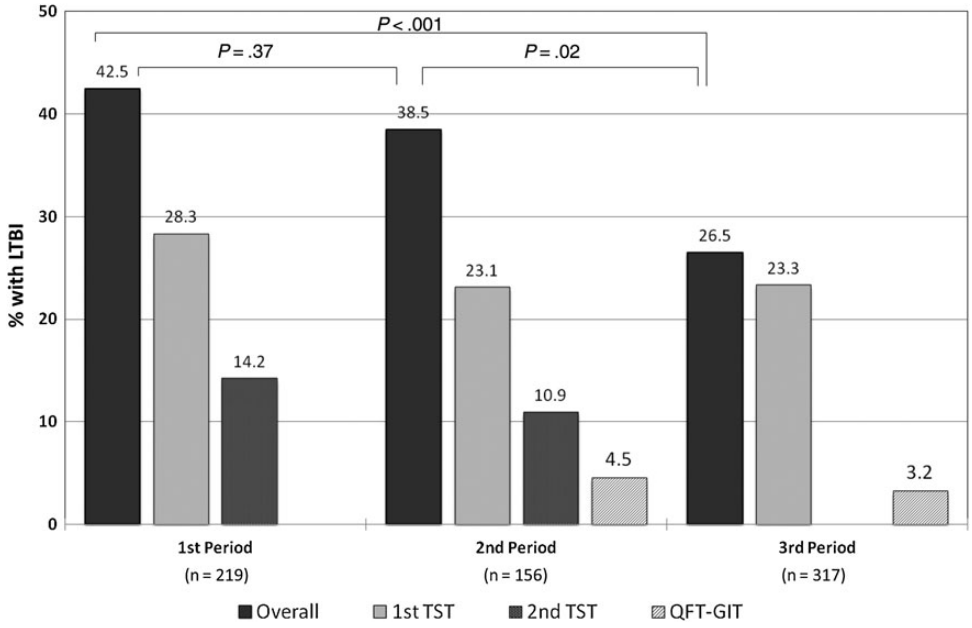

Figure 3. Prevalence of latent tuberculosis (LTBI) in 692 patients according to the 3 study periods. Bars denote the overall proportion of LTBI, and the individual contribution of the first tuberculin skin test (TST), second TST, and QuantiFERON-TB Gold In-Tube (QFT-GIT), respectively.

negative LTBI tests and developed culture-confirmed pulmonary and disseminated tuberculosis, respectively, in the first year on treatment; the remaining patient received a 4-month course of rifampin and developed pleural tuberculosis 3 months after the LTBI therapy. The fourth patient developed pleural tuberculosis in the first year after negative LTBI screening and had never received anti-TNF agents (Supplementary Table 2).

At the 2-year follow-up, the incidence of active tuberculosis was 2.47 cases per 1000 patient-years (95% CI, .79–5.97 per 1000 patient-years) for the whole cohort. Comparison between exposed and nonexposed cohorts found no significant differences (2.85/1000 and 1.77 cases/1000 patient-years, respectively; $P = .74$; Table 3).

After adjusting for sex, age, type of anti-TNF, tuberculosis risk factors, and baseline immunosuppressant agents, anti-TNF treatment was not associated with a higher risk of developing tuberculosis (relative risk, 1.02 [95% CI, .11–9.85]). In addition, differences in risk between periods for patients exposed to anti-TNF agents were not significant ($P = .94$).

DISCUSSION

We report the results of a comprehensive clinical program for tuberculosis prevention in patients receiving anti-TNF therapy. The key findings can be summarized as follows: (1) although anti-TNF–associated tuberculosis can be prevented, certain risk remains in the first year of therapy; (2) the single-step TST plus QFT-GIT strategy offered a substantial reduction in LTBI treatment proportion compared with the 2-step TST-based strategies; and (3) the systematic periodic retesting of patients for LTBI does not appear to be needed following negative testing prior to anti-TNF therapy.

Table 1. Prevalence of Latent Tuberculosis by Tuberculin Skin Test and QuantiFERON-TB Gold In-Tube According to BCG Vaccination Status

Test	BCG Vaccinated no./No. (%; 95% CI)	Non-BCG Vaccinated no./No. (%; 95% CI)	*P* Value
Overall TST	82/196 (41.8; 35.2–48.8)	138/496 (27.8; 24.1–31.9)	<.01
1st TST	66/196 (33.7; 27.4–40.6)	106/496 (21.4; 18–25.2)	<.01
2nd TST	16/52 (30.8; 20.0–44.3)	32/225 (14.2; 10.3–19.4)	<.01
QFT-GIT	32/159 (20.1; 14.6–27.0)	53/314 (16.9; 13.1–21.4)	.39

Abbreviations: BCG, Bacillus Calmette-Guerin; CI, confidence interval; no./No., number of patients with positive results/number of patients on whom the test was carried out; QFT-GIT, QuantiFERON-TB Gold In-Tube; TST, tuberculin skin test.

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Table 2. Concordance Between Tuberculin Skin Test and QuantiFERON-TB Gold In-Tube

Test	BCG Vaccinated	Non-BCG Vaccinated
QFT-GIT with 1st TST (n = 468)		
No.	159	309
Agreement, % (95% CI)	75 (67–81)	91 (87–94)
κ (95% CI)	0.38 (.23–.53)	0.69 (.58–.79)
QFT-GIT with 2nd TST (n = 117)		
No.	27	90
Agreement, % (95% CI)	78 (57–91)	86 (76–92)
κ (95% CI)	0.13 (0–.56)	0.30 (.02–.59)
QFT-GIT with either 1st or 2nd TST (n = 468)		
No.	159	309
Agreement, % (95% CI)	73 (65–80)	90 (86–93)
κ (95% CI)	0.36 (.22–.51)	0.67 (.57–.78)

Five patients with indeterminate QFT-GIT were excluded from the concordance analysis.

Abbreviations: BCG, Bacillus Calmette-Guerin; CI, confidence interval; QFT-GIT, QuantiFERON-TB Gold In-Tube; TST, tuberculin skin test.

Our findings showed that the incidence of tuberculosis among patients exposed to anti-TNF agents was comparable to that of nonexposed patients. However, 3 of the 4 tuberculosis cases diagnosed during the study period occurred in the exposed cohort before the completion of the first year on anti-TNF treatment. Two of them had no evidence of LTBI infection at baseline, which strongly suggests reactivation of undetected LTBI. Recently, a large study evaluating golimumab targeted patients for preventive therapy based on TST plus QFT-GIT. In this study, 0.3% of patients with negative baseline results for both tests developed tuberculosis during the first year of follow-up [10]. These findings are in accordance with our results, showing that anti-TNF–associated tuberculosis can be greatly reduced, but not completely prevented [30].

Table 3. Incidence of Active Tuberculosis During the First 2 Years of Follow-up

Population	Observation Period, Patient-years	Cases of Tuberculosis	Incidence Rate /1000 Patient-years (95% CI)	*P* Value
Whole cohort (n = 726)	1616.5	4	2.47 (.79–5.97)	
Nonexposed	565.5	1	1.77 (.09–8.7)	.74*
Exposed	1051.4	3	2.85 (.73–7.77)	
1st period (n = 194)	380.1	1	2.63 (.13–13.0)	.94**
2nd period (n = 130)	255.5	1	3.91 (.20–19.3)	
3rd period (n = 218)	415.9	1	2.40 (.12–11.9)	

Abbreviation: CI, confidence interval.

* Difference between exposed and nonexposed cohort.

** Difference between 3 periods in exposed patients.

The single-step TST plus QFT-GIT strategy used in the last period led to a substantial reduction in the proportion of patients put on preventive treatment as compared to the previous 2-step TST approaches, without increasing tuberculosis incidence. The reduction is even more striking if the epidemiology profile of the cohort in the last period is taken into account. Because a larger proportion of patients came from tuberculosis-endemic countries, a higher prevalence of LTBI might be expected. Thus, this decrease was likely the result of avoiding the false-positive diagnoses linked to the 2-step TST practice. This assumption is supported by several findings. There was poor agreement between QFT-GIT and the second TST (more discordant results with positive TST and negative QFT-GIT) in comparison with the first TST. In addition, there was an independent association between BCG vaccination and both second TST positivity (boosting effect) and discordant results (TST positive/QFT-GIT negative).

As for the use of IGRAs, 11.8% of patients diagnosed with LTBI in the present study were only identified by QFT-GIT, which represented a 13.4% increase in the overall diagnoses. Conversely, considering QFT-GIT as the only diagnostic method in the third period would have supposed a further reduction in LTBI diagnoses of 41.6%. However, the major concern when using only IGRAs is the potential risk of developing tuberculosis in TST responders with a negative IGRA test. Five recent studies have assessed the risk of tuberculosis in anti-TNF candidates in whom LTBI diagnosis and the decision for treatment was based on IGRAs regardless of the TST results [22, 31–34]. None of the 136 patients with a negative IGRA and positive TST developed tuberculosis after 1 year minimum follow-up. Nonetheless, in a large international study of anti-TNF candidates, 2 of 150 patients with TST positive/QFT-GIT negative results developed active tuberculosis. To note, both patients were from high-prevalence tuberculosis countries [35, 36].

Our data do not support the systematic periodic retesting of patients with negative screening results at baseline. After a median follow-up of almost 5 years, no tuberculosis cases occurred beyond the first 12 months of anti-TNF therapy. Furthermore, periodic TST would increase the number of false-positive results, particularly in BCG-vaccinated patients. Concerning IGRAs, Zwerling et al described unexpectedly high conversion rates in healthcare workers without either conversion with the TST or exposure to a known source of tuberculosis, suggesting that these apparent conversions may not reflect new tuberculosis infections [37]. As a consequence, the indiscriminate retesting of patients with negative LTBI tests at baseline may lead to further unnecessary treatments. In view of our results, retesting should be based on individual risk assessment for tuberculosis infection.

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The main strengths of our research are the homogeneity of the series and the long follow-up assessment period. Patients were prospectively enrolled and evaluated under the same clinical program for prevention of tuberculosis. In contrast to other series, our results cannot be biased by diverse diagnostic and treatment strategies and underreporting. Tuberculosis preventive therapy was instituted and controlled by the evaluating team at the Tuberculosis Unit, where active measures are systematically taken to promote and control adherence to preventive therapy, as described elsewhere [38]. Our study, however, has limitations that deserve further comment. First, the estimates of tuberculosis incidence are not precise due to the small number of events. Second, 54 patients were lost to follow-up, and some cases of active disease may have been missed in this group. However, underestimation is unlikely as they were lost after a median follow-up of 3 years. Third, 2 of the 4 active tuberculosis cases were not culture confirmed. Although their clinical features made tuberculosis the most likely cause of their disease, the final diagnosis could not be proved.

In summary, 3 relevant conclusions can be drawn from this study. First, although anti-TNF–associated tuberculosis can be greatly reduced, a certain risk remains during the first year of treatment. Second, the 2-step TST approach for LTBI screening prior to anti-TNF therapy is no longer justified. Third, systematic periodic retesting for LTBI in patients with negative test results prior to anti-TNF therapy is not required.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online (http://cid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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Potential conflicts of interest. M. S. has collaborated with Alere Healthcare SLU by giving talks on IGRAs. All other authors report no potential conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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1. Immunosuppressed population

1.2 Transplant candidates

Short Report

OPEN

Immunodiagnostic Tests' Predictive Values for Progression to Tuberculosis in Transplant Recipients

A Prospective Cohort Study

Laura Muñoz, MD,[1,6] Aina Gomila, MD,[1] Susana Casas, PhD,[2] José Castellote, PhD,[3] Montserrat Arnan, PhD,[4] Antoni Rafecas, PhD,[5] and Miguel Santin, PhD[1,6]

Background. Little is known about the predictive value for progression to tuberculosis (TB) of interferon-γ release assays and how they compare with the tuberculin skin test (TST) in assessing the risk of TB infection in transplant recipients. **Methods.** We screened 50 liver transplant (LT) and 26 hematopoietic stem cell transplant (HSCT) recipients with both QuantiFERON-TB Gold In-tube (QFT-GT) and TST and prospectively followed them for a median of 47 months without preventive chemoprophylaxis. **Results.** In the LT cohort, 1 in 22 (4.5%) QFT-GT–positive patients developed posttransplant TB, compared with none of the QFT-GT–negative patients. In the HSCT cohort, none of the 7 QFT-GT–positive patients developed TB, whereas 1 case (5.3%) progressed to active TB among the 19 QFT-GT-negative patients. Comparable results were obtained with the TST: in the LT group, 1 of 23 TST-positive and none of the 27 TST-negative patients developed TB; and in the HSCT group, none of the 8 TST-positive and one of the 18 TST-negative patients progressed to active TB. **Conclusions.** In this cohort of transplant recipients, the positive predictive value of QFT-GT for progression to active TB was low and comparable to that of TST. Although the risk of developing TB in patients with negative results at baseline is very low, some cases may still occur.

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Transplant recipients are at increased risk for tuberculosis (TB) compared to the general population,[1] although its risk varies with the type of transplant and the endemic TB burden.[2-4] In low-prevalence regions, transplant-associated TB mostly arises from the reactivation of a latent TB infection (LTBI), which can be effectively prevented with proper treatment.[5] Therefore, guidelines strongly recommend screening and treatment for LTBI for transplant candidates.[1,6,7]

The tuberculin skin test (TST) has been the reference method for targeting TB chemoprophylaxis. However, its low sensitivity in immunosuppressed patients and its limited ability to identify patients at higher risk of reactivation compromise its reliability in transplant candidates. These limitations, together with the prevailing lack of awareness of the risk of active TB and a fear of isoniazid toxicity,[8] make physicians not offer universal LTBI treatment in this population.

The T cell–based interferon-γ release assays (IGRAs) have been increasingly used for detecting LTBI in many clinical scenarios. Although published data suggest that IGRAs might perform better than TST in immunocompromised patients, such as transplant candidates,[9] little is known about their ability to predict posttransplant TB.[10-14]

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This study aimed to assess the usefulness of the Quanti - FERON-TB Gold In-tube (QFT-GT) for predicting the development of active TB in comparison to the TST in patients undergoing liver transplant (LT) and hematopoietic stem cell transplant (HSCT) in a low-TB burden setting (17.3 per 100,000 population).[15]

MATERIALS AND METHODS

We performed a prospective cohort study to evaluate the performance of TST and QFT-GT for detecting LTBI in consecutive candidates to LT and HSCT between July 2008 and July 2010 at Duran i Reynals and Bellvitge University Hospitals in Barcelona (Spain). All patients provided written informed consent before enrolment, and the ethics committee approved the study.

Transplant candidates were referred to the TB unit for clinical assessment and were asked to enrol after active TB had been excluded. In accordance with current guidelines, we followed a symptom-driven diagnostic workup for ruling out active TB in our cohort. All patients were screened for respiratory and had a chest x-ray performed. Because all our patients were asymptomatic and there were no concerning radiographic findings, no microbiologic testing was necessary to evaluate for active TB infection.

Experienced staff took a blood sample for QFT-GT testing, and TST was administered immediately after. For the TST, any induration 5 mm or greater was considered positive.[7] If the TST result was negative, another TST was administered within a week to assess any booster effect. After testing, patients were referred back to their treating physicians, who decided whether to treat them or not. In LT candidates, the risk of liver toxicity was considered to outweigh the potential benefit of chemoprophylaxis regardless of the TST or QFT-GT results. The lack of specific guidelines and the priority of treating a hematologic malignance also meant no LTBI treatment for patients in the HSCT cohort.

By December 31, 2013, we checked on the statuses of patients by reviewing their medical charts and contacting their physicians. We focused on transplant procedure and the development of active TB and death. A definitive TB diagnosis was defined as the isolation of *Mycobacterium tuberculosis* complex in clinical samples, or a positive molecular test result and response to specific treatment. The incidence of active TB was calculated both as a cumulative incidence and as a density incidence (events per person-year) with 95% confidence intervals (95%CI). The positive/negative predictive values for TB progression for each test were defined as the proportion of patients with positive/negative results who did/did not develop TB within the follow-up period, respectively.

RESULTS

The initial cohort included 90 patients with end-stage liver disease and 27 patients with hematologic malignancies that were considered for LT and HSCT, respectively. However, of the patients with end-stage liver disease scheduled for LT, 24 died before transplantation, 16 improved without LT, and 50 (55.6%) eventually received LT by the follow-up date. All 27 patients with hematologic malignancies received HSCT but we excluded 1 candidate because he was screened for LTBI after transplantation.

The baseline characteristics of both cohorts are summarized in Table 1. The prevalence of LTBI according to the

TABLE 1.

Baseline Characteristics of Liver and Hematopoietic Stem Cell Transplant Recipients

Baseline Characteristics	Liver Transplant, n = 50 (%)	Hematopoietic Stem Cell Transplant, n = 26 (%)	*P*
Age: median (IQR), y	57.5 (51-64)	52.0 (39-60)	<0.01
Male sex	38 (76)	8 (30.8)	<0.01
Spanish born	45 (90)	23 (88.5)	0.56
Risk factors for TB	5 (10.0)	4 (15.4)	0.37
Birth/residence in a high-prevalence country	2 (4.0)	3 (11.5)	0.22
Exposure to active TB	2 (4.0)	1 (3.8)	0.73
Occupational exposure	1 (2.0)	—	—
Immunosuppressive treatment in the previous 6 months	3 (0.06)	24 (92.3)	<0.01
BCG scar	15 (30.0)	8 (30.8)	0.95
Primary reason for transplant			
Alcoholic or hepatitis virus cirrhosis	24 (48.0)	—	—
Hepatocellular carcinoma	20 (40.0)	—	—
Other liver diseases	6 (12.0)	—	—
Acute leukemia	—	6 (23.1)	—
Lymphoma	—	10 (38.5)	—
Multiple myeloma	—	8 (30.8)	—
Others	—	2 (7.6)	—
TST result			
Positive	23 (46.0)	8 (30.8)	0.2
Negative	27 (54.0)	18 (69.2)	0.2
QFT-GT result			
Positive	22 (44.0)	7 (26.9)	0.15
Negative	26 (52.0)	19 (73.1)	0.08
Indeterminate	2 (4.0)	—	—

BCG indicates Bacillus Calmette-Guerin.

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QFT-GT was 44% and 26.9% in the LT and HSCT cohorts, respectively; 2 patients in the LT (2.6%) had indeterminate results due to low production upon stimulation with phytohemagglutinin. Regarding TST, 23 patients (46%) in the liver cohort and 8 (30.8%) in the HSCT cohort presented with positive reactions. Correlation of LTBI tests' results and traditional TB risk factors are shown in Table 2. Median time from LTBI screening and transplantation was 15 days (interquartile range [IQR], 8-23).

The LT cohort was followed up for a median of 47.5 months (IQR, 35.0-53.9) after transplantation, over which period, 7 patients died, none was lost to follow-up, and 1 patient developed TB (incidence rate, 0.6 per 100 person-years; 95% CI, 0.3-28.3). He was a 67-year-old man with hepatocellular carcinoma and positive QFT-GT and TST at baseline, who presented with abdominal pain and anorexia 11 months after an orthotopic LT. A computed tomography scan showed an ileocecal mass and a subsequent biopsy confirmed granulomatous inflammation. Culture from colonic and liver biopsies yielded *Mycobacterium tuberculosis* complex, and he made a complete recovery after a 9-month regimen of rifabutin, isoniazid, and levofloxacin.

The HSCT cohort was followed up for a median of 47.51 months (IQR, 27.0-57.5), and of the 26 participants, 7 patients died, 2 were lost to follow-up (after 113 and 370 days), and 1 patient developed TB (incidence rate, 1.1 per 100 person-years; 95% CI, 0.05-5.4). This patient was a 46-year-old woman who had tested negative for TST and QFT-GT before receiving an allogeneic HSCT for acute leukemia. Three months after transplantation, she developed multiple organ dysfunction, which was attributed to progression of the leukemia. She died 15 days after being admitted to the intensive care unit. A skin biopsy culture revealed infection with *M. tuberculosis* complex. Table 3 shows the incidence rates for TB and the predictive values for progression to TB according to each diagnostic test.

DISCUSSION

This study aimed to determine the ability of the QFT-GT to predict the development of active TB in patients undergoing LT and HSCT. Our findings reveal a poor positive predictive value of QFT-GT for progression, which is comparable to that of the TST. Only 1 (4.5%) QFT-GT-positive patient among LT recipients, and none of the QFT-GT-positive HSCT recipients, developed TB within 3.5 years of transplantation. However, the risk of developing active TB among QFT-GT-negative patients was minimal.

To our knowledge, only 4 studies have reported the incidence of active TB in solid organ transplant recipients screened with IGRAs.[10-13] The incidence of TB in IGRA-positive patients could only be assessed in 2 that did not offer preventive therapy.[10,11] Kim et al[10] reported 4 cases of TB among 71 T-SPOT.TB-positive patients in a prospective cohort of 272 kidney transplant recipients, whereas Lange et al[11] found no cases of active TB among 25 QFT-GT-positive solid organ transplant recipients. Furthermore, no cases of TB occurred among the combined 409 IGRA-negative transplant patients in these retrospective studies. On the contrary, in their series of 633 and 87 patients, Jeong et al[13] and Theodoropoulos et al[12] described 1 (1.1%) and 2 (0.3%) cases of TB in QFT-GT–negative transplant patients, respectively. Although there is limited data available on IGRAs in HSCT recipients,[11,14] these also report low positive and very high negative predictive values for progression to active TB.

A recent study assessed the TST and both IGRAs to identify patients at risk for TB in different groups of immunocompromised patients in Europe.[16] Although it included a large number of solid organ transplant and HSCT recipients, both LTBI tests were carried out after the transplant procedure. Their results, including a high unexpected rate of indeterminate results, are therefore not comparable with the present study.

There is no consensus on whether TST or IGRAs should be used to assess the risk of transplant-associated TB and ultimately prevent it.[6] This uncertainty may be linked to the scarcity of longitudinal data of simultaneous screening with both tests, together with the inability of either test to predict the development of active TB.[16-18] In our study, incidence of TB and predictive values for TB progression either in the LT cohort or the HSCT cohort did not differ significantly with the 2 tests. Although 2 series of kidney and HSCT patients reported a higher incidence of TB in IGRA-positive than in TST-positive patients, the differences were not statistically significant.[10,14] These results are consistent with those reported in a previous meta-analysis, in which IGRAs showed a modest, but higher positive predictive value for TB progression than the TST with and without risk stratification.[17]

TABLE 2.

Correlation of QFT-GT and TST Testing and Traditional TB Risk Factors for Both Cohorts

		Liver Transplant Candidates				HSCT Candidates			
		QFT-GT				QFT-GT			
		Pos	Neg	Indet	Global	Pos	Neg	Indet	Global
TST	Positive	18[a]	4	1	23	5	3	0	8
	No. Risk F	2[1,2]	2[1,4]	—		1[2]	1[1]	—	
	Negative	4	22	1	27	2	16[b]	0	18
	No. Risk F	—	2[2,3]	—		—	2[1]	—	
	Global	22	26	2	50	7	19	0	26

[a] Case 1: a 67-year-old Spanish man developed disseminated TB 387 days after LT (and 532 days after LTBI screening). He had no specific TB risk factors and had tested positive for TST and QFT while awaiting liver transplantation for hepatocellular carcinoma.

[b] Case 2: a 46-year-old Spanish woman developed disseminated TB 100 days after HSCT (and 116 days after LTBI screening). He had tested negative for both TST and QFT. She had no specific risk factors for TB. The reason for practicing an unrelated allogeneic bone marrow transplant was an acute leukemia.

Indet, indeterminate. No. Risk F: Number of patients with risk factors for TB, as described: being born in a high-prevalence TB country[1], previous TB close contact[2], health worker[3], (ex-) intravenous drug user[4].

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TABLE 3.

The Incidence of Active Tuberculosis, and Positive Predictive Value of QFT-GT and TST in Liver and Hematopoietic Stem-Cell Transplant Recipients

	Liver Transplant (n = 50)	Hematopoietic Stem Cell Transplant (n = 26)
QFT-GT-positive	22	7
Median follow-up, mo	37.9 (35.8-54.2)	55.9 (33.0-57.1)
Person-years, p-y	81.5	25.9
TB cases	1	0
Incidence rate of TB cases per 100 p-y (95%CI)	1.2 (0.1-6.1)	—
PPV (95% CI)	4.5 (0.8-21.8)	—
QFT-GT-negative	26	19
Median follow-up, mo	42.0 (30.7-51.2)	47.3 (29.1-55.9)
Person-years, p-y	83.3	65.4
TB cases	0	1
Incidence rate of TB cases per 100 p-y (95%CI)	—	1.5 (0.1-7.5)
NPV (95%CI)	100 (87.1-100)	94.7 (75.4-99.1)
TST-positive	23	8
Median follow-up, mo	44.8 (35.4-52.1)	51.6 (24.8-59.1)
Person-years, p-y	75.0	29.3
TB cases	1	0
Incidence rate of TB cases per 100 p-y (95%CI)	1.3 (0.1-6.6)	—
PPV (95%CI)	4.4 (0.8-21.0)	—
TST-negative	27	18
Median follow-up, mo	50.8 (34.2-56.7)	47.5 (27.0-57.5)
Person-years, p-y	99.6	62.1
TB cases	0	1
Incidence rate of TB cases per 100 p-y (95% CI)	—	1.6 (0.1-8)
NPV (95% CI)	100 (87.5-100)	94.4 (74.2-99.0)

PPV indicates positive predictive value; NPV, negative predictive value.

The major advantage of IGRAs over the TST in healthy people in low-prevalence settings is that it can reduce the number of people considered for preventive chemotherapy without increasing the risk of subsequent active TB.[19] Although this characteristic cannot be applied to immunocompromised patients, in whom IGRAs may not save LTBI diagnostics as compared with TST, the negative predictive value of IGRAs for progression to active TB is consistently high in this population, and probably better than that of the TST.[17]

Therefore, with the current data available, the choice of TST or IGRAs for screening transplant candidates should be based on the expected specificity in each setting, operational factors, logistics, patients' preferences, and cost; and always keeping in mind that a negative result does not rule out the future risk of developing TB.[16]

The main limitation of our study is the small sample size and the low progression rate during follow-up, both of which preclude an accurate estimation of the incidence and predictive values for progression to active TB.

In conclusion, the rate of posttransplant TB among QFT-GT-positive patients was both low and comparable to that of the TST in this cohort of LT and HSCT recipients. Our results add to the evidence that IGRAs are poor at predicting the development of active TB in transplant recipients.

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2. Systematic review

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Interferon-γ release assays versus tuberculin skin test for targeting people for tuberculosis preventive treatment: An evidence-based review

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T-SPOT.TB;
Systematic review

Summary *Objectives:* To assess whether Interferon-γ release assays (IGRAs) reduce the number of people considered for tuberculosis (TB) preventive treatment without increasing subsequent active disease.
Methods: Longitudinal studies with both tuberculin skin test (TST) and IGRAs were identified through a PubMed search. Reductions in diagnosis of TB infection and increases in incident TB in people considered not infected, using IGRAs either instead of TST or as a confirmatory test (two-step approach), were assessed.
Results: In comparison with TST alone, the pooled reductions in diagnosis of TB infection obtained with IGRAs were 16.7% and 5.8% at 5 and 10 mm cut-offs respectively, and 24.5% and 12.4% at 5 and 10 mm respectively with the two-step approach. Compared with TST alone, incident TB among people considered not infected increased with the two-step approach (0.94% with T-SPOT®.TB and 1.1% with QuantiFERON®-TB Gold In-Tube) in one of seven studies in high-income countries. In middle- and low-income countries, two of four studies presented increases (0.08 and 0.03 per 100 patient-years respectively) with the two-step approach.
Conclusions: In high-income countries, the use of IGRAs, either instead of TST or as confirmatory test reduces the number of people considered for preventive treatment, without a significant risk of subsequent active disease.
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Introduction

The detection and treatment of latent tuberculosis infection (LTBI) is an important component of the tuberculosis (TB) elimination strategy worldwide.[1] This is particularly so in low burden TB countries, in which most new cases occur due to reactivation of a past acquired infection.[2] However, while the effectiveness of this practice has been demonstrated,[3,4] the number of people who must be treated to prevent a single TB case is disproportionally high because of the low ability of the tuberculin skin test (TST) to predict development of active TB.[5–7] Furthermore, the long treatment duration and occurrence of adverse events, as well as the need for regular monitoring during treatment, frequently lead to suboptimal adherence to treatment and low completion rates.[8]

T-cell-based interferon-γ release assays (IGRAs) arose as a promising alternative to the old TST. Unfortunately, despite their higher specificity[9] and better association with risk factors for acquisition of *Mycobacterium tuberculosis* infection,[10] their accuracy for predicting the development of active TB is low – at best, only slightly better than TST.[6,7] In a systematic review (SR) by Rangaka et al.,[6] compared with test-negative results, the incidence rate ratio (IRR) for incident TB was 2.11 (95%CI 1.29–3.46) for IGRA-positive and 1.60 (95%CI 0.94–2.72) for TST-positive at 10 mm cut-off. In another SR, Diel et al.[7] found a positive predictive value of 2.7% (95%CI 2.3–3.2) for IGRAs and 1.5% (95%CI 1.2–1.7) for TST. Nevertheless, since the proportion of positive results obtained with IGRAs is usually lower than with TST, and considering the very low risk of developing TB with a negative test,[6,7] their use may still be preferable, since they substantially reduce the number of people put on treatment.

We aimed to ascertain whether using an IGRA either in place of TST or to confirm a positive TST might reduce the number of people considered for preventive treatment without leading to a significant increase in the risk of subsequent active TB.

Methods

This systematic review was conducted in accordance with the PRISMA statement.[11]

Search strategy and study selection

We searched PubMed for relevant original articles, using a pre-established combination of terms: "tuberculosis", "QuantiFERON-TB", "T-SPOT.TB", "interferon-gamma assay", "predictive value", "incident tuberculosis" and "tuberculin skin test", as listed in titles, abstracts or text words. The search was limited to studies in humans published in English or Spanish between January 1, 2005 and May 31, 2012. We also reviewed citations of the original and review articles, and guidelines for additional references. We selected longitudinal studies of healthy or immunosuppressed individuals, adults or children, in which patients were screened for LTBI with TST and an IGRA (commercial available tests [QuantiFERON®-TB Gold In-Tube (QFT-GIT) and T-SPOT®.TB] or in-house developed Elispot) either together or as a confirmatory test of a positive TST result, which also provided results on incident TB in people left untreated. We excluded studies with the old version of the ELISA assay (QuantiFERON®-TB Gold), studies in which IGRAs were not performed according to the manufacturer's instructions, and studies presenting non-original data, conference abstracts, editorials, reviews and guidelines.

Quality assessment

We checked the quality of the studies with the QUADAS check list,[12] and a modified Newcastle-Ottawa scale for non-randomized observational studies,[13] adapted from Rangaka et al.[6]

Data extraction

The two authors (L.M. and M.S.) independently in duplicate conducted the study selection and compiled the data using a standardized data extraction sheet. Discrepancies were resolved by discussion and consensus. The following data were recorded: author, date of the study and year of publication, country, design (prospective/retrospective), population of the study, prevalence of BCG vaccination, IGRA evaluated (ELISA, Elispot, in-house, commercial), cut-off for positivity of TST, TST and IGRA results, development of incident active TB and length of follow-up.

Data synthesis and analysis

We looked at the reduction in the proportion of individuals diagnosed with TB infection and the increase in rates of incident active TB by using an IGRA either as alternative to TST or as a confirmatory test (two-step approach) with respect to the standard practice (TST alone). To do so, we compared the number of diagnoses of TB infection with TST (TST-positive), with IGRAs (IGRA-positive) and with TST followed by an IGRA (TST-positive/IGRA-positive). Reduction in diagnosis of TB infection with IGRAs was represented by the subset of individuals with discordant TST-positive/IGRA-negative results. Increases in rates of active TB with the alternative approaches to TST alone would be represented by individuals with a negative IGRA who developed TB during the follow-up, and who would have been captured by TST.

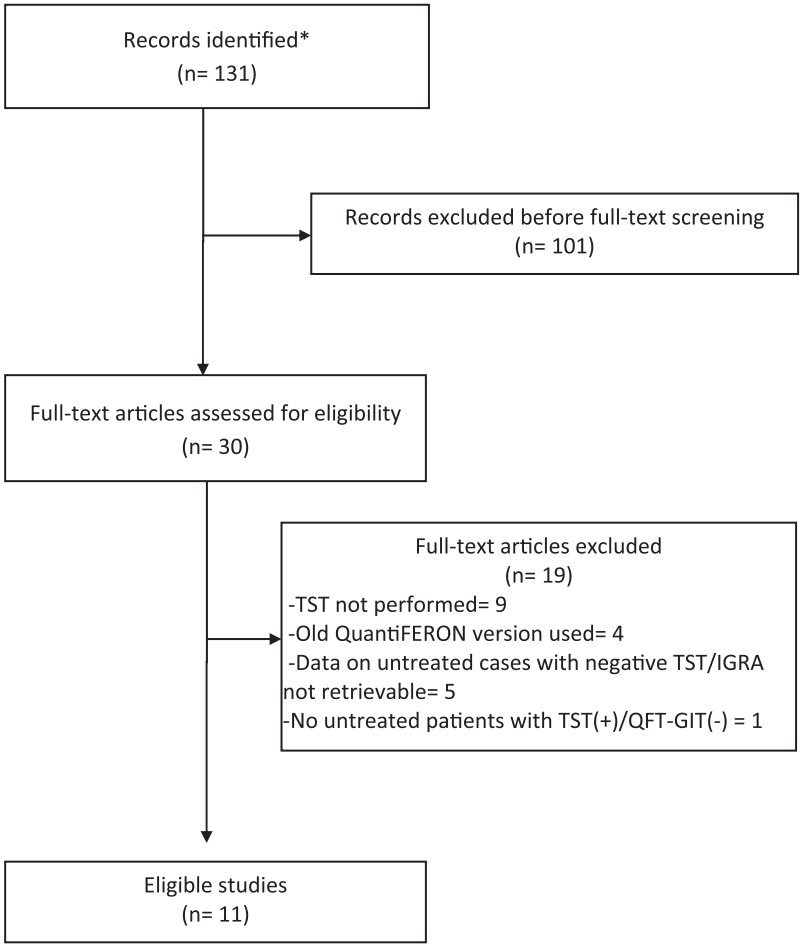
Rates of incident TB are given as presented in the original papers, either as cumulative incidence or person-years incidence rates (density incidence). The results of both outcomes were stratified for countries income status according to the World Bank Classification.[14] Pooled estimates were only obtained for reduction in diagnosis of TB infection by either alternative to TST alone (IGRA instead of TST or as a confirmatory test of TST) according to the TST cut-off for positivity (5 or 10 mm). Overall pooled estimates were precluded by the distinct cut-offs used for TST. A random-effects synthesis model meta-analysis was used to pool the effect across the studies. Heterogeneity was quantified by the I^2 statistic. The analyses were performed with MetaAnalyst software.[15]

Results

Characteristics of the studies

Of the 131 citations identified, 11 were eligible for the analysis (six studies with QFT-GIT,[16,18,20,21,23,26] two with commercial T-SPOT.TB,[19,25] two with in-house Elispot,[22,24] and one that included both QFT-GIT and T-SPOT.TB[17]) (Fig. 1 and Table 1 of the supplementary material). For the study including QFT-GIT and T-SPOT.TB,[17] results of the two tests were reported separately. In two studies,[16,17] an IGRA was performed as a confirmatory test in individuals with positive TST. Seven studies were conducted in high-income,[16–21,23] three in middle-income countries,[22,25,26] and one in a low-income country.[24] Five studies followed up TB case contacts,[16–18,22,24] three followed up patients with immune-mediated inflammatory diseases (IMID) screened prior to anti-TNF therapy,[19,20,23] one followed up a cohort of patients with silicosis,[25] one followed up a cohort of asylum seekers,[21] and one followed up adolescents in a high-burden TB area.[26] Preventive treatment was offered to individuals with a positive IGRA regardless of TST result, in six studies[16,18–21,23]; to individuals with positive TST in two,[22,25] and no treatment in three.[17,24,26] For the two studies in which TST was used as the diagnostic test to institute preventive treatment, the group of individuals who refused it was the subset of our analysis (untreated individuals with negative TST and positive TST/negative IGRA).[22,25]

Five studies met the 14 quality indicators,[16,18,22,24,25] and the other six met all but one.[17,19–21,23,26] As for the assessment of incident TB, length of follow-up was adequate in most cases (at least two years in eight studies[16–18,22–26]) and the samples evaluated were representative of the population at risk in all the studies. However, the quality was constrained by the lack of independent blind assessment, incorporation of either IGRA or TST into the reference standard for the diagnosis, and the low proportion of culture-confirmed diagnoses of TB cases. Five studies stated the time period elapsed from screening to diagnosis of active disease to define incident TB[17,21,24–26] (three months in



* Identified from Medline and two systematic reviews[6, 7] on the predictive value of IGRAs for incident tuberculosis.

Figure 1 Flowchart for study selection.

two studies, two months in two, and from day 0 in one other), and six did not report it.[17–20,22,23] However, of these six studies, the cases recorded occurred after three months in three studies, and no cases occurred in the other three (Table 2, supplementary material).

Prevalence of LTBI according to TST and IGRA results

Prevalence of LTBI with TST was higher than with IGRAs in eight of the nine studies in which the two tests were performed in all patients.[18–22,24–26] In the other study, conducted in a high-income country with IMID patients,[23] IGRA yielded a slightly higher rate of positive results than TST (Table 3, supplementary material).

Overall, using an IGRA test to screen TB infection would have achieved a reduction in diagnosis rates of TB infection, either by replacing TST (nine studies[18–26] with a total of 9304 patients) or as a confirmatory test for TST (11 studies[16–26] with a total of 11,996 patients) (Tables 1 and 2). The pooled reduction in the diagnosis by using an IGRA in place of TST varied according to the TST cut-off used (pooled 16.7% [95%CI 0.6–32.8; I^2 = 98.6%], and 24.5% [95%CI 13.0–36.1; I^2 = 97.9%] when IGRA was used instead of TST and as a confirmatory test respectively using a 5 mm cut-off, and 5.8% [95%CI −4.1 to 15.8; I^2 = 54.8%], and 12.4% [95%CI 4.0–20.9; I^2 = 90.9%] when IGRA was used instead of TST and as a confirmatory test respectively using a 10 mm cut-off).

Incident active TB in individuals without TB infection according to the TST and IGRA results

To assess whether the reduction in the diagnosis of TB infection and the secondary increase in the number of individuals left untreated might lead to a rise in rates of subsequent active TB, we looked at the development of active disease in people considered non-infected by any of the three screening approaches (TST, IGRA in place of TST, and IGRA as a confirmatory test for TST).

High-income countries

Increase in incident TB was assessed in seven studies,[16–21,23] which included 4560 individuals (2616 had a negative TST result, 1458 of 2179 who had undergone the test had a negative IGRA result, and 3533 had either a negative TST or a positive TST/negative IGRA result). There was a slight increase in the incidence of subsequent active TB with the TST/IGRA approach in one study[17] (0.94% and 1.1% with T-SPOT.TB and QFT-GIT respectively) with respect to TST (Table 3). In this study, conducted in the Netherlands[17] with immigrant TB contacts born in high burden countries, both IGRAs were only performed in individuals with a positive TST result. This approach missed two individuals using T-SPOT.TB, and three individuals using QFT-GIT who developed active TB during a two-year follow-up period, and who would have been considered infected by the TST result. The contacts who later developed active TB had their IGRA test performed shortly after the diagnosis of the index case (5, 19 and 34 days for the three patients with negative QFT-GIT, and 5 and 34 days for the two patients with negative T-SPOT.TB), and the test was not repeated later. There was no increase in incident TB in the other six studies.[16,18–21,23]

Middle- and low-income countries

Increase in incident TB was assessed in four studies,[22,24–26] which included 7586 individuals (3714 had a negative TST result, 4049 had a negative IGRA result, and 4645 had either a negative TST or a positive TST/negative IGRA result). There was a slight increase in incident TB with the TST/IGRA approach in two of the four studies (Table 4).[24,26] In one of them, conducted in TB contacts in the Gambia,[24] the two-step approach would have missed four individuals who were later diagnosed with active TB, and who would have been considered infected by the TST result, which represented an increase of 0.08 per 100 pyrs. The other

Table 1 Prevalence of TB infection with IGRAs either in place of TST or as a confirmatory test compared to TST alone. High-income countries.

Reference (Year)	Population	TST cut-off	Reduction in diagnosis of TB infection (%; 95%CI)[a]	
			IGRA vs TST	TST/IGRA vs TST
Song[16] (2007)	TB case-contacts	≥10 mm	NA	67 (3.7; 1.5–5.9)
Kik[17] (2009)[b]	TB case-contacts	≥5 mm	NA	118 (27.3; 20.7–33.4)
Kik[17] (2009)[c]	TB case-contacts	≥5 mm	NA	167 (38.6; 32.3–44.4)
Diel[18] (2010)	TB case-contacts	≥5 mm	410 (45.5; 41.4–49.4)	412 (45.7; 41.4–49.4)
Laffite[19] (2009)	Anti-TNF candidates	≥10 mm	10 (21.7; 3.3–38.4)	10 (21.7; 3.3–38.4)
Garcovich[20] (2011)	Anti-TNF candidates	≥5 mm	3 (6.8; −7.3 to 21.3)	3 (6.8; 0–19.9)
Chang[23] (2011)	Anti-TNF candidates	≥10 mm	−1 (1.2; −14.4 to 16.7)	16 (19.3; 5.0–32.5)
Harstad[21] (2010)	Asylum seekers	≥6 mm	178 (22.0; 17.3–26.6)	208 (25.7; 21.0–30.2)

TB = tuberculosis; TST = Tuberculin Skin Test; IGRA = Interferon Gamma Release Assay; QFT-GIT = QuantiFERON-TB Gold In-tube; T-SPOT = T-SPOT.TB; TST/IGRA denotes sequential approach (positive TST followed by IGRA as a confirmatory test); NA = not applicable (IGRA performed only to individuals with positive TST); TNF = tumor necrosis factor.

[a] Reduction in the number of diagnoses of TB infection by using an IGRA test either in place of TST (IGRA vs TST) or as a complementary test for a positive TST result (TST/IGRA vs TST), as alternative strategies to TST alone.

[b] With T-SPOT.

[c] With QFT-GIT.

Table 2 Prevalence of TB infection with IGRAs either in place of TST or as a confirmatory test compared to TST alone. Middle- and low-income countries.

Reference (Year)	Population	TST cut-off	Reduction in diagnosis of TB infection (%; 95%CI)[a]	
			IGRA vs TST	TST/IGRA vs TST
Hill[24] (2008)	TB case-contacts	≥10 mm	53 (3.2; −0.1 to 6.5)	230 (14.0; 10.8–17.1)
Bakir[22] (2008)	TB case-contacts	≥5 mm	47 (16.4; 9.4–23.2)	73 (25.4; 19.2–31.2)
Leung[25] (2010)	Patients with silicosis	≥5 mm	10 (4.1; −4.4 to 12.6)	37 (15.4; 6.6–23.8)
Mahomed[26] (2011)	Adolescents with no prior TB	≥5 mm	225 (4.3; 2.4–6.2)	511 (9.7; 7.8–11.6)

TB = tuberculosis; TST = Tuberculin Skin Test; IGRA = Interferon Gamma Release Assay; T-SPOT = T-SPOT.TB; QFT-GIT = QuantiFERON-TB Gold In-tube; TST/IGRA denotes sequential approach (positive TST followed by IGRA as a confirmatory test).
[a] Reduction in the number of diagnoses of TB infection by using an IGRA test either in place of TST (IGRA vs TST) or as a complementary test for a positive TST result (TST/IGRA vs TST), as alternative strategies to TST alone.

study, which involved adolescents in South Africa,[26] found a slight rise in rates of subsequent active TB with the TST/IGRA approach (0.03 per 100 pyrs).

Discussion

In this systematic review we assessed whether using IGRAs either in place of TST or to confirm its positivity might reduce the number of diagnoses of latent TB, and therefore considered for preventive treatment, without increasing the risk of incident active TB. The available evidence, although scarce, strongly suggests that in immunocompetent people in high-income countries IGRAs can replace TST or being used as a confirmatory test to reduce the number of people considered for preventive treatment, without increasing subsequent active disease. In the Netherlands study[17] the two IGRAs missed some few recently exposed close contacts who later developed TB. However, they were administered only once, and shortly after the diagnosis of the index case; since IGRAs have a "window period" after exposure, like TST,[27] it is plausible that the contacts with initial negative IGRA results would have been captured if the test had been repeated a few weeks later.

Which of the two alternative approaches — IGRA either in place of TST or as a confirmatory test — is better for screening people at risk is a matter of debate. In immunocompetent individuals, in whom TST sensitivity is roughly comparable to that of the IGRAs, a two-step approach may be appropriate.

Table 3 Incident TB in individuals considered not infected according to the TST and IGRA results. High-income countries.

Reference (Year)	Population	Follow-up	Incident TB in individuals without TB infection n/N (Incidence)[a]			Increase in incidence[b]	
			TST (TST-ve)	IGRA (IGRA -ve)	TST/IGRA (TST-ve and TST+ve/IGRA-ve)	IGRA vs TST	TST/IGRA vs TST
Song[16] (2007)	TB case-contacts	2 yrs	10/1556 (0.6%)	N.A.	10/1623 (0.6%)	N.A.	No
Kik[17] (2009)[c]	TB case-contacts	2 yrs	0/94	N.A.	2/212 (0.94%)	N.A.	Yes (0.94%)
Kik[17] (2009)[d]	TB case-contacts	2 yrs	0/94	N.A.	3/261 (1.1%)	N.A.	Yes (1.1%)
Diel[18] (2010)	TB case-contacts	Up to 4 yrs	2/346 (0.6%)	0/750	2/756 (0.3%)	No	No
Garcovich[20] (2011)	Anti-TNF candidates	1 yr	0/39	0/44	0/42[c]	No	No
Chang[23] (2011)	Anti-TNF candidates	2 yrs	0/65	0/64	0/64	No	No
Laffite[19] (2009)	Anti-TNF candidates	1.5 yrs	0/28	0/38	0/38	No	No
Harstad[21] (2010)	Asylum seekers	23–32 months	0/394	0/562	0/601	No	No

n/N = cases of incident TB/individuals considered not infected by each of the three approaches; TST = tuberculin skin test; IGRA = interferon-gamma release assays; QFT-GIT = QuantiFERON-TB Gold In-tube; T-SPOT = T-SPOT.TB; TST/IGRA denotes sequential approach (positive TST followed by IGRA as a confirmatory test); N.A. = Not applicable; TNF = tumor necrosis factor.
[a] Cumulative incidence (%) or incidence rate per 100 pyrs.
[b] Increase in incidence among individuals considered not infected by using an IGRA test either in place of TST (IGRA vs TST) or as a complementary test for a positive TST result (TST/IGRA vs TST), as alternative approaches to TST alone.
[c] With T-SPOT.
[d] With QFT-GIT.

Table 4 Incident TB in individuals considered not infected according to the TST and IGRA results. Middle- and low-income countries.

Reference (Year)	Population	Follow-up	Incident TB in individuals without TB infection n/N (Incidence)[a]			Increase in incidence[b]	
			TST (TST-ve)	IGRA (IGRA -ve)	TST/IGRA (TST-ve and TST+ve/IGRA-ve)	IGRA vs TST	TST/IGRA vs TST
Hill[24] (2008)	TB case-contacts	2 yrs	10/990 (0.55/ 100 pyrs)	10/1043 (0.50/ 100 pyrs)	14/1320 (0.63/100 pyrs)	No	Yes (0.08/100 pyrs)
Bakir[22] (2008)	TB case-contacts	2 yrs	2/194 (1.03%)	1/241 (0.41%)	2/267 (0.70%)	No	No
Leung[25] (2010)	Patients with silicosis	1–5 yrs	4/80 (5.0%)	1/90 (1.11%)	4/117 (5.0%)	No	No
Mahomed[26] (2011)	Adolescents with no prior TB	2–4 yrs	12/2350 (0.22/ 100 pyrs)	13/2575 (0.22/ 100 pyrs)	16/2861 (0.25/100 pyrs)	No	Yes (0.03/100 pyrs)

n/N = cases of incident TB/individuals considered not infected by each of the three approaches; TST = tuberculin skin test; IGRA = interferon-gamma release assays; QFT-GIT = QuantiFERON-TB Gold In-tube; T-SPOT = T-SPOT.TB; TST/IGRA denotes sequential approach (TST followed by IGRA as a confirmatory test); N.A. = Not applicable.

[a] Cumulative incidence (%) or incidence rate per 100 pyrs.

[b] Increase in incidence among individuals considered not infected by using an IGRA test either in place of TST (IGRA vs TST) or as a complementary test for a positive TST result (TST/IGRA vs TST), as alternative approaches to TST alone.

After an initial TST, an IGRA would reduce the number of individuals considered for preventive treatment and follow-up. The lower the expected rate of infection in the population screened, the more cost-effective this strategy will be. In contrast, in populations with an expected high prevalence of infection, it is likely that replacing TST with an IGRA may be safe and more cost-effective.

In immunosuppressed populations, the present review identified three small longitudinal studies with IMID patients screened for LTBI before starting anti-TNF agents,[19,20,23] in which none of 29 patients with positive TST/negative IGRA and none of 146 with negative IGRA, regardless of TST results, had developed active TB after at least 12 months of follow-up. These results, though promising, should be confirmed in larger cohort studies. As the initial TST in immunosuppressed patients may miss some individuals who would not have the chance of being captured by the IGRA, the two-step approach may not be an appropriate strategy in these patients. Our review did not identify any similar studies in HIV-infected patients. However, the high negative predictive value of QFT-GIT reported in a large cohort[28] suggests that IGRAs could replace TST for detecting latent infection in HIV-seropositive people.

Results obtained for high-income countries are not applicable to low-income high TB burden settings. In two large cohorts in the Gambia[24] and South Africa,[26] a proportion of individuals who later developed active TB were missed by using an IGRA in place of TST or in a two-step fashion. The results of this analysis, together with the modest predictive value of IGRAs, which does not add meaningful contribution to TST, and the high TB burden favoring re-infection, bear out the recommendations of the World Health Organization (WHO), which advise against the use of IGRAs for identifying people at risk in these settings.[29] Therefore, implementation of IGRAs in constrained-resource settings for screening people at risk of developing active TB should be based on operational factors and cost rather than on clinical benefits.

This systematic review has limitations. First, most studies have important shortcomings related to the longitudinal assessment of the development of active TB. Moreover, in two studies, there was a self-selection bias due to the fact the cohort of analysis comprised people who refused preventive treatment. Second, the limited data on immunosuppressed people precludes drawing any firm conclusions regarding these populations.

In summary, IGRAs substantially reduce the number of diagnoses of TB infection, and therefore, the number of people considered for preventive treatment. Among TB contacts in high-income settings, using an IGRA in place of TST or as a confirmatory test does not increase the risk of subsequent active TB. Among immunosuppressed patients, replacing TST with an IGRA may be appropriate. However, more evidence is needed because of the limited available evidence. In low-income high TB burden countries, there is an increase in the incidence of active TB among people with negative IGRA compared to those with negative TST, which argues against the use of IGRAs in these settings.

Appendix A. Supplementary material

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jinf.2012.12.005.

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3. Contact tracing studies

3.1 Longitudinal cohort study

QuantiFERON®-TB Gold In-Tube for contact screening in BCG-vaccinated adults: A longitudinal cohort study

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Running head: IGRAs in BCG-vaccinated contacts

Keywords: IGRA, QuantiFERON-TB Gold In-Tube; tuberculin skin test, contact tracing, risk of TB

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UNDER REVIEW (Plos One)

Abstract

Objective. To assess the utility of QuantiFERON®-TB Gold In-tube (QFT-GIT) for targeting preventive therapy in BCG-vaccinated contacts of tuberculosis (TB), based on its high specificity and negative predictive value for development of TB.

Methods. We compared two screening strategies for TB contact tracing in two consecutive periods: the tuberculin skin test (TST) period, when all contacts were screened with the TST alone; and the QFT-GIT period, when BCG-vaccinated contacts underwent TST and QFT-GIT. Diagnosis of TB infection among BCG-vaccinated contacts relied on TST ≥5 mm in the TST period, while in the QFT-GIT period either a positive QFT-GIT or a TST ≥15 mm was required.

Measurements and main results. Six hundred and sixty-one contacts were compared. In the QFT-GIT period there was a reduction in diagnoses of TB infection (77.4% vs. 51.2%; p <0.01) and preventive therapy prescribed (62.1% vs. 48.2%; $p = 0.02$) among the 290 BCG-vaccinated contacts. After a median follow-up of 5 years, cumulative incidences of TB were 0.62 and 0.29 in the TST and QFT-GIT periods respectively ($p = 0.59$).

Conclusions. In BCG-vaccinated TB contacts, the addition of QFT-GIT safely reduced TB diagnosis and treatment rates without increasing the risk of subsequent active TB.

Introduction

Detection and treatment of recently infected people is an essential measure of tuberculosis (TB) control in low-prevalence countries [1]. Up to approximately ten years ago, the diagnosis of TB infection relied exclusively on the tuberculin skin test (TST). A positive TST response indicates infection with *Mycobacterium tuberculosis* indirectly, by measuring the delayed-type hypersensitivity response to the intradermal injection of a mixture of wall antigens, the so-called PPD (purified protein-derivate), which is shared by many mycobacteria species and the Bacillus Calmette–Guérin (BCG) strain [2]. The main limitations of the TST for targeting preventive therapy among the contacts of patients with pulmonary TB (TB contacts) are its low specificity and poor ability to identify those likely to develop active disease [3]. Thus, a high number of TB contacts need to be treated to prevent a case of TB in clinical practice.

By contrast to the TST, the interferon-γ release assays (IGRAs), the *in vitro* immunodiagnostic tests based on *M. tuberculosis* complex-specific antigens, have no cross-reactivity with the BCG-vaccine strains and most non-tuberculous mycobacteria [4-6]. After more than a decade, evidence indicates that, at best, the ability of these tests to predict the development of TB is only a little better than that of the TST [7, 8]. Nevertheless, IGRAs yield fewer positive results than TST, are more specific, and have shown a high negative predictive value for better selecting those immunocompetent individuals who will not develop TB; thus, their use for targeting TB contacts for preventive therapy, especially in BCG-vaccinated subjects, may still be preferable to TST and also more cost-effective [9].

In 2007, the QuantiFERON®-TB Gold In-tube (QFT-GIT) test was implemented in our center. Shortly after, our TB Unit modified its internal protocol for contact tracing by adding the QFT-GIT to the ongoing TST-based strategy for screening and informing treatment decisions in BCG-vaccinated contacts of TB. Here, we report our experience with this

practice. We hypothesized that using the QFT-GIT to target TB contacts would reduce the number of individuals diagnosed with, and treated for, TB infection compared with the previous TST-only strategy without an increased risk of subsequent active TB.

Methods

Design, setting, and study population

A retrospective comparative study of two screening strategies for TB contact tracing was performed at the TB Unit of a teaching hospital for adults in Barcelona (Spain) between January 2006 and December 2010. The Ethics Committee of Bellvitge University Hospital approved the study (PR269/11).

We included immunocompetent contacts older than 15 years who had no history of TB infection and whose index case had culture-proven non-MDR pulmonary TB. As part of routine clinical practice, medical histories, BCG-vaccination status (vaccine scar), treatment, adverse events and adherence to therapy had been gathered prospectively.

Screening strategies and preventive therapy

We compared two consecutive 30-month periods: TST period (January 2006 to May 2008), and QFT-GIT period (June 2008 to December 2010). In both periods, active TB was ruled out through symptom-guided interview and chest X-ray. In the TST period there was no difference in contact management regarding BCG-vaccination status: the screening was performed with TST, and non-responders underwent a second test after the window period (8 weeks). Preventive therapy was prescribed if TST was ≥5 mm by 48–72 hours after administration. In the QFT-GIT period, two different strategies were used according to BCG-vaccination status. While non-BCG contacts were screened only with TST, as in the TST-

period, BCG-vaccinated contacts were simultaneously screened using both the QFT-GIT assay and the TST. In this group preventive therapy indication was established by either a positive QFT-GIT assay or a TST result ≥15 mm. If the QFT-GIT assay was negative and the TST was <15 mm, a second QFT-GIT assay was performed 8 weeks later. Treatment regimens included 6–9 months of treatment with isoniazid (INH) as the first-choice option, or 4 months with rifampicin (RMP) or 3 months with RMP plus INH as alternative regimens. While on treatment, contacts had regular appointments at the TB unit. There, both blood tests for monitoring liver function and adherence assessments were carried out. The latter included the Eidus-Hamilton test [10] for those taking INH and the simple checking of urine color for those on rifampicin.

Follow-up

In 2015, the vital status and development of TB were checked among all contacts by retrospective review of the electronic medical records of both the Hospital and local primary care services, which were available online. If no data were available for the last 6 months or before the contact completed at least 5 years of follow-up, individuals were contacted by phone and briefly interviewed using a pre-designed questionnaire form. If the contact was not reachable, they were considered lost to follow-up. Contacts were censored at the time of active TB diagnosis, death, loss to follow-up, or after 5 years of follow-up, whichever came first.

Data Analysis

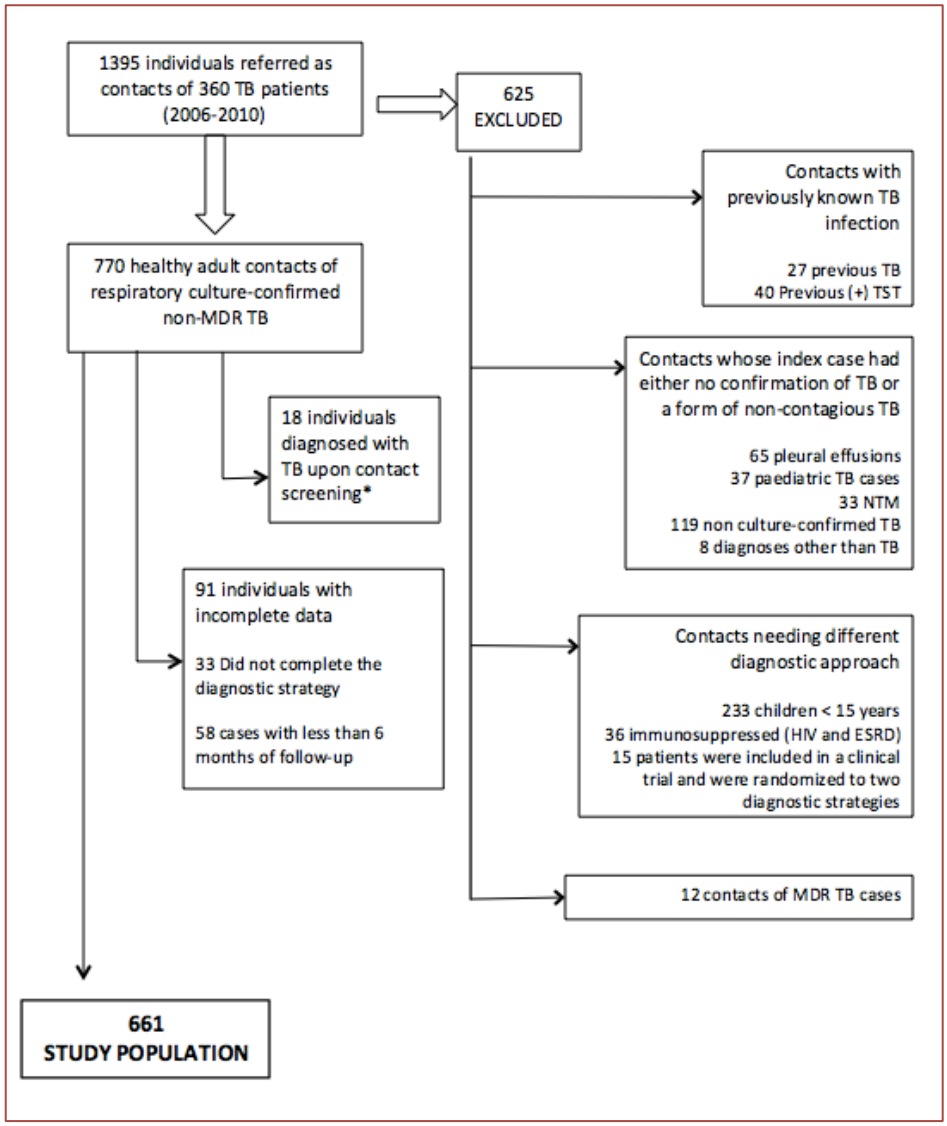
Incidence was given as the density of incidence (TB cases per person-time). Continuous variables were presented as medians (interquartile ranges) and compared with the Student *t*

test or the Mann–Whitney *U* rank test, as appropriate. Differences in categorical variables were assessed with the χ^2 test. All statistical analyses were two-tailed, and a *p*-value <0.05 was considered statistically significant. Analyses were performed with IBM® SPSS® Statistics for Macintosh, Version 22.0 (IBM Corp., Armonk, NY; released 2013) and the OpenEpi software (Open Source Epidemiologic Statistics for Public Health) [11].

Results

During the study period, 1395 contacts of 360 index cases were evaluated, and 661 were included in the analysis (321 in the TST period and 340 in the QFT-GIT period). The selection of eligible contacts is summarized in Fig 1, and the baseline characteristics of the cohort by study period is shown in Table 1. The QFT-GIT period included a higher proportion of foreign-born individuals (p <0.01), close contacts (p <0.01), and BCG-vaccinated subjects (p = 0.01).

Figure 1. Chart of the contacts included



TB: tuberculosis; HIV: human immunodeficiency virus; MDR: multi-drug resistant; ESRD: end-stage renal disease; TST: tuberculin skin test; NTM: Non-tuberculous mycobacteria
[a]12 and 6 patients in the first and second period, respectively.

Table 1. Baseline characteristics and immunodiagnostic test results by study period

	TST period (n= 321)	QFT-GIT period (n= 340)	*p*
Gender, man; n (%)	151 (47.0)	157 (46.2)	0.82
Age; median (IQR)	37 (25.5-48.5)	33.5 (21-46)	0.08
Foreign-born; n (%)	72 (22.4)	148 (43.5)	<0.01
-Latin America; n (% of foreign-born)	40 (55.6)	94 (63.5)	--
-North Africa	9 (12.5)	22 (14.9)	--
-Sub-Saharan Africa	1 (1.4)	15 (10.2)	--
-India/Pakistan	1 (1.4)	12 (8.1)	--
-South-East Asia	3 (4.2)	2 (1.4)	--
-Eastern Europe	18 (25)	3 (2)	--
Close contact[a]; n (%)	170 (53.0)	222 (65.3)	<0.01
Index case with positive smear; n (%)	229 (71.3)	228 (67.1)	0.24
BCG-vaccination; n (%)	124 (38.6)	166 (48.8)	0.01
1st TST-positive; n (%)	194 (60.4)	168 (49.4)	<0.01
TB infection diagnosis; n (%)	223 (69.5)	184 (54.1)	<0.01
BCG-vaccinated	96/124 (77.4)	85/166 (51.2)	<0.01
Non-BCG-vaccinated	127/197 (64.5)	99/174 (56.9)	0.14
Preventive therapy prescribed; n (%)	186 (57.9)	171 (50.3)	0.05

IQR: interquartil range, BCG: Bacillus Calmette-Guérin, TST: tuberculin skin test.
[a] Exposure to the index case was stratified as close (household or daily ≥6 hours of exposure), frequent (daily <6 hours of exposure), and occasional (no household or daily exposure, and <2 hours of exposure each time).

TB Infection diagnosis and preventive therapy

Regarding diagnosis, 407 of 661 contacts (61.6%) were diagnosed with TB infection according to the definition in each period (69.5% in the TST period and 54.1% in the QFT-GIT period; p <0.01). Of the 290 BCG-vaccinated contacts in both periods, 181 (64.5%) were diagnosed with TB infection (77.4% in the TST period and 51.2% in the QFT-GIT period; p <0.01). Table 2 shows the results of both tests for TB infection diagnosis in the 166 BCG-vaccinated patients in the QFT-GIT period. As for the 371 non-BCG contacts, 226 (60.9%) were diagnosed with TB infection, with no significant differences between periods (64.5% in the TST period and 56.9% in the QFT-GIT period; $p = 0.14$).

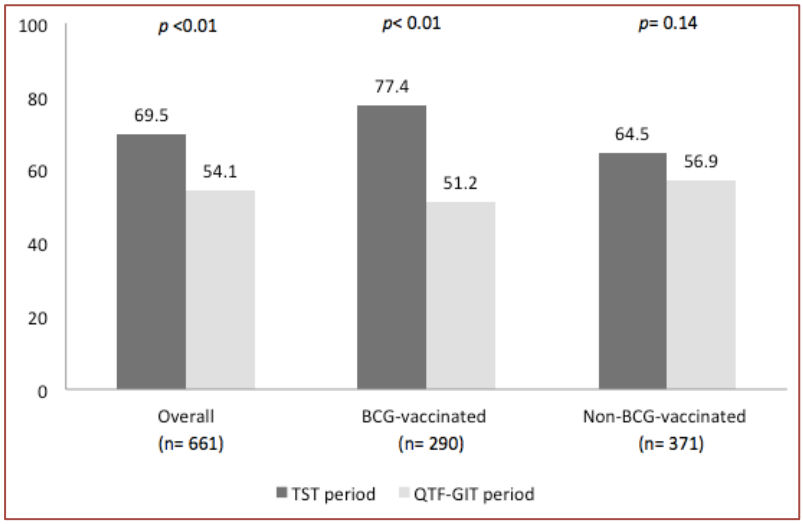
Table 2. TST and QFT-GIT results of BCG-vaccinated patients in the second period

		QFT-GIT		
		Positive	Negative	
TST	Positive	55 (40 (73%) patients with TST ≥15 mm)	44 (21 (48%) patients with TST ≥15 mm)	99
	Negative	9	58	67
		64	102	166

TST: tuberculin skin test; QFT-GIT: QuantiFERON®-TB Gold In-Tube.

While there was a higher proportion of diagnosis of TB infection among BCG-vaccinated contacts than among non-BCG-vaccinated contacts in the TST-period (77.4% and 64.5% respectively; $p = 0.01$), there was no such difference in the QFT-GIT period, when TB infection was diagnosed in 51.2% and 56.9% of BCG-vaccinated and non-BCG-vaccinated contacts respectively; $p = 0.29$) (Fig 2).

Figure 2. Proportion of TB infection diagnosis by study period and BCG-vaccination status



As regards treatment, 357 courses of preventive treatment were prescribed. There were 50 contacts diagnosed with TB infection that were not recommended treatment: 37 (16.6%) and 13 (7%) in the TST and QFT-GIT-periods respectively. Eight contacts refused treatment. The most common regimen was 6-month INH (n = 275), followed by INH for 9 months (n = 47), rifampicin for 4 months (n = 19), and combination therapy with INH-RMP for 3 months (n = 8). Among the 330 INH-based regimens, 19 individuals (5.8%) developed toxicity and required drug withdrawal; 14 of them (73.7%) completed treatment with RMP. Overall, 290 (81.2%) contacts completed a whole course of treatment (77.9% and 87.1% in the TST and QFT-GIT periods, respectively).

Development of active tuberculosis

The outcomes of the 661 contacts are shown in Table 3. Information was retrievable from the electronic medical records for 616 (93.2%) individuals, and another 45 (6.8%) were contacted by phone.

Table 3. Final disposition and incidence of active TB by study period

	TST period (N = 321)	QFT-GIT period (N = 340)	*p*
Died[a]	11	13	0.79
Lost to follow-up before 5 years	14	54	<0.01
Median follow-up, years (IQR)	3.5 (2.0-4.2)	3.6 (2.7-4.4)	0.5
Follow-up (5 years maximum)			
Median (IQR), years	5 (N.A)	5 (4.9-5.0)	
Patient-years	1581.84	1595.15	
Incident TB cases	2	1	0.96
Cumulative incidence, %	0.62	0.29	0.59
Density of incidence, (TB cases x 1 000 p-years (95%CI))	1.26 (0.21-4.18)	0.63 (0.03-3.09)	0.62

TB: tuberculosis. TST: tuberculin skin test; QFT-GIT: QuantiFERON®-TB Gold In-Tube. N.A: non-applicable.[a] Died of non-TB related causes.

Over the median follow-up period of 5 years, three contacts developed active TB: two screened in the TST period and one screened in the QFT-GIT period. Table 4 shows their main features.

Table 4. Descriptive features of the TB cases diagnosed during the follow-up period

	TST period		QFT-GIT period
Time from the first TB infection screening (years)	4.5	4.2	3.3
Description and relationship with the index case	Man, 51 Spanish BCG-vaccinated Occasional relationship	Woman, 22 Bolivian BCG-vaccinated Close contact	Woman, 26 Spanish Not-BCG-vaccinated Close contact
TB infection screening	Negative TST and booster. No TB infection. No treatment.	Positive TST (16 mm). TB infection diagnosis and treatment	Positive TST (13 mm); switched from negative after the window period) TB infection diagnosis and treatment
Risk factor for developing TB	No risk factors	Abandoned preventive treatment in the first month (pregnancy)	Lack of adherence to treatment (6-month isoniazid)
Form of TB	Pulmonary Upper lobes.	Pulmonary Upper lobes.	Pulmonary Upper lobes.

BCG: Bacillus Calmétte-Guerin; IC: Index case; TST: tuberculin skin test; TB: tuberculosis.

Discussion

The results of this observational study support our hypothesis that the use of QFT-GIT for targeting BCG-vaccinated TB contacts for preventive therapy is as effective as a TST-based strategy for preventing subsequent development of TB, while allowing a substantial reduction of treatment prescriptions.

In 2008, we found that we were treating a huge proportion of BCG-vaccinated individuals (three out of every four contacts), and therefore decided to update the screening strategy. In

June of that year we introduced the QFT-GIT assay as part of our routine assessment of BCG-vaccinated TB contacts, in line with current knowledge at that time. Despite the conservative approach of treating close contacts with TST ≥15 mm and negative QFT result, we attained a significant reduction of 26% in TB diagnoses among BCG-vaccinated contacts, without increasing the risk of active TB. This outcome is consistent with the findings of longitudinal studies in other countries with low and intermediate incidences of TB and high vaccination rates [12-14]. Although the effect of BCG vaccination on the TST's specificity should not last over 10 years if BCG was received in infancy, as it used to until 1978 in Spain, and BCG would unlikely have a major influence in TST results in adults [15], obvious differences have been reported to date in TST results and BCG vaccination status [16].

In a German study of close contacts of smear-positive TB patients (n = 954), of the 495 BCG-vaccinated contacts who were TST-positive, 83% were ≥5 mm and 31% were ≥10 mm, while only 17% had a positive QFT-GIT assay result [12]. After at least two years of follow-up, none of the 413 TST-positive/QFT-GIT-negative untreated contacts had developed active TB. In a French study of 687 TB contacts, of the 300 TST-positive contacts (≥10 mm), only 106 (35%) had positive QFT-GIT results [13]. Two contacts developed active TB after 3 years, one of whom had a discordant TST-positive/QFT-GIT-negative result (negative predictive value for the QFT-GIT assay of 99.8%). In another retrospective study from South Korea, which included 1826 high-school student contacts, of the 270 TST-positive contacts (≥10 mm), 203 (75%) had positive QuantiFERON-Gold (QFT-G) results, but none of the 67 TST-positive/QFT-G-negative untreated contacts progressed to active TB [14]. Conversely, a Dutch study, which included foreign-born close contacts of smear-positive TB patients with high BCG-vaccination rates (81%), showed that using the QFT-GIT assay for preventive therapy decision, resulted in three missed contacts who had positive TST results and subsequently progressed to active TB [17]. However, in that study, QFT-GIT was performed

only once, and shortly after the diagnosis of the index case. Therefore, it is plausible that these cases could have been captured if retested a few weeks later.

Despite the reported differences, the results of the present and the three previous studies indicate that the QFT-GIT assay is safe for targeting preventive therapy to fewer contacts. As for cost-effectiveness in contact screening, the benefits of applying QFT-GIT as either a confirmatory test or in place of the TST is also currently unknown. Some heath economic models have explored this issue. Given the similar sensitivities of QFT-GIT and TST for TB infection in immunocompetent individuals and the higher specificity of QFT-GIT, despite its higher testing costs, some models indicate that IGRA-based strategies might be the most cost-effective option when a high pre-test probability is expected (>59%) [18]. However, in cases where the estimated probability is lower, performing the QFT-GIT only in TST-positive contacts would probably be most cost-effective, as it would significantly reduce the number of QFT-GIT tests, and thus the overall testing costs. In our study, the BCG-vaccinated group in the second period increased from 38% to almost half the cohort of contacts; the saving of 26% of preventive therapies, blood tests and follow-up visits, as well as the avoidance of unnecessary risk, justify the change in the protocol.

The present study also provides two relevant findings related to the screening and treatment of TB infection among contacts. First, there was a non-negligible TB prevalence of 2.3% at the time of the first appointment in the TB Unit among the 770 individuals with a recent infection in our cohort (12 and 6 patients in each period, respectively); indeed, this confirms the importance of contact tracing for finding new cases and providing early treatment to avoid TB transmission [19]. Second, a remarkably high proportion of individuals (81.2%) completed a full course of preventive therapy without serious adverse events. In 5% of cases, foreseeable liver toxicity was detected early and reversed by prompt drug withdrawal. Our experience confirms that high completion rates are possible when well-trained staff deliver

comprehensive health education about treatment and toxicity following systematic interviews and providing written information [20].

Although the single-center design might theoretically be considered a limitation, in fact it is one of the main strengths of the study because it guarantees the homogeneity of the series. Since contacts were prospectively evaluated under the same clinical program, our results were not biased by diverse diagnosis and treatment strategies. Indeed, it was the evaluating team who treated the index cases and their contacts, prescribed preventive therapy, systematically took active measures to promote and control adherence to treatment, and looked for the follow-up of the contacts. While there are larger cohorts, they usually come from regional databases or the fusion of several databases coming from regions with different TB prevalence, with different diagnostic and therapeutic approaches and no complete data on BCG-vaccination [21]. This paper provides well-documented evidence on the secure saving of unnecessary treatments of TB contacts after the implementation of an IGRA. Although differences in LTBI diagnosis could be attributed to a difference in the cut-off of the TST (5 mm in the first period and 15 mm in the second), QFT-GIT contributed in the decision-making by means of its high negative predictive value [7].

The other strength of the study is the long-term assessment for TB development. These five years of follow-up include the highest risk period for developing active TB after being infected, which has been classically established in two years [22]. Had we chosen a two-year period, we would have missed the three new cases of TB.

Despite its strengths, this study also has limitations that deserve further comment. First, the retrospective design: development of active TB was evaluated by passive monitoring of contacts, who were assessed by reviewing clinical charts and phone interviews. This may have resulted in some missed cases of active disease, although specific features of TB (lack of mention in clinical notes equals lack of active TB) and the fact that active cases would

have been referred to our Centre do make this unlikely. Moreover, we did a cross-match with the contacts in our cohort and the detection-system of new TB cases in Catalonia from 2006 to 2015. Second, there were differences in the follow-up at 5 years, probably because of the high proportion of immigrants in the second period (45.9%), who went back to their countries as a consequence of the economic crisis in our country. However, the mean follow-up period was almost 4 years (3.99; SD 1.08), which means that most of the high-risk period of developing TB was passed before leaving Spain. Third, there were very few contacts that progressed to active in both periods; as a consequence, wide confidence intervals impaired a proper comparison of TB incidence between the two periods. Fourth, since we did not genotype the causative strain of the three "assumed" cases of incident secondary TB, we cannot exclude the possibility of reinfection by a different strain.

In conclusion, the results of this study add evidence on the benefit of implementing QFT-GIT to target BCG-vaccinated contacts for preventive therapy. This approach reduces exposure to unnecessary treatment without increasing the risk of subsequent active TB. Prospective cohort studies with health economic data are needed to determine whether this strategy is suitable and cost-effective for the management of non-BCG-vaccinated contacts, and other risk groups for active TB.

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3. Contact tracing studies

3.2 Clinical Trial: the OPTIMIST study

QuantiFERON®-TB Gold In-Tube as a confirmatory test for tuberculin skin test in tuberculosis contact tracing: A non-inferiority clinical trial

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Research in context

Evidence before this study: Some observational studies have shown that, compared to the tuberculin skin test (TST), interferon-γ release assays (IGRA) may reduce the number of diagnoses of tuberculosis infections in people at risk. However, so far there is no reliable evidence to confidently base preventive therapy on IGRAs. As a consequence, there are large discrepancies in guidelines and position statements for their use across epidemiologically similar settings.

Added value of this study: This is the first clinical trial comparing two diagnostic strategies, with and without an IGRA, for targeting preventive therapy in contact investigations of tuberculosis. This trial demonstrates that a two-step strategy of TST confirmed by QFT-GIT allows avoiding a significant proportion of unnecessary preventive treatments, without increasing the risk of subsequent active disease in household contacts of tuberculosis.

Implications of all available evidence: The results of this trial support previous evidence on the higher accuracy of IGRAs over TSTs, and reassure their use for targeting preventive therapy in contact investigations of tuberculosis in low-incidence settings.

SUMMARY

Background. Interferon-γ release assays have better specificity than the tuberculin skin test (TST) for the diagnosis of tuberculosis infection and high negative predictive value for the development of tuberculosis disease. We hypothesized that a sequential strategy using TST followed by confirmation with the whole-blood interferon-γ release assay QuantiFERON®-TB Gold In-Tube (QFT-GIT) would narrow the target population for preventive therapy in contact tracing of tuberculosis without increasing the risk of subsequent active disease.

Methods. We conducted an open-label, randomized trial, to test the non-inferiority of a two-step strategy with the tuberculin skin test followed by QFT-GIT as a confirmatory test (the TST/QFT arm) to the standard TST-alone strategy (TST arm) for targeting preventive therapy in healthy household adult contacts of tuberculosis in 12 Spanish hospitals. Randomisation was stratified by centre in a 1:1 allocation ratio, using a computer-generated randomization list. The primary endpoint was the development of tuberculosis in a two-year period of follow-up, with a non-inferiority margin of 1.5 percentage points. We performed both intention-to-treat and per-protocol analysis. This study was registered with Clinicaltrials.gov, number NCT01223534.

Findings. From September 23rd, 2010 to February 3rd, 2014 a total of 871 contacts were randomized. There were 79 dropouts; 792 and 492 individuals were included in the intention-to-treat and per-protocol analyses, respectively. Four contacts in the TST arm and two in the TST/QFT arm developed tuberculosis. In the modified intention-to-treat analysis, this accounted for 0.99% in the TST arm and 0·51% in the TST/QFT arm (-0.48% difference; 97.5% confidence interval [CI], -0.90% to 1.86%); in the per-protocol analysis, the corresponding rates were 1.67% and 0.82% in the TST and TST/QFT arms, respectively (-0.85% difference; 97.5% CI, −1.43% to 3.14%). Of the 792 contacts analysed, 65.3% in the

TST arm and 42.2% in the TST/QFT arm were diagnosed with tuberculosis infection (23.1% difference; 97.5% CI, 16.4% to 30.0%).

Interpretation. In low-incidence settings, screening household contacts with the tuberculin skin test and using QFT-GIT as a confirmatory test is not inferior to TST-alone for preventing active tuberculosis, allowing a safe reduction of preventive treatments.

Funding. This is an investigator-initiated trial. The study was supported by a competitive grant of the Spanish Government. Alere™, the Spanish distributor of QFT-GIT (Cellestis Limited, currently owned by Qiagen) provided the participating centres with QFT-GIT blood-collecting tubes.

Introduction

According to the World Health Organization (WHO), tuberculosis (TB) tops the causes of death from infectious diseases worldwide.[1] While prompt diagnosis and proper treatment are essential to tackle the TB epidemic in high-incidence settings, an additional important measure for TB control in low-incidence countries is treating latently-infected people who are at risk of developing active disease.[1] Recently acquired TB infection, from individuals with infectious pulmonary disease, is known to pose a high risk of progression to active disease.[2] Therefore, tracing the contacts of patients with pulmonary TB has become the central intervention for identifying and treating recently infected people, and ultimately preventing the development of new cases of TB.[3] Unfortunately, because of the low ability of the tuberculin skin test (TST) to predict active TB, this practice requires treating a high number of at-risk people to prevent a single case of disease.[4,5]

T-cell-based interferon-gamma release assays (IGRAs) were first developed a decade ago, at which time they represented a promising alternative to the TST; however, they too are limited by their poor ability to predict future active TB, being only slightly better than TST at best.[6–8] Although this dampened initial expectations, IGRAs may still be preferable to the TST because they may reduce the number of contacts put on treatment without increasing subsequent active TB.[7] Although supported by several longitudinal studies in low-incidence settings,[9–11] the true benefit of the IGRA-based screening strategies over the standard TST-based strategy has not been demonstrated in randomized clinical trials.

In this clinical trial, we assessed the non-inferiority to traditional testing of a sequential strategy using the TST followed by confirmation with the QuantiFERON®-TB Gold In-Tube (QFT-GIT) test when targeting preventive therapy in household contacts of patients with TB.

We hypothesized that such a strategy should narrow the target population compared with the standard TST-based strategy, without increasing the risk of subsequent active TB.

Methods

Study design

The OPTIMIST study (Clinicaltrials.gov number: NCT01223534) was an investigator-initiated, randomized, open-label, controlled clinical trial that we conducted at 12 public hospitals in Spain. The aim of the study was to test the non-inferiority of a sequential strategy of TST followed by QFT-GIT as a confirmatory test against a standard TST-alone strategy when targeting preventive therapy for household contacts of patients with TB.

The trial was conducted in accordance with the Declaration of Helsinki, and was reported in agreement with the key methodological items of the CONSORT (Consolidated Standards of Reporting Trials) statement. The research ethics committee at each participating site approved the study protocol, with Bellvitge University Hospital acting as Research Ethics Coordinator (ref AC111/09). The study Protocol can be found online (Supplementary material).

Participants

Participants were considered eligible if they were healthy adult household contacts of patients with pulmonary or laryngeal TB and if they provided written informed consent. Contacts whose index case finally had either no culture-confirmed TB or a multi-resistant strain were withdrawn from the study. Exclusion criteria included any immunosuppressive conditions and previous diagnosis of TB infection.

Randomisation

Participants were randomly allocated to either a TST-alone strategy (the TST arm) or a two-

step TST/QFT-GIT strategy (the TST/QTF arm). Randomisation was stratified by center in a 1:1 allocation ratio, using a computer-generated randomization list integrated into the eCRF. None of the investigators could access the list.

Procedures

Participants allocated to the TST/QFT arm were first tested with TST, and responders (≥5 mm) underwent a QFT-GIT test for confirmation. Diagnosis of TB infection was based on the QFT-GIT result. By contrast, diagnosis of TB infection was based only on the TST result in the TST arm. Participants with negative TST or QFT-GIT results, who had been tested two months before the last contact with the index case, also underwent a second test eight weeks later (Figure 1). All contacts diagnosed with TB infection were given isoniazid 300 mg daily for six months, or rifampicin 600 mg daily for four months if they did not tolerate isoniazid or if the related index case's culture yielded an isoniazid-resistant strain. Adherence was measured at each appointment as follows: by detection of N-acetyl isoniazid in the urine, using the Eidus-Hamilton test,[12] for isoniazid; by the orange color of urine in case of rifampicin; and by returned pill count in each visit. Development of active TB was assessed by clinical evaluation and chest x-ray at 24 months. When there was clinical suspicion of TB, respiratory samples were taken for smear stain and culture, and chest x-rays were taken. Follow-up visits were scheduled twice a year for two years, irrespective of whether participants received preventive therapy.

Outcomes

The primary study endpoint was the proportion of contacts developing active TB during the 24 months after randomization. Diagnosis of active TB was considered definitive if *Mycobacterium tuberculosis* was isolated in clinical samples, highly probable if there were compatible clinical data and a positive molecular test in respiratory samples or sterile fluids

without positive culture, or probable if there were suggestive clinical and radiographic signs without microbiological confirmation and a favorable response to specific therapy. Strains of secondary TB cases were matched with those of their index case through MIRU-VNTR **(**Mycobacterial Interspersed Repetitive Units**-**Variable Number of Tandem Repeats), which was performed by amplifying the 24 MIRU-VNTR loci as described elsewhere.[13]

The secondary endpoint was the proportion of contacts diagnosed with TB infection.

The safety and tolerability of preventive therapy were assessed on the basis of symptoms or signs during treatment (clinical assessment and blood tests at baseline and after 1 and 3 months of treatment, and whenever patients presented with new symptoms suggestive of drug toxicity).

Statistical analysis

We designed the trial to assess the non-inferiority of the TST/QFT arm with respect to the TST arm for preventing development of active TB. Non-inferiority was defined as the upper limit of the one-sided, 97.5% confidence interval (CI) of the difference in TB incidence between the TST/QFT and the TST arms, being less than 1.5 percentage points. Based on an expected 0.5% risk of progression to active disease within the first two years after the randomization, 348 patients was the number of contacts required in each group to achieve a power of 80% to demonstrate the non-inferiority hypothesis. To account for a possible 20% patient loss to follow-up in each group, we planned to enroll 870 contacts overall. This sample size was also sufficient to detect a minimum expected effect size of 10% reduction in the proportion of TB infection diagnosis, assuming a TST positive rate of 45%.

Pre-specified subgroup analyses were performed according to Bacillus Calmette-Guérin (BCG) vaccination status. We added a post-hoc analysis to improve the expression of the

savings in unnecessary treatments from one strategy to another, which was expressed as the number needed to screen (NNS). This measure was an estimate of the number of contacts needed to be screened in the TST/QFT arm to avoid one unnecessary treatment in the TST arm. Thus, it is the reciprocal of the absolute risk reduction (1/absolute risk reduction) of being diagnosed with TB infection.

We performed both modified intention-to-treat and per-protocol analyses. The modified intention-to-treat population included all participants who completed the allocated diagnostic strategy and had at least one follow-up assessment. The per-protocol population was restricted to participants who adhered to the clinical trial instructions as established in the protocol in terms of preventive therapy completion, proven adherence and schedule punctuality. The protocol specified an interim analysis two years after the inclusion of the 400th patient, and we established that the trial would stop if the lower 97.5% CI for the estimated incidence of TB was strictly to the left of the non-inferiority margin. After reviewing the interim analysis, the data and safety monitoring committee recommended continuing the study as planned.

The baseline characteristics and outcome measures were compared by the χ^2test for categorical variables, and Student *t*-test or nonparametric Mann-Whitney *U* test for continuous variables, as appropriate. The incidence of active TB was reported as the cumulative incidence and incidence rate (per 100,000 person-years). All Statistical analyses were carried out with R (version 3.2.5 for Windows).

An independent data monitoring committee, the Central Clinical Research Unit in Clinical Trials (UCICEC) had access to all data and oversaw the trial. The Clinical Trials and Statistical Unit at Bellvitge University Hospital-IDIBELL downloaded and analyzed the data contained in

the electronic case report forms (eCRF) following the data analysis plan.

Role of the funding source. Alere™, the Spanish distributor of QFT-GIT (Cellestis Limited, currently owned by Qiagen) provided the participating centers with QFT-GIT blood-collecting tubes, but had no role in the design of the trial, data analysis, or interpretation of the results.

Results

From September 23, 2010 to February 3, 2014 we enrolled 871 subjects in the study: of these, 438 were allocated to the TST arm and 433 were allocated to the TST/QFT arm. After excluding 79 (9.1%) participants, 792 (90.9%) were included in the modified intention-to-treat analysis, and 482 (55.3%) qualified for the per-protocol analysis. The study ended in February 2016 when the last recruited patient attended the last follow-up visit. The flow of patients through the study is shown in figure 2.

Both arms were well balanced in terms of baseline demographic characteristics, relationship with the index case, and clinical features of the index cases (Table 1).

During the follow-up period, six contacts developed active TB, four in the TST arm and two in the TST/QFT arm. All six cases occurred before the sixth month from their baseline appointment. Full details of the TB cases are provided in Table 2. By the modified intention-to-treat analysis, the cumulative incidences of active TB were 0.99% (97.5% CI, 0.34% to 2.85%) and 0.51% (97.5% CI, 0.12 to 2.17) in the TST and the TST/QFT arms, respectively, giving a difference in the TB incidence rate of −0.48 (97.5% CI, −0.90 to 1.86). The results of the per-protocol analysis were consistent with of the modified intention-to-treat analysis, giving a cumulative incidence of active TB of 1.67% (97.5% CI, 0.58 to 4.76) and 0.82% (97.5% CI, 0.19 to 3.45) in the TST and the TST/QFT arms, respectively; the difference in TB

incidence rate was −0.85 (97.5% CI, −1.43 to 3.14) (Figure 3 and Table 3). Three of the six active TB cases in the trial were culture-confirmed, and MIRU-VNTR based genotyping analysis showed identical patterns between their isolates and those from their respective index cases.

In the modified intention-to-treat analysis, TB infection was diagnosed in 263 of 403 contacts (65.3%) in the TST arm, and 164 of 389 contacts (42.2%) in the TST/QFT arm, giving a difference of 23.1% (95% CI, 16.4% to 30.0%). In the per-protocol analysis, 155 of 239 (64.9%) and 104 of 243 (42.8%) were diagnosed with TB infection in the TST and TST/QFT arms respectively, giving a difference of 22.1% (95% CI, 13.5% to 30.9%).

We included 869 subjects in the safety analysis. There were 54 adverse events in 49 patients (28 and 21 for the TST and TST/QFT arms respectively; p=0.4). Regarding the 429 subjects who were given preventive therapy, 42 (9.8%) experienced treatment-related adverse events, the most frequent being liver toxicity related to isoniazid (28 events; 6.8%). Details of these adverse events are provided in the Supplementary Appendix, Table S1.

The analysis stratifying for BCG-vaccination status showed a difference in TB infection diagnoses (between the TST and the TST/QTF arms) of 34.5% (95% CI, 22.5% to 46.5%) in BCG-vaccinated contacts, and 19.5% (95% CI, 10.9% to 28.0%) among non-BCG-vaccinated contacts in the modified intention-to-treat analysis. The corresponding data for the difference between the two arms in the per-protocol analysis were 31.9% (95% CI, 16.9% to 47.0%) among BCG-vaccinated contacts and 18.9% (95% CI, 7.77% to 30.1%) among non-BCG-vaccinated contacts (Figure 4). After adjusting for contagiousness of the index case (sputum smear status and cavitation on chest x-ray), time (cumulative hours per week), and degree of exposure (intimate versus frequent) as covariates, assignation to the TST/QFT arm remained as an independent predictor of reduction in the proportion of diagnosed TB

infections (Tables 4-5).

In the modified intention-to-treat analysis, for every five contacts screened in the two-step TST/QFT arm, one unnecessary treatment could be avoided (NNS=5 ([1/(0.65 – 0.42)= 4.38]). If only the BCG-vaccinated population was considered, the NNS would fall to 3 ([1/ (0.79 – 0.44)= 2.86]) (Supplementary Appendix; Figure S1)

Discussion

In this trial, we demonstrated that using the QFT-GIT as a confirmatory test following a positive TST for targeting preventive therapy in household TB contacts is not inferior to TST-alone strategy for the prevention of subsequent active TB, while it provides the advantage of reducing the number of preventive treatments.

According to the WHO, detection and treatment of TB infection by contact investigation to identify recently exposed people is a main goal of the global strategy to eliminate TB in low-incidence countries by 2035.[14] Therefore, optimizing the screening strategies and promoting compliance with preventive therapy schemes is essential. Since their introduction, IGRAs have been increasingly used for screening adult TB contacts. However, a lack of compelling evidence for their superiority over TST has led to a diversity in the recommendations given in guidelines and position statements; instead, these have relied on reasons other than the evidence-based balance between the benefits and harms of the tests and strategies.[15–20] Some guidelines favor the use of either the TST or IGRA,[15,16,18] whereas others recommend primary testing with the TST, and only retesting TST reactors with an IGRA.[17,19,20]

The two-step approach, aimed at reducing false-positive TST results —especially in BCG-vaccinated individuals— is supported by both observational studies[10,21–23] and favorable

cost-effectiveness analyses in low-incidence countries.[24–26] A large German study which tested close contacts of TB, showed a substantial reduction in the number of diagnoses of TB infection with QFT-GIT (17%) compared with the TST at 10 mm cut-off (31%), and none of the untreated contacts with TST-positive and QFT-GIT-negative results developed TB after at least two years of follow-up.[21] Similarly, 35% of TST-positive contacts in a French study had a positive QFT-GIT, and only one contact (0.2%) with a TST-positive and QFT-GIT-negative result developed active TB after three years of follow-up.[23] In a study of TB contacts in high school students in South Korea, the addition of QFT-Gold (QFT-G) as a confirmatory test for the TST reduced the number of preventive treatments, with none of the TST-positive/QFT-G-negative contacts progressing to active disease.[22] In a Dutch study using an IGRA to confirm TST results, three of nine foreign-born contacts who subsequently developed active TB were missed by the QFT-GIT (they were TST-positive but QFT-GIT-negative).[10] Now, for the first time, a randomized controlled clinical trial provides solid evidence showing the benefit of including IGRA tests to avert over-diagnosis and avoidable treatments when testing adult contacts of patients with TB in a low-incidence setting. Our results showed that for every five contacts screened with the two-step strategy, one unnecessary preventive treatment could be avoided. This benefit was achieved not only in the subset of BCG-vaccinated contacts, in which it may be expected, but also among non-vaccinated contacts. Indeed, several analyses, including those of contacts with the highest risk profiles (i.e. intimate contacts of smear-positive index cases who presented with cavitation on chest x-ray), still showed a significant reduction in the proportion of TB infection diagnosis when screened with the two-step approach.

Our results provide reassurance that using QFT-GIT in TB contact tracing is effective and safe. However, we have not established whether this is the most convenient strategy or whether

the QFT-GIT should entirely replace TST in terms of cost-effectiveness. In the setting of well-selected high-risk contacts, which implies a high estimated pre-test probability, screening with the QFT-GIT alone might be amore cost-effective option, despite the higher unit cost. Conversely, if there is a low pre-test probability, performing the QFT-GIT in TST reactors would probably be most convenient because this strategy would reduce the overall number of QFT-GIT tests, and therefore, testing costs.

Our trial has weaknesses that deserve further comment. First, we did not include a third arm of screening with the QFT-GIT alone, which would have helped us to establish either using QFT-GIT as a confirmatory test or replacing the TST is better strategy. Unfortunately, the necessary size of the sample was prohibitive and could not be achieved in a reasonable period. Second, the two-year observational period may have been insufficient to assess the real risk of developing TB. Indeed, this is a common limitation of most contact-tracing studies looking at the risk of TB development. However, because the first two years after recent exposure constitute the period of highest risk,[2] our results are comparable with previous observational studies.

We concluded that in low-incidence settings of TB, using QFT-GIT to confirm positive TST reactors allows a significant reduction of TB infection diagnoses and preventive therapy prescriptions without increasing the risk of active disease.

Contributors

MS conceived and designed the trial. The first two authors (LM and MS) interpreted the analysis. LM wrote the first draft of the manuscript. The rest of the authors recruited subjects for the trial and collected data. All authors reviewed the manuscript critically for important intellectual content; gave the final approval of the version to be published, and

agreed to be accountable for all aspects of the work, ensuring that questions related to the accuracy or integrity of any part of the work were appropriately investigated and resolved.

Declarations of interest

MS has received travel reimbursement and fees by giving talks on IGRAs at symposia sponsored by Alere Healthcare S.L.U. (supplier of QuantiFERON-TB Gold In-Tube for Spain).

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Tables

Table 1. Baseline characteristics of participants and relationship with their index cases

	Intention-to-treat population		Per-protocol population	
	TST arm (n=403)	**TST/QFT arm (n=389)**	**TST arm (n=239)**	**TST/QFT arm (n=243)**
Participant characteristics				
Gender, male; n (%)	181 (44.9)	169 (43.4)	105 (43.9)	108 (44.4)
Age, years; median (IQR)	39.4 (30.2–54.0)	39.5 (30.3–53.2)	40.4 (30.5–54.5)	42.0 (31.1–56.5)
Spanish origin; n (%)	306 (75.9)	292 (75.1)	197 (82.4)	202 (83.1)
BCG-vaccination*; n (%)	114 (28.3)	108 (27.8)	76 (31.8)	72 (29.6)
Index case data				
Cavitation on X-ray; n (%)	208 (51.6)	210 (54.0)	123 (51.0)	139 (56.7)
Smear-positive sputum; n (%)	313 (77.7)	295 (75.8)	171 (71.0)	179 (73.1)
Weeks since symptom onset; median (IQR)	8 (4–15)	8 (4–16)	8 (4–13)	10 (4–20)
Contact characteristics				
Intimate; n (%)	249 (61.8)	226 (58.1)	158 (65.6)	146 (59.6)
Daily contact >12 h/d; n (%)	121 (30.0)	110 (28.3)	84 (35.2)	78 (32.1)
Days of exposure; median (IQR)	108.0 (59.0–170.0)	121.0 (74.0–180.0)	121.0 (74.0–174.0)	127.0 (87.5–183.5)

*Missing data on BCG-vaccination status (16 and 22 subjects in the TST and TST/QFT arms respectively.
IQR: Interquartile range; TB: tuberculosis; BCG: Bacillus Calmette-Guérin; TST: tuberculin skin test. QFT-GIT: QuantiFERON®-TB Gold In-Tube.TST arm: TB infection diagnosis relies on TST results exclusively. TST/QFT arm: TB infection diagnosis relies on QFT-GIT results in TST positive contacts (confirmatory test), or in a negative TST

Table 2. Description of the active tuberculosis cases developed during the study period

	TST arm				TST/QFT arm	
Sex, Age (years)	Woman, 31	Woman, 20	Man, 60	Woman, 38	Man, 56	Man, 41
TB infection at screening	Positive TST	Positive TST	Negative TST	Negative 1stTST	Negative 1stTST	Negative 1stTST
Preventive therapy (PT)	Isoniazid 6 m*	Isoniazid 3 m*	No	No	No	No
Time from screening (days)	304 (4 months after PT)	91 (While on PT)	182 (Scheduled visit)	60 (Window period)	60 (Window period)	60 (Window period)
Type of TB	Pleural	Pleural	Pulmonary	Pulmonary	Pulmonary	Pulmonary
Tests at active TB diagnosis	N.A	N.A	Negative TST and QFT-GIT	Positive TST	Positive TST and QFT-GIT	Positive TST and QFT-GIT
Diagnosis	Definitive Sputum culture†	Probable Lymphocytic exudative pleural effusion with normal ADA activity. Response to TB treatment	Definitive Sputum culture†	Definitive Sputum culture†	Probable Response to TB treatment	Probable Response to TB treatment

*months. †In the three culture-confirmed cases of tuberculosis, MIRU-VNTR genotyping showed the involvement of the same strain as their respective index cases. TB: tuberculosis; TST: tuberculin skin test. QFT-GIT: QuantiFERON®-TB Gold In-Tube. TST arm: TB infection diagnosis relies on TST results exclusively. TST/QFT arm: TB infection diagnosis relies on QFT-GIT (QuantiFERON®-TB Gold In-Tube) results in TST positive contacts (confirmatory test), or in a negative TST; ADA: adenosine deaminase; MIRU-VNTR: Mycobacterial Interspersed Repetitive Units-Variable number tandem repeats; N.A: non applicable.

Table 3. Participants' disposition and development of active disease at the end of the study

	Intention-to-treat population		Per-protocol population	
	TST arm (n=403)	TST/QFT arm (n=389)	TST arm (n=239)	TST/QFT arm (n=243)
Follow-up (days); median (IQR)	735.0 (720.0–791.0)	735.0 (727.0–773.0)	735.0 (728.0–755.0)	735.0 (729.0–749.0)
Dead	2	3	0	0
Lost to follow-up	67	45	-	-
Active TB	4	2	4	2
Cumulative incidence	0.99 (0.34–2.85)	0.51 (0.12–2.17)	1.67 (0.58–4.76)	0.82 (0.19–3.45)
Incidence rate (n x 100,000 p/y)	505.5 (110.6–954.5)	252.1 (21.1–629.8)	820.0 (180.4–1557.8)	405.5 (33.9–1012.8)

IQR: Interquartile range; TB: tuberculosis; TST: tuberculin skin test. TST arm: TB infection diagnosis relies on TST results exclusively. TST/QFT arm: TB infection diagnosis relies on QFT–GIT (QuantiFERON® -TB Gold In-Tube) results in TST positive contacts (confirmatory test), or in a negative TST.

Table 4. Variables associated with diagnosis of tuberculosis infection in household contacts of pulmonary tuberculosis

	Intention-to-treat population				Per-protocol population			
Variable	Unadjusted		Adjusted		Unadjusted		Adjusted	
	OR	CI95%	OR	CI95%	OR	CI95%	OR	CI95%
Allocation to the TST/QFT arm	0.39	0.29–0.52	0.37	0.26–0.50	0.41	0.28–0.58	0.37	0.25–0.56
Index case with positive sputum smear	2.53	1.81–3.58	2.28	1.55–3.38	2.94	1.95–4.48	2.68	1.67–4.33
Index case with cavitation on chest X-ray	2.04	1.54–2.71	1.71	1.24–2.36	2.02	1.41–2.91	1.54	1.0–2.36
Intimate contact with the index case	1.59	0.83–1.89	1.71	1.24–1.98	1.69	1.17–2.46	1.79	1.19–2.72
Symptomatic weeks of the index case								
4-11 vs. <4 weeks	1.25	0.83–1.89	1.27	0.82–1.98	1.53	0.97–2.44	1.39	0.85–2.30
>11 vs. <4weeks	1.77	1.14–2.76	1.64	1.01–2.66	1.62	1.02–2.58	1.48	0.90–2.47

TST: tuberculin skin test. QFT-GIT: QuantiFERON®-TB Gold In-Tube. OR: Odds Ratio. CI: Confidence interval.
TST/QFT arm: TB infection diagnosis relies on QFT-GIT (QuantiFERON®-TB Gold In-Tube) results in TST positive contacts (confirmatory test), or in a negative TST.

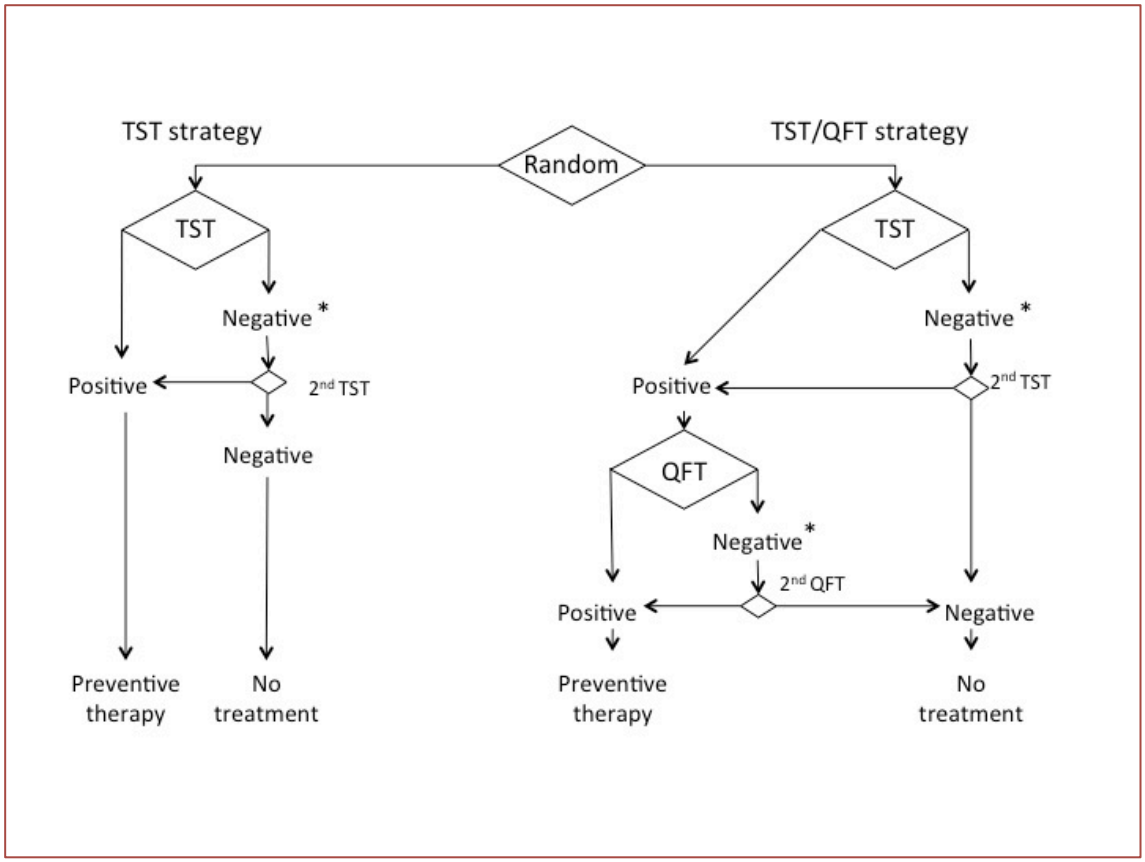
Table 5. Differences in diagnosis of tuberculosis infection between TST and TST/QFT arms stratified for risk

High risk group*								
	Intention-to-treat Population			Chi-Square test	Per-protocol Population			Chi-Square test
ARM	TST arm	TST/QFT arm		*p*	TST arm	TST/QFT arm		*p*
Diagnosis of TB infection	89	64	153		53	44	97	
No TB infection	28	42	70		15	22	37	
	117	106	223	0.014	68	66	134	0.18
Non-High risk group								
	Intention-to-treat Population			Chi-Square test	Per-protocol Population			Chi-Square test
ARM	TST arm	TST/QFT arm		*p*	TST arm	TST/QFT arm		*p*
Diagnosis of TB infection	174	100	274		102	60	162	
No TB infection	112	183	295		69	117	186	
	286	283	569	0.00	171	177	348	0.00

High-risk group incudes intimate contacts of index cases with both cavitary lesions and positive smear sputum.TB: tuberculosis; TST: tuberculin skin test. QFT-GIT: QuantiFERON® -TB Gold In-Tube; TST/QFT arm: TB infection diagnosis relies on QFT-GIT (QuantiFERON® -TB Gold In-Tube) results in TST positive contacts (confirmatory test), or in a negative TST.

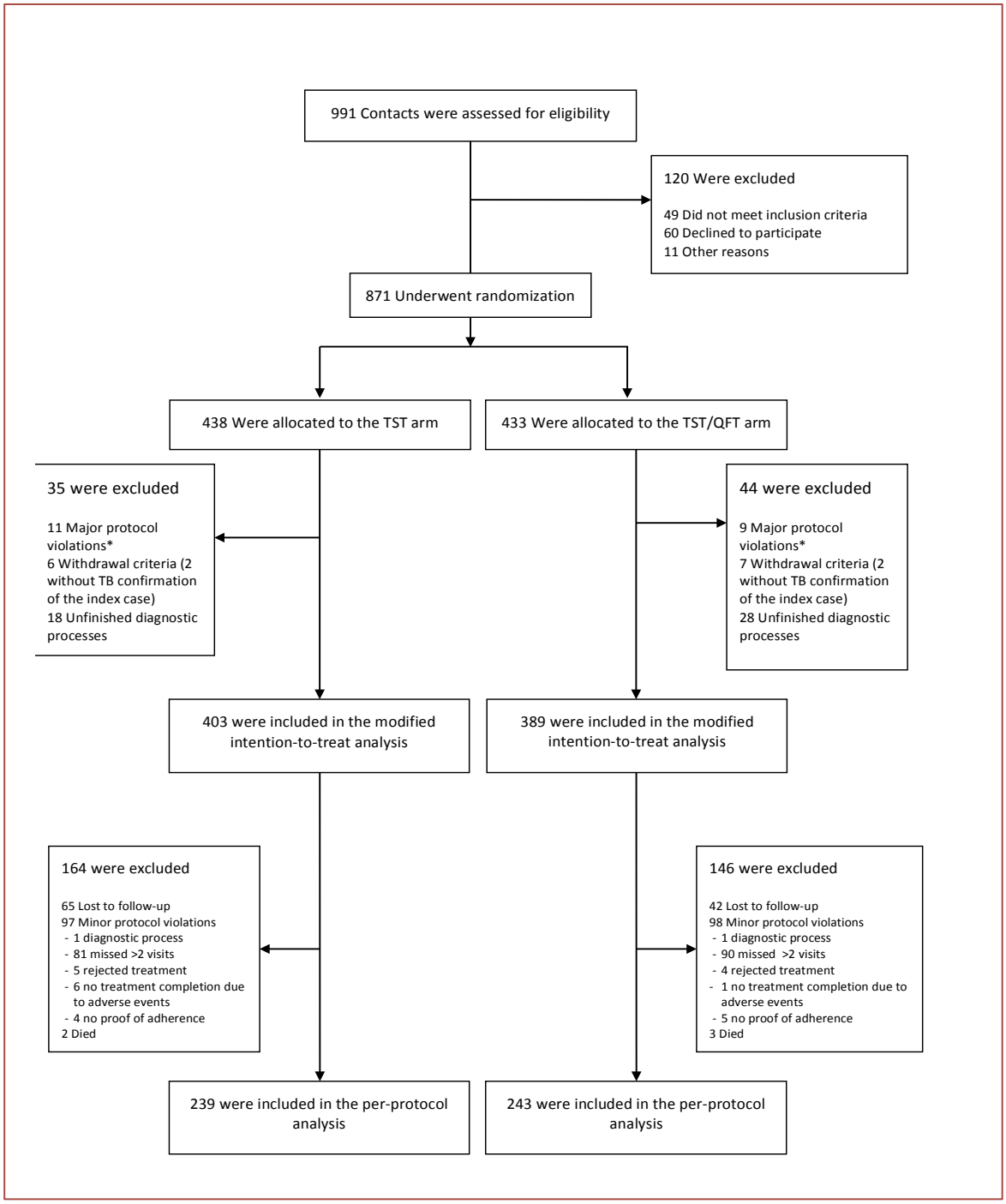
Figures

Figure 1. Overview of the diagnostic strategies



TST: tuberculin skin test. QFT-GIT: QuantiFERON®-TB Gold In-Tube.
* Negative responders were tested again (after eight weeks) so as to avoid the window period. Only if their first appointment was after two months of their last contact with the index case was there no need for a second test.

Figure 2. Study enrolment, randomization, and follow-up of 991 participants assessed



TB: tuberculosis. TST arm: TB infection diagnosis relies on TST results exclusively. TST/QFT arm: TB infection diagnosis relies on QFT-GIT (QuantiFERON®-TB Gold In-Tube) results in TST positive contacts (confirmatory test), or in a negative TST.

* Two subjects (one per arm) were randomized twice. The real number of contacts to be assessed is 869.

Figure 3. Differences in tuberculosis incidence between TST and TST/QFT arms by both modified intention-to treat and per-protocol analyses

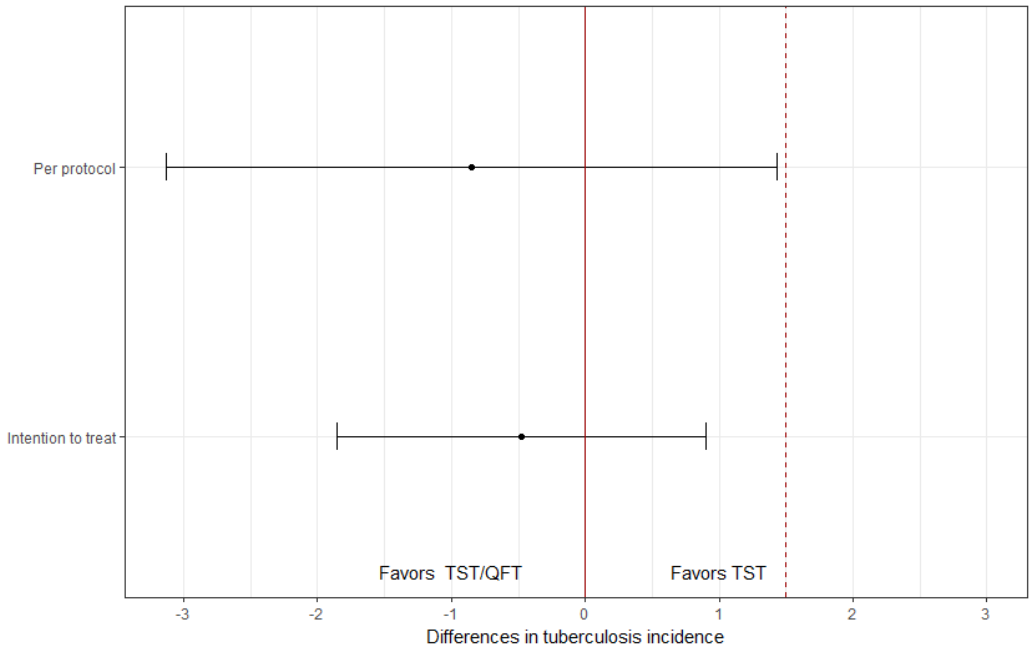


Figure 4. Proportion of participants with tuberculosis infection per arm in the modified intention-to-treat population (A) and in the per-protocol population (B)

Figure 4A.

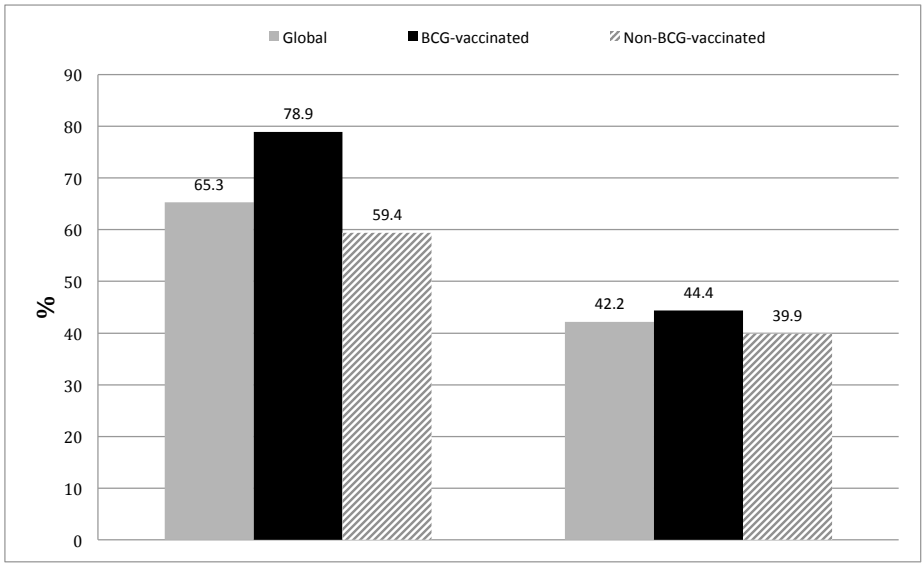
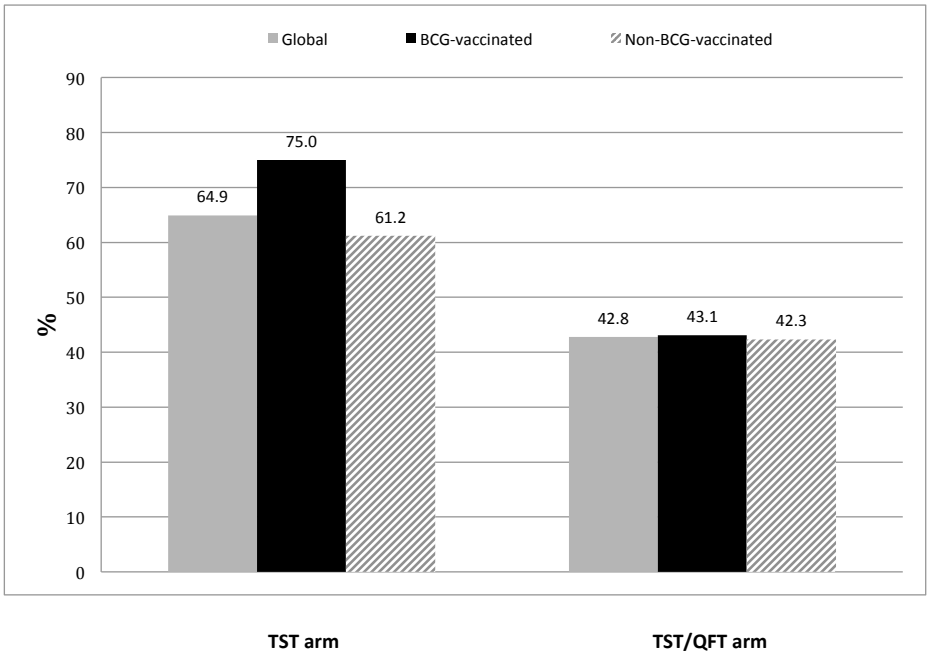


Figure 4B.



BCG: Bacillus Calmette-Guérin. TST arm: Tuberculin Skin Test arm. TST/QFT arm: two-step Tuberculin Skin Test, followed by QuantiFERON®-TB Gold In-Tube, as a confirmatory test.

* Differences between arms for both modified intention-to-treat and per-protocol analyses (Global $p< 0.001$; BCG-vaccinated $p< 0.001$, and Non-BCG-vaccinated $p= 0.001$).

Figure S1 Risk of diagnosis of TB infection and active disease in both arms.

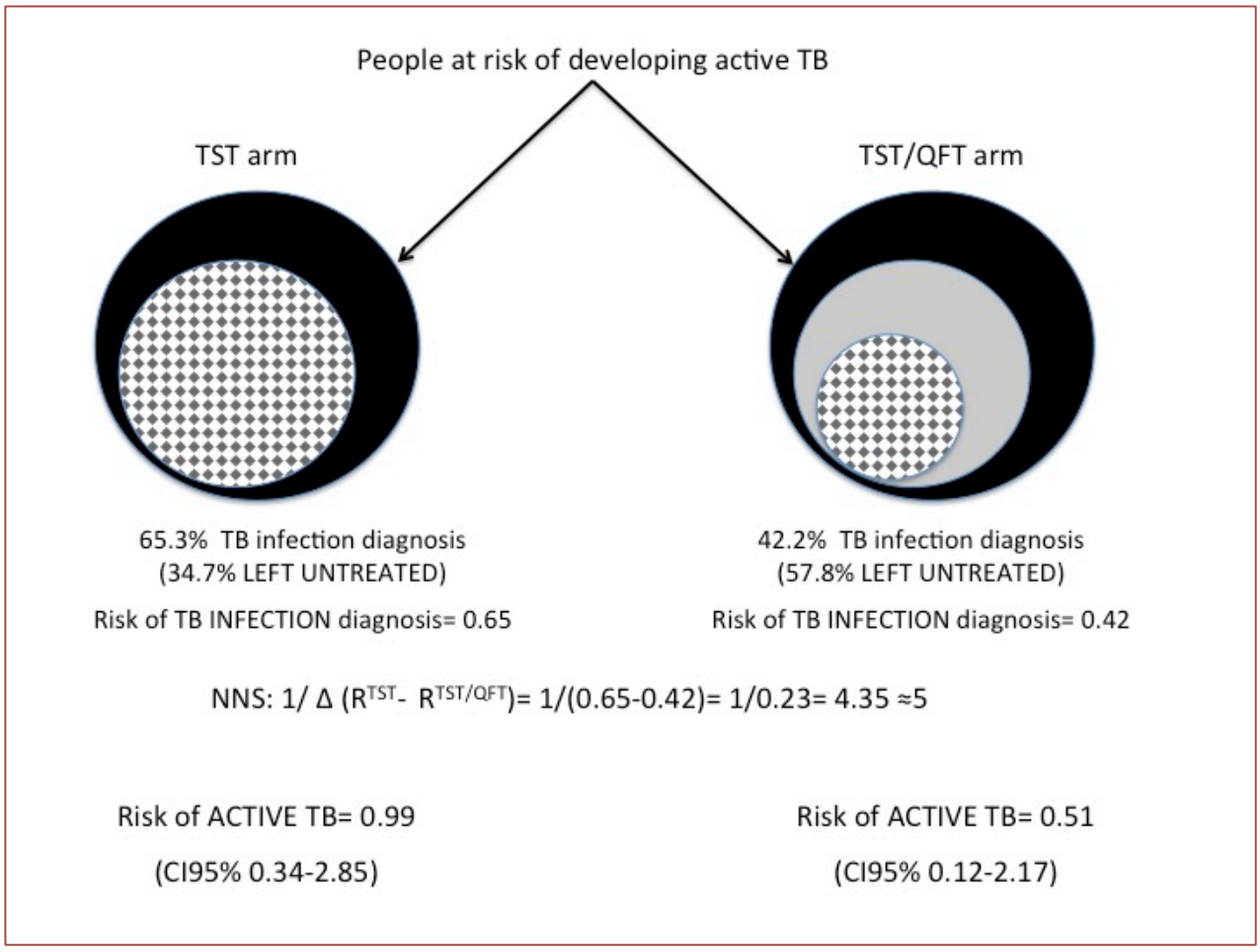
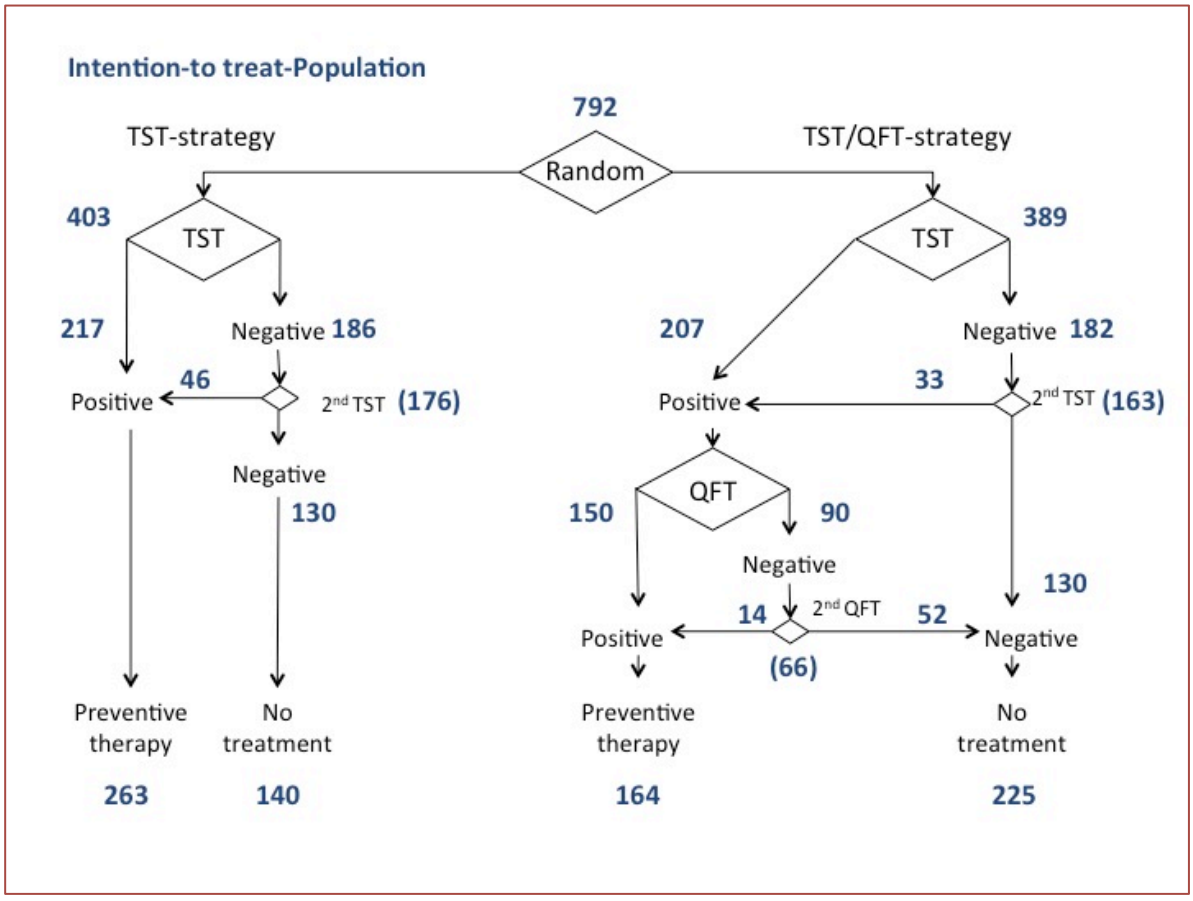
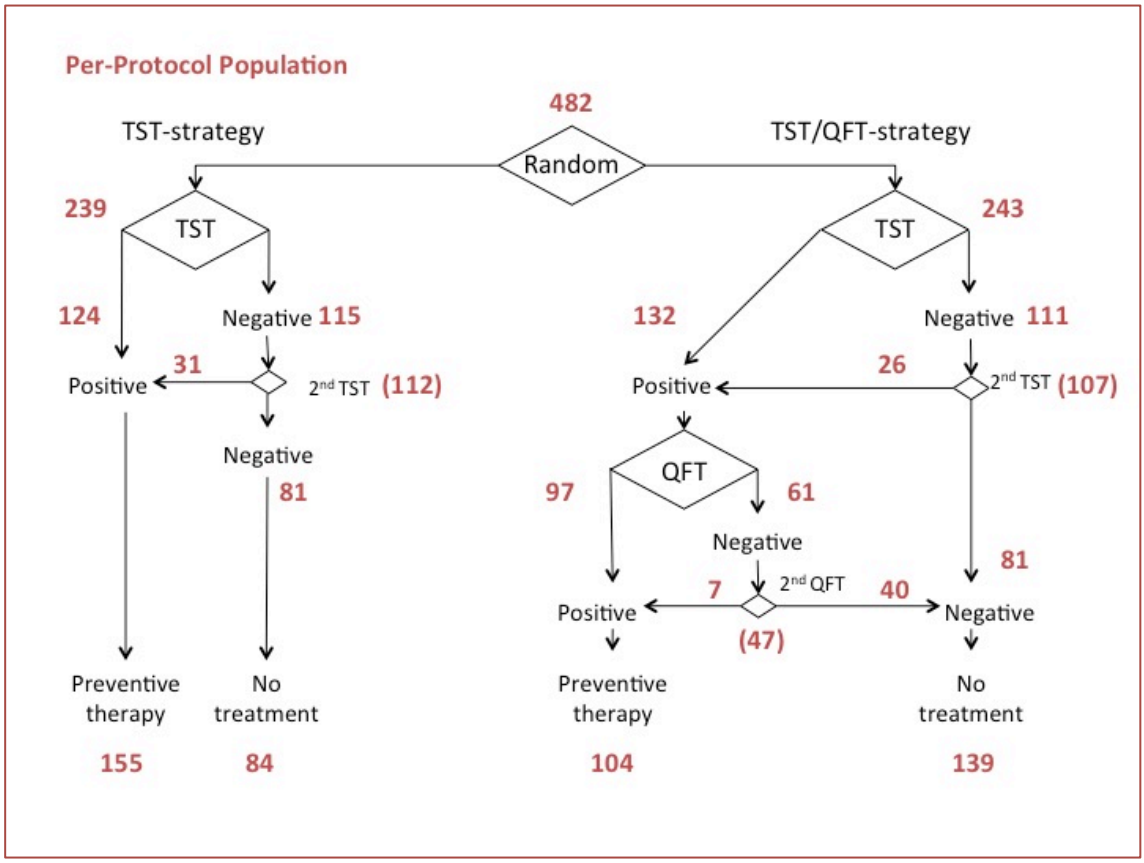


Figure S2:
A. Contacts included in both arms by intention-to treat analysis



B. Contacts included in both arms by per-protocol analysis



The numbers in brackets represent the number of contacts that underwent a second test. The ones who did not have a test repeated had already exceeded the window period when that test was carried out.

DISCUSSION

In low-incidence countries as a whole, the main contribution to the *End TB strategy* consists of proper screening and LTBI treatment for individuals at higher risk for developing active tuberculosis.[113] Unfortunately, until now diagnostic tests are not accurate enough to select which individuals are at higher risk of developing active tuberculosis. Clearly defined groups have been defined as the riskiest, but not all individuals in those groups would develop tuberculosis if left untreated, moreover, treating them all would not imply zero-risk for subsequent active development of the disease. It is not only important to detect new cases of LTBI. Preventive therapy should be completed in order to be effective. Currently available treatments are lengthy, not free from side effects, and require compulsory appointments to check adherence and tolerance for the treatment to be safe and effective. While less toxic and easier to adhere and complete regimens are under investigation, making the most of available tests can also enhance better management of latent tuberculosis infections.

The aim of this dissertation is to improve diagnostic strategies in each risk group. Two different groups and different strategies have been considered: immunosuppressed individuals in which higher sensitivity is preferred, and healthy subjects after being exposed to *Mycobacterium tuberculosis* complex, in which specificity is preferable.

As for groups with impaired immunity, the observational, longitudinal study to check the results of our modified IGRA-containing protocol of LTBI diagnosis in candidates to anti-TNF agents showed three main results. First, anti-TNF–associated tuberculosis can be greatly reduced when a comprehensive protocol for screening and treating LTBI is applied, although a certain risk remains during the first year of treatment. Second, this risk is not related to a lack of sensitivity in diagnostic tests. On the contrary, we

discourage the use of a two-step TST-strategy as a way to increase the number of diagnosis, although that is what the Spanish guidelines currently recommend. In our series, an independent association between BCG vaccination and both second TST positivity (boosting effect) and discordant results (TST positive/QFT-GIT negative) was found, suggesting that the second test increased false positive results. The withdrawal of a second TST offered a substantial and safe reduction in LTBI diagnosis and treatment as compared to the previous two-step TST-based strategies, with no more risk of subsequent active tuberculosis. Third, the systematic periodic retesting of patients for LTBI does not appear to be needed following negative testing prior to anti-TNF therapy. This common question, shared by most clinicians, had until now remained unsolved in the literature.

Regarding another group of immune-impaired patients, we reported the results of a small cohort of liver transplant candidates and individuals waiting for haematological stem cell transplantation, with a log follow-up period. We were able to calculate the predictive values of both TST and QFT-GIT, as no patient was offered preventive treatment, according to the indications of the transplant team. The rate of post-transplant tuberculosis among QFT-GIT-positive patients was both low and comparable to that of the TST in this cohort. Our results added to the evidence that IGRAs are poor at predicting the development of active tuberculosis in transplant recipients, as did the previous four reports of transplant individuals tested with IGRAs at the time it was published. Therefore, with the currently available data, the choice of TSTs or IGRAs for screening transplant candidates should be based on the expected specificity in each

setting, operational factors, logistics, patients' preferences, and cost; always keeping in mind that a negative result does not rule out the future risk of developing tuberculosis.

As for the other group at risk, people recently exposed to an active case of tuberculosis, we confirmed our hypothesis of the safe reduction of preventive treatments when QFT-GIT is added to the diagnostic strategy. First, our experience at the TB Unit after the introduction of QFT-GIT to test BCG-vaccinated contacts showed a significant reduction of diagnoses and thus preventive treatments as compared to the immediate previous period. A remarkably high proportion of individuals (>80%) completed a full course of preventive therapy without serious side effects. Our experience confirms that high completion rates are possible when well-trained staff deliver comprehensive health education about treatment and toxicity. Second, we tested the same hypothesis in a large multicentre clinical trial, the OPTIMIST study. We demonstrated that using the QFT-GIT as a confirmatory test following a positive TST for targeting preventive therapy in household tuberculosis contacts is not inferior to TST-alone strategy for the prevention of subsequent active disease, while it provides the advantage of reducing the number of preventive treatments. For the first time, a randomized controlled clinical trial has provided solid evidence showing the benefit of including IGRA tests to avert over-diagnosis and avoidable treatments when testing adult contacts of patients with TB in a low-incidence setting. This benefit was achieved not only in the subset of BCG-vaccinated contacts, in which it may be expected, but also among non-vaccinated contacts.

The Optimist Study has contributed to the reinforcement and creation of highly specialized TB Units, well trained in contact tracing studies of TB in Spain, which is an undeniable added value of this trial.

These results are expected to remodel the Spanish guidelines and include IGRAs in the recommendations of contact tracing studies of tuberculosis. Other settings with similar epidemiologic profile may also benefit from these outcomes. Although some societies and institutions have already implemented IGRAs in their day-to-day practice, these results may encourage those who still have not to add IGRAs to LTBI screening protocols, as there is now strong evidence of their benefits.

This dissertation has several limitations that deserve comment. First, diagnostic properties, especially predictive values, are closely related to the target disease's prevalence in each setting. Different regions may not benefit from this LTBI approach, mainly if a high pre-test probability is expected. Second, a proper cost-effectiveness analysis might be needed in each type of risk-population to demonstrate the superiority of IGRA-based strategies on a large scale. Third, our cohort of transplant recipients was small and very few cases of active tuberculosis developed, so incidence rates showed huge confidence intervals. Moreover, liver transplant recipients constitute the less immunosuppressed group of solid-organ transplantations; thus, our results cannot be extrapolated to kidney, heart and lung transplant recipients.

Several questions remain to be addressed in further research. The cost-effectiveness of each strategy needs to be assessed ahead of implementation. Other clinical trials comparing results after IGRA-based decisions in other risk groups will be needed in order to establish good quality evidence for the implementation of IGRAs. Countries

with different epidemiologic profiles may need their own trials to better ascertain the best strategy in terms of efficacy and cost-effectiveness. The new-generation QFT-GIT[77] and new-TST (C-Tb)[93] may need to be tested in high-risk populations, not only to compare its performance with QFT-GIT and T-SPOT.TB, but also to make decisions based on its results so as to achieve patient-important outcomes.

CONCLUSIONS

The results of these studies provide significant evidence on the benefits of several IGRA-including diagnostic strategies in populations at risk for developing active tuberculosis. Such gain is achieved by safely avoiding unnecessary preventive treatments through a better targeting of individuals at risk.

First, in patients about to receive anti-TNF agents:

1. Anti-TNF-associated tuberculosis can be greatly reduced through a comprehensive clinical program. However, a certain risk remains during the first year of treatment.
2. The two-step TST approach for latent tuberculosis infection screening prior to anti-TNF therapy signifies a substantial increase in the proportion of LTBI diagnoses and treatment prescriptions, with no effect on the incidence of subsequent active tuberculosis. Therefore, this strategy, which is currently recommended in the Spanish guidelines, is no longer justified.
3. Systematic periodic retesting for latent tuberculosis infection in candidates to anti-TNF agents with negative LTBI test results at baseline is not required.

Second, in liver and HSCT transplant receptors:

The rate of post-transplant tuberculosis among QFT-GIT positive patients was both low and comparable to that of the TST in this cohort of liver transplant and haematological stem cell transplantation recipients. Therefore, the choice of TSTs or IGRAs for screening transplant candidates should be based on the expected specificity in each setting, operational factors, logistics, patients' preferences, and cost.

Third, in healthy individuals with recent exposure to active tuberculosis:

The use of QuantiFERON®-TB Gold In-Tube to confirm positive TST reactors allows for a significant reduction of tuberculosis infection diagnoses and preventive therapy prescriptions, without increasing the risk of active disease, in low-incidence settings of tuberculosis.

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ANNEXES

1. Study Protocol (NCT01223534)

Comparison of two strategies for therapeutic decision-making in tuberculosis contact tracing: a standard strategy based on tuberculin skin test (TST) alone vs. TST combined with QuantiFERON®-TB Gold In-Tube (QFT).

Protocol identifying number

EudraCT no. 2009-017430-49
Promotor code: QFT-ECC-01
ClinicalTrials.gov identifier: NCT01223534

VERSION 6 (April 1st, 2011)

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ABBREVIATIONS

AE	*Adverse event*
AEMPS	*Agencia Española del Medicamento y Productos Sanitarios*
AR	*Adverse reaction*
BCG	*Bacillus Calmette-Guérin*
CRF	*Case Report Form*
EC	*Ethics Committee*
ICF	*Informed Consent Form*
ICH	*International Conference of Harmonisation*
IFN	*Interferon*
ITT	*Intention-to-treat*
LTBI	*Latent tuberculosis infection*
IGRAs	*IFN-gamma release assays*
MTC	*Mycobacterium tuberculosis complex*
NTM	*Non-tuberculous mycobacteria*
PCR	*Polymerase Chain Reaction*
PI	*Principal Investigator*
PIS	*Participant/ Patient Information Sheet*
PP	*Per-protocol*
PPD	*(tuberculin) Purified Protein Derivate*
QFT	*QuantiFERON®-TB Gold In-Tube*
SAE	*Serious Adverse Event*
SSI	*Statens Serum Institute*
TB	*Tuberculosis*
TST	*Tuberculin Skin Test*
TU	*Tuberculin Unit*

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INTRODUCTION

Study Rationale And Scientific Background

Contact investigation of tuberculosis (TB) cases is the most readily available intervention to identify recently infected individuals and has been considered as an essential component of the tuberculosis control and elimination strategy in most low-incidence countries.[1]

Diagnosis of tuberculosis infection is currently based on tuberculin skin test (TST).[2,3] However, TST has two main limitations: low specificity due to cross-reaction with BCG and non-tuberculous mycobacteria (NTM) antigens, and low sensitivity in subjects with impaired cellular immunity. In routine clinical practice, a positive TST in people with significant contact with a tuberculosis case is considered indicative of recent infection and preventive treatment is offered to avert development of active disease. This strategy avoids leaving untreated people at risk, but overestimates recent infection and leads to overtreatment.

To overcome the drawbacks of TST, new in vitro assays based on interferon-gamma (IFN-gamma) release in response to specific Mycobacterium tuberculosis (MTC) antigens have been developed.[4] Two IFN-gamma release assays (IGRAs) are currently available: the T-SPOT.TB (Oxford Immunotec, Abingdon, UK), which is based on the enzyme-linked immunospot (ELISpot) assay; and the whole blood-based QuantiFERON®-TB Gold In-Tube (QFT) (Cellestis Ltd, Carnegie, Australia), which use ELISA to detect IFN-gamma in the culture supernatant. IGRAs have better specificity and equal or greater sensitivity for the detection of tuberculosis and latent tuberculosis infection (LTBI), as well as a better correlation with the degree of exposure to a source of tuberculosis.

IGRAs are good markers of recent infection and represent a promising technique for investigating tuberculosis contacts. Clinical experience, particularly with QFT, suggests that people positive on the QFT test have a higher risk of developing active disease than those who are negative, regardless of the TST result.[5,6] In a prospective study by Diel et al 601 adult household contacts were evaluated with TST and QFT simultaneously, and preventive treatment was only offered to those with positive QFT result.[5] Over a three-year follow-up,

6 of 41 (15%, all of whom refused treatment) developed tuberculosis. In contrast, none of 181 subjects who had negative QFT and positive TST, and were therefore not treated, developed tuberculosis.

In a recent update of this cohort with a further 816 close contacts, none of the 824 untreated contacts who were QFT negative (410 of them were TST positive) developed active TB over the 3.7± 0.92 person-years of follow-up, yielding a negative predictive value of 100%.[7] Of the 1033 contacts, 19 untreated contacts developed active disease (they all had been QFT positive and had refused treatment).

These results suggest that a positive QFT result may identify better subjects at higher risk of developing active disease after close contact with a tuberculosis case, whereas risk of progression for those with negative QFT may be very low, regardless of their TST result. However, other authors have got different results, turning this matter into a conflicting evidence.[5,8]

Even though IGRAs are being increasingly used in clinical practice, and though several scientific societies have adopted them as the reference method to guide therapeutic decisions, the best strategy in the context of contact tracing remains to be defined.[9] Important questions remain to be answered, particularly the significance of discordant results between IGRAs and TST, in terms of the long-term development of tuberculosis.

The purpose of this study is to assess the appropriateness of a sequential strategy of TST followed by QFT for diagnosing tuberculosis infection and for deciding preventive treatment in close contacts of tuberculosis. This strategy will be compared with the standard practice for diagnosis of tuberculosis infection, that is, TST alone.

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OBJECTIVES AND PURPOSE

PRIMARY OBJECTIVE

To prove that the incidence of tuberculosis among subjects evaluated by TST combined with QFT is not higher than among subjects evaluated by TST alone.

To prove that the proportion of subjects diagnosed with tuberculosis infection, and therefore treated, will be lower among subjects evaluated by TST combined with QFT than among subjects evaluated by TST alone.

SECONDARY OBJECTIVES

To compare both strategies in terms of health resources and expenditures.

TRIAL DESIGN

STATEMENT OF THE ENDPOINTS

Primary endpoints

1. Development of active tuberculosis (differences in incidence in a two-year follow-up period). Diagnosis of TB will be based on these definitions:
    - Definitive diagnosis: isolation of MTC in clinical samples.
    - High probability diagnosis: suggestive symptoms of TB and a positive result of a molecular test in respiratory samples or sterile fluids (PCR).
    - Probable diagnosis: suggestive clinical and radiographic signs of TB without microbiological confirmation and a favorable response to specific therapy.
2. Proportion of treated patients (diagnosed with LTBI) in both groups.

Secondary endpoints

1. Crude mortality, related to the trial or its drugs.
2. Adverse effects: If a patient develops severe hepatic toxicity (grade 3-4 of the *National Cancer Institute Common Toxicity Criteria* version 3.0), drugs will be stopped. In case of immunoallergic phenomena, if the reaction is early, mild and transient, treatment won't be stopped. However, if toxicity is sustained, regardless of severity, the responsible drug will be withdrawn.
3. Treatment withdrawal due to toxicity or non-compliance: A proper compliance is defined as taking all prescribed medications for the length of time necessary (6-month treatment for isoniazid or 4 months for rifampin).
4. Health resources by means of direct costs.

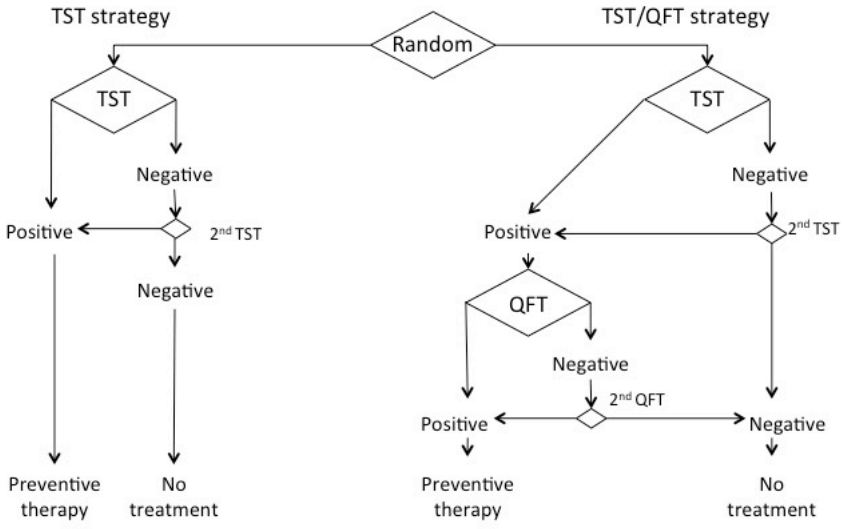
DESIGN

Prospective, randomized, non-blinded, multicenter study with parallel group design, comparing two therapeutic strategies according to two different diagnostic approaches of tuberculosis infection in close contacts.

RANDOMIZATION

Eligible patients (who meet all of the inclusion criteria and none of the exclusion criteria) will be randomly assigned to one of the two study arms. Randomization will be performed stratifying by center in a 1:1 allocation ratio. The randomization list will be computer-generated by version 9.2 from SAS system for Windows (Copyright© 2002-2008 by SAS institute Inc.).

STUDY SCHEMA



Having ruled out active disease, patients will be assigned to arm A or arm B.

All participants will be given TST, and only those patients in ARM B with a positive TST will have QFT tested.

The window period will be avoided by repeating TST or QFT if less than 8 weeks have elapsed since the last contact with the index case and the first test is negative.

Definition of LTBI and thus, decision to treat will depend on the assigned arm:

ARM A (standard practice): treatment decisions will rely on TST result.

ARM B (experimental practice): treatment decisions will rely on QFT result.

DESCRIPTION OF THE PRODECURES: DIAGNOSTIC TESTS AND TREATMENT

1. Tuberculin skin test (TUBERCULIN PPD RT/23 2TU)

Descriptive name of medicine:

Tuberculin Purified Protein Derivative (PPD) for intradermal (Mantoux) test.

Composition:

Sterile solutions in concentrations of 2 TU PPD, wholly prepared from a master seed lot of *Mycobacterium tuberculosis.* The tuberculin dilutions are prepared with phosphate buffered saline containing 0,01% chinosol (potassium hydroxyquinoline sulphate) as preservative and 0,005% Tween 80 (polysorbatum 80) as stabiliser. The antigen fulfills the World Health Organization requirements for PPD.

Identification:

Colourless solution in 1,5 mL or 5 mL clear vials sealed with a rubber stopper and flip-off aluminium overseal.

Pharmacological action of the medicine:

The active substances are the proteins of tubercle bacilli which cause induration as a specific skin response of the delayed type at the site of injection in individuals infected with tuberculosis. Induration may be palpable as early as 5 hours after injection, but normally reaches a peak only after 48 to 72 hours. The reaction subsides over the course of several days.

Indications:

The intradermal (Mantoux) skin test employing PPD RT/23 is used as an aid in the diagnosis of tuberculosis infection. Reactivity to the test may be depressed or suppressed if the individual is suffering from advanced tuberculosis disease, acute viral infection (including immunization with live viral vaccine during past 14 days), or overwhelming bacterial infection. Patients receiving corticosteroids or other immunosuppressive agents, or who are suffering from malignant conditions, may also react poorly to the tuberculin test.

Contraindications:

Known hypersensitivity to the test, such as may occur in individuals who are known tuberculin reactors and who have been repeatedly tested with tuberculin, or in persons who have previously suffered from tuberculosis.

Dosage and directions for use:

Where clinical tuberculosis is suspected, or where testing for an immune response following vaccination is the objective, the test may be performed with 2 TU in order to limit outspoken local reaction. Where used for epidemiological purposes, such as to define the tuberculin test response profile in a cross-section of the population, 2 TU is used.

The test is made by intradermal injection of exactly 0,1 mL of the tuberculin solution. It is essential that the injection be given in the upper layer of the skin, since a possible positive reaction will be difficult to interpret if the tuberculin is injected too deeply. A suitable injection will result in the formation of a white papule about 10 mm in diameter, which will remain visible for approximately 10 minutes. The most suitable site for the test is the middle third of the flexor side of the lower arm. A disposable 1.0 mL graded tuberculin syringe fitted with a 26 gauge short barrel needle should be used for the injection. Syringes used for tuberculin tests must not be used more than once.

Interpretation of the test:

The test is judged 48 to 72 hours after injection. The transverse diameter of the induration is measured in millimeters, excluding any surrounding erythematous zone. An induration ≥ 5 mm should be considered positive in contact tracing.

Side-effects and special precautions:

Pain and pruritus may occur at the injection site, with vesiculation, ulceration, or necrosis in highly sensitive persons. If given to patients with tuberculosis, a severe reaction may occur. Discomfort at the test site may be relieved by cold packs or topical glucocorticoid ointment or cream. Transient bleeding may occur at the site of injection, but is of no significance. Adrenaline should be immediately available in the event of an anaphylactic or an acute hypersensitivity reaction occurring. An immediate, local inflammatory type reaction may occur due to the constituents of the diluent. Allergic reactions to tuberculin tests have been reported. Less frequently, swelling of the lymph nodes may occur. Patients should not rub or massage the injection site.

(Information from Statens Serum Institut (SSI); Copenhagen. Denmark)

2. QuantiFERON®-TB Gold In-Tube

QuantiFERON®-TB Gold In-Tube (QFT) is an *in vitro* diagnostic test using a peptide cocktail simulating ESAT-6, CFP-10 and TB7.7 (p4) proteins to stimulate cells in heparinised whole blood. Detection of interferon-γ (IFN-γ) by Enzyme-Linked Immunosorbent Assay (ELISA) is used to identify *in vitro* responses to these peptide antigens that are associated with *Mycobacterium tuberculosis* infection. QFT is an indirect test for *M. tuberculosis* infection (including disease) and is intended for use in conjunction with risk assessment, radiography and other medical and diagnostic evaluations.

The QFT test is a test for Cell Mediated Immune responses to peptide antigens that simulate mycobacterial proteins. These proteins, ESAT-6, CFP-10 and TB7.7 (p4), are absent from all BCG strains and from most non-tuberculosis mycobacteria with the exception of *M. kansasii, M. szulgai* and *M. marinum.*

Individuals infected with *M. tuberculosis* complex organisms usually have lymphocytes in their blood that recognize these and other mycobacterial antigens. This recognition process involves the generation and secretion of the cytokine, IFN-γ. The detection and subsequent quantification of IFN-γ forms the basis of this test. A high determination of IFN-γ suggests TB infection.

3. Isoniazid

Isoniazid is the hydrazide of isonicotinic acid and is one of the primary drugs for TB treatment. The activity of isoniazid is limited to the mycobacteria of the *M. tuberculosis* complex; it is bactericidal for rapidly dividing organisms and bacteriostatic for "resting" bacilli. The probable mechanism of action is the inhibition of the biosynthesis of mycolic acids, a component of the mycobacterial cell wall.

Administration on the trial:

Isoniazid will be the first therapeutic option for all participants. A single 300 mg tablet of Cemidon® will be provided during 6 months, given on an empty stomach. The total amount of tablets taken per patient should be 168. If treatment is stopped for any reason for a period lower than 2 weeks, isoniazid should be taken again until the completion of the 168 doses. If the period without treatment overcomes a fortnight, treatment should begin again, no matter the number of previous dosages.

4. Rifampicin

Rifampin is a semi-synthetic rifamycin derivative that is highly active against mycobacteria, most gram-positive bacteria, and some gram-negative bacteria. It is bactericidal for both intracellular and extracellular microorganisms. By inhibiting prokaryotic DNA-dependent RNA polymerase, it suppresses the early elongation of the nucleotide chain in RNA synthesis.

Administration on the trial:

Rifampicin will be used as a second-line treatment. A single 600 mg tablet of Rimactan® or Rifaldin® will be provided during 4 months, given on an empty stomach. The total amount of tablets taken per patient should be 112. If treatment is stopped for any reason for a period lower than 2 weeks, rifampin should be taken again until the completion of the 112 doses. If the period without treatment overcomes a fortnight, treatment should begin again, no matter the number of previous dosages.

DATA RECORDED FROM PARTICIPANTS

The master file will contain each of the documents required in the guidelines for Good Clinical Practice (CPMH/ICH/135/95).

The trial staff will ensure that the participants' anonymity is maintained. The participants will be identified only by a participant ID number on the Case Report Form (CRF) and any electronic database. All documents will be stored securely and only accessible by trial staff and authorized personnel.

The CRF will include demographic data, medical history, information regarded to the index case and the diagnostic tests performed to rule out LTBI. In case of needing treatment, information about its compliance and adverse effects will be also detailed.

See attachment 1 "Schedule of events" for the complementary tests needed.

END OF TRIAL

The end of the trial is the date of the last visit of the last participant.

STUDY POPULATION

INCLUSION CRITERIA

1. Age ≥18 years.
2. Household contact of a patient with pulmonary and/or laryngeal TB (defined as positive smear or positive nucleic acid amplification test, and/or positive culture).
3. Written consent given.

EXCLUSION CRITERIA

1. Pregnant or breast-feeding women.
2. Immunosuppressive conditions, as one of the following:
   - Known HIV infection
   - Treatment with corticosteroids (at least 10 mg/d)
   - Transplant recipients taking immunosuppressive drugs
   - Treatment with tumor necrosis alpha inhibitors
   - Active cancer under treatment with chemotherapy
   - Uncompensated cirrhosis
   - Renal failure in hemodialysis
3. Prior TB or positive TST.
4. Abnormal chest X-ray with apical fibro-nodular changes typical of healed TB.
5. Isoniazid or rifampin resistant strain (index case).
6. Contraindication to both isoniazid and rifampin.

SUBJECT WITHDRAWAL CRITERIA

Each participant has the right to withdraw study at any time. In addition, the investigator may discontinue a participant from the study at any time if the investigator considers it necessary for any reason including:

- An adverse event requiring discontinuation of the study medication, mainly liver toxicity due to isoniazid or rifampicin.
- Lost to follow up (not attendance to two consecutive visits).
- Indeterminate result of QFT after repeating it twice.
- Isoniazid or rifampin resistant strain (index case).
- No culture-confirmed TB case (index case).

The type and timing of the data to be collected for withdrawn subjects will be the same as the subjects which continue running the study.

TRIAL INTERVENTION

SCREENING FOR LTBI, TREATMENT AND FOLLOW-UP

All household members of a TB patient will be invited to participate. They should meet all inclusion criteria and none of the exclusion ones. Having ruled out active disease (with a clinical and image evaluation with chest X-ray), and after obtaining written informed consent, participants will undergo randomized to:

ARM A (standard practice)

Treatment decisions will be based on TST result: in case of a positive result, treatment will be given.

In case of a negative result, if less than 8 weeks have elapsed since the last contact with the index case, TST will be repeated in order to avoid window period. If it turns positive, treatment will be given.

ARM B (experimental practice).

Procedures in this arm will also include TST. As in Arm A, in case of a negative result, if less than 8 weeks have elapsed since the last contact with the index case, TST will be repeated in order to avoid window period.

Those subjects with a positive TST will have QFT done. Treatment decisions will be based on QFT results: in case of a positive result, treatment will be given.

If QFT is done after a first positive TST and it results in a negative determination, QFT should be repeated 8 weeks later, in order to overpass the window period.

Diagnostic tests

TST: The standard Mantoux test consists of an intradermal injection of 2 TU of Statens Serum Institute (SSI) tuberculin RT 23 in 0.1 ml solution for injection. The solution is injected intradermally (between the layers of dermis) and read 48 to 72 hours later. An induration ≥ 5 mm will be considered as a positive reaction, thus pointing out TB infection.

QFT: QuantiFERON®-TB Gold In-Tube (Cellestis) will be done following the manufacturer's instructions. It consists on a negative control tube (without antigen) and a tube with specific antigens of MTC (ESAT6, CFP10 and TB7.7). After blood collection, as soon as possible, and within 16 hours, both tubes will be incubated at 37ºC for 16-24 hours and then centrifuged at 2000-3000 g (RCF) for 15 minutes. Afterwards, concentration of IFNγ will be measured on 200 μl of plasma from each tube by means of ELISA. Results will be calculated using QuantiFERON®-TB Gold In-Tube Analysis Software. A positive, negative or indeterminate result will be obtained. In case of an indeterminate one, the test should be repeated. If it remains indeterminate, the subject will be withdrawal from the trial, and the therapeutic decision will relay on the TST.

Treatment of patients with LTBI

See Section 4.5 “Description of the diagnostic tests and treatment”, on pages 13-14.

Follow-up of patients

Visits listed below are protocol-specified study visits for all recruited subjects:

- Baseline visit: in which PI tells the patient whether he/she requires treatment or not, based on the results of the diagnostic test in each arm. By this time, a clinic interview to rule out active TB symptoms and a chest X-ray will have been carried out.
- Months 6, 12, 18 and 24: clinical assessment.
- Months 6 and 24: chest X-ray.

In addition, people taking medication will also undergo the following visits and complementary tests:

- Baseline visit: patients diagnosed with LTBI will have blood drawn for CBC with differential and platelet count, serum or plasma creatinine, and liver function tests.
- Months 1 and 3: an extra visit for compliance, adverse event assessment and blood analysis (with the same measured parameters as in baseline visit) will be carried out.

See Attachment 1 “Schedule of events” for more details.

Monitoring subject compliance

In case of taking isoniazid, compliance will be measured with the Eidus-Hamilton test, which detects N-acetyl isoniazid in urine and confirms that the drug is being ingested: with little drops of two reagents added to urine, a red colour develops.

In case of taking rifampin, the orange colour of urine will confirm the compliance.

Both tests will be done in the outpatient clinic during routine visits. Missing tables will also be accounted.

DRUGS AND DIAGNOSTIC TESTS: STORAGE AND DISTRIBUTION

TST: PPD RT/23 should be stored away from light, and at a temperature between 2°C and 8°C. Once a vial of PPD RT/23 tuberculin has been opened, its contents should be used within 8 hours. Any remaining liquid should be discarded.

QFT: Store blood collection tubes at 4°C to 25°C. The shelf life of the QuantiFERON®-TB Gold blood collection tubes is 15 months from the date of manufacture when stored at 4°C to 25°C.

Drugs (Isoniazid and Rifampin) should be stored in a cool, dry place. Each batch of tablets will be labeled in the Pharmacy Department and then carried to the outpatient clinic where participants will be attended. PI in each center will record the batch number and its expiration date, in order to warrant sample traceability as RD 223/2004 establishes, with the rest of data required.

MEDICATIONS PERMITTED AND NOT PERMITTED DURING THE TRIAL

In case of patients taking isoniazid, they should not be treated with carbamazepine and disulfiram, because of the potentiation of its action.
In case of patients taking rifampin, all the following drugs are not permitted: Protease inhibitors, delavirdine, cyclosporine, tacrolimus, itraconazol and ketoconazol.

ASSESSMENT OF EFFICACY

EFFICACY PARAMETERS

- The primary endpoint will be development of active disease after a two-year period of follow-up.
- The secondary endpoint will be the health resources used and costs.

METHODS AND TIMING FOR ASSESSMENT

- Development of active disease will be assessed through clinical evaluation and chest X-ray following the schedule of events. In case of clinical suspicion, respiratory samples will be taken for microbiological analysis.
- Health resources will be computed by recording number of visits and complementary tests needed in each case.

ASSESSMENT OF SAFETY

SAFETY AND TOLERABILITY ENDPOINTS

- TST and QFT: the safety and tolerability endpoint in both diagnostic tests will be the measures taken in order to remove the local discomfort that these tests may cause.
- Drugs: the primary endpoint for analysis of safety and tolerability is discontinuation of assigned treatment for any reason. Other aspects that will be assessed as secondary endpoints include mortality, the occurrence of 3 and 4 grade toxicities (according to modified *National Cancer Institute Common Toxicity Criteria* Version 3.0.) and the rates and types of toxicity thought possibly, probably, or definitely related to the drug by the investigator.

METHODS AND TIMING FOR ASSESSMENT

- TST and QFT: any incidence and injury caused by any diagnostic test will be recorded.
- Drugs: in those subjects receiving treatment, tolerability will be assessed in each visit during the treatment period by means of clinical interview and checking of normal liver parameters in the blood analysis, following the schedule of events.

ADVERSE EVENT REPORTING

All symptoms and laboratory findings will be graded according to severity using the modified *National Cancer Institute Common Toxicity Criteria* Version 3.0.

DEFINITIONS

- An adverse event (AE) is defined as any unintended or abnormal clinical observation that is not of benefit to the patient.
- A serious adverse event (SAE) is defined as any experience that is fatal or life-threatening, is persistently or significantly disabling (as determined by the principal investigator), requires inpatient hospitalization, or prolongation of hospitalization, is a congenital anomaly, or overdose of study drugs. Any Grade 4 toxicity is considered a SAE.

REPORTING

Any Serious Adverse Event (this includes any Grade 4 toxicity) must be reported to the sponsor within 48 hours of the site's awareness of the event. The initial communication will be followed by a detailed report.

Those adverse events that are serious, unexpected, and possibly, probably, or definitely related to the study drugs, will be reported by the sponsor to the Health Authority in Spain (AEMPS) in the form of a written Safety Report within a 15-day period of time.

AEMPS should also be notified of any death or life-threatening adverse event before the first 7 days of the notification to the sponsor. The initial communication will be followed by a detailed report in an 8-day period of time. These SAE will be reported using the European standard electronic format.

Similarly, the sponsor will report every SAE to the Reference Ethics Committee and the rest of Ethics Committees involved, with the same time limit as the reports to AEPMS.

In addition, the following adverse events must be reported on an Adverse Event Report Form:

- new medical diagnosis (at the time of enrollment, if the patient already has a medical diagnosis whose signs or symptoms worsen during the study to a Grade 3 or 4, this is an adverse event that must be reported)
- any grade 3 adverse event
- study drug discontinuation, temporary or permanent, due to an adverse event
- pregnancy

CLINICAL MANAGEMENT OF ADVERSE EVENTS

In general, for grade 1 toxicities, the patient will be followed carefully and the study drugs will be continued. For grade 2 toxicities, the patient will be followed more carefully, with additional laboratory and/or clinic visits as necessary, and the study drugs may be temporarily held at the investigator's discretion. For any grade 3 toxicity that, in the principal investigator's judgment is due to study drug(s), the causative study drug(s) should be held. The clinician should rule out other possible causes of the symptoms before discontinuing study medication. When possible, concomitant medications should be held first at the discretion of the principal investigator if he/she suspects they are contributing to the toxicity. Depending on the nature and severity of the toxicity, the degree to which it resolves, and/or the emergence of alternative explanations for the toxicity or the subject's deterioration, the study drugs(s) may be restarted at the discretion of the investigator. For any recurring grade 3 or grade 4 toxicities, the study drugs should be temporarily held and may be permanently stopped at the discretion of the investigator. For thrombocytopenia (platelet count < 75,000/cu mm) attributed to rifampin, rifamycins should generally not be restarted. For other grade 3 or 4 rifamycin-attributable toxicities, rifamycins may be held and may be permanently stopped at the discretion of the investigator.

Any patient with grade 4 renal, hepatic, cardiac or hematological toxicity will have his or her study medications held immediately. The laboratory test or clinical finding in question

will be reassessed as soon as possible. The repeat test will guide management of the event as follows:

- If the repeat assessment shows toxicity of grade 3 or lower, and if the patient has continued to receive study drugs between the two testing dates, then the patient will be managed according to the appropriate toxicity level of the repeat test.
- If the repeat test shows toxicity of grade 3 or lower, and if the patient has not received study drugs between the two testing dates, then the patient will be managed at the discretion of the investigator with regard to the re-administration of study drugs, and otherwise according to the toxicity level of the repeat test.
- If the repeat test shows grade 4 toxicity, then the patient will be permanently discontinued from study medications. The patient will continue to be followed in the study.

For other grade 4 toxicities, the study drugs will be temporarily held and may be restarted or permanently stopped at the discretion of the investigator.

If a patient develops hepatic toxicity requiring study drug discontinuation, the following evaluation will be undertaken: assessment for history of injection or non-injection drug use, alcohol ingestion, use of other hepatotoxic drugs, and performance of serologic tests for viral hepatitis (IgM antibody for Hepatitis A, Hepatitis B surface antigen, IgM antibody to Hepatitis B core antigen, antibody to Hepatitis C).

DISCONTINUATION OF STUDY DRUGS DUE TO ADVERSE EVENTS

Certain events or conditions may necessitate temporary or permanent discontinuation of the study medication. Patients who experience such events or conditions, however, will still be "on study" and will be followed until study completion. Any patient for whom the study medication is temporarily discontinued will be restarted on study medication as soon as possible. If study drugs are permanently discontinued, further LTBI therapy may be administered at the investigator's discretion. These patients will be followed in the study according to the guidelines and time-points established in the protocol.

Temporary Discontinuation

Criteria for temporary discontinuation of study therapy

- Development of a toxicity that, depending on its nature and severity, requires temporary discontinuation of the study medication until the toxicity resolves as indicated in the preceding toxicity management section.
- Development of another medical condition that makes the administration of the study drug inadvisable. The decision to discontinue temporarily the study medication in this situation will be at the investigator's discretion. The period during which the patient is off study medication will be as short as clinically possible.

Permanent Discontinuation

Criteria for permanent discontinuation of study therapy

- Development of a toxicity that warrants permanent discontinuation of any study drug.
- The patient refuses further study therapy.
- It is the investigator's judgment that it is no longer in the best interest of the patient to continue study therapy.
- Termination of the study.

If a patient refuses further study therapy, the patient will be treated with a non-study regimen, but will continue with scheduled follow-up study visits. If a patient withdraws consent, the patient will be treated with a non-study regimen, and all study-specific follow-up will stop.

STATISTICAL METHODS AND SAMPLE SIZE

STATISTICAL ANALYSIS

The primary efficacy analyses will be performed in the per-protocol (PP) population, though a sensitivity analysis will be performed on intention-to-treat (ITT) population. The ITT population will consist of all randomized subjects, while the PP population will include ITT patients who comply with the protocol. Safety analyses will be performed on all randomized patients (ITT population)

The main point of analysis will be the difference between the incidences of tuberculosis in both arms, under the hypothesis of non-inferiority. Incidences will be compared with a unilateral confidence interval of 97.5% for the difference. The number of treated patients within each treatment arm will be similarly analyzed. The difference in health resources and costs between the two arms will be the secondary point of evaluation.

The baseline characteristics and the rest of outcome measures in the study will be compared between study arms by means of χ^2 test for categorical variables, and for continuous variables, by means of t-Student test or nonparametric Mann-Whitney tests (if normality of the variable can be assumed). The incidence of tuberculosis will be reported as cumulative incidence and as the incidence rate (cases per 100,000 person-years). *P* values are based on two-tailed with a significance level <0.05. Statistical analysis will be carried out with SPSS (version 15 for Windows).

SAMPLE SIZE CALCULATION

The sample size was calculated to demonstrate the non-inferiority of the experimental strategy (TST and QFT) with respect to standard practice (TST alone) in terms of preventing TB development and reducing the amount of TB infection diagnosis in the experimental group. Assuming an alpha of 0.025 and a beta of 0.20, a loss to follow-up of 20% and an

incidence of tuberculosis in the first two years of household contacts of TB patients of 0.5% (Diel et al, ARCCM 2008), 870 individuals are needed (435 per arm). This sample size is also sufficient to detect a 10% minimal difference in the number of subjects needing treatment between the two groups (assuming the same error types), thus covering the required size to account for the two primary endpoints.

INTERIM ANALYSES AND STOPPING RULES

An interim analysis will be performed two years after the inclusion of the 400th patient in order to discard lack of efficacy of the experimental strategy (higher incidence of TB in the group with less treated patients). Our “stopping rule” establishes that the trial would stop if the 97.5% confidence interval for the estimated incidence of TB lies strictly to the left of the non-inferiority margin.

ETHIC, DEONTOLOGICAL AND REGULATORY CONSIDERATIONS

The Investigator will ensure that this study is conducted in accordance with the principles of the Declaration of Helsinki, ICH Guidelines for Good Clinical Practice and RD 223/2004 (article 7), and in full conformity with relevant regulations

The protocol, informed consent form (ICF), participant information sheet (PIS) and any applicable documents will be submitted to an appropriate Ethics Committee (EC) and Regulatory Authority (AEMPS) for written approval.

All substantial amendments to the original approved documents will be also sent to an appropriate Ethics Committee (EC) and Regulatory Authority (AEMPS) for written approval.

INFORMED CONSENT

The participant must personally sign and date the latest approved version of the informed consent form before any study specific procedures are performed.

Written and verbal versions of the Participant Information Sheet (PIS) and Informed Consent Form (ICF) will be presented to the participants detailing no less than: the exact nature of the study; the implications and constraints of the protocol; the known side effects and any risks involved in taking part. It will be clearly stated that the participant is free to withdraw from the study at any time for any reason without prejudice to future care, and with no obligation to give the reason for withdrawal.

The participant will be allowed as much time as wished to consider the information, and the opportunity to question the Investigator or other independent parties to decide whether they will participate in the study. Written Informed Consent will then be obtained by means of participant dated signature and dated signature of the person who presented and obtained the informed consent. The person who obtained the consent must be suitably qualified and experienced, and have been authorized to do so by Principal Investigator. A copy of the signed ICF will be given to the participants. The original signed form will be retained at the study site.

SUBJECT CONFIDENTIALITY

The trial staff will ensure that the participants' anonymity is maintained, as *Ley de protección de datos de carácter personal (15/99)* orders. The participants will be identified only by a participant ID number on the CRF and any electronic database. All documents will be stored securely and only accessible by trial staff and authorized personnel. The study will comply with the Data Protection Legislation, which requires data to be anonymized as soon as it is mandatory to do so.

INSURANCE

An insurance policy has been arranged with Zurich® to cover study participants.

AGREEMENT TO PUBLISH

Miguel Santín MD, PhD as Sponsor of the trial agrees to make publicly available the main study results, regardless of their outcome.

FUNDING

This trial has received a grant from *Ministerio de Sanidad, Política Social e Igualdad (Convocatoria SAS 2481/2009 from September 17th)*, with a global amount of 106.000 €, approved on January 2010. A deferral has been granted to ensure the feasibility of the trial on December 30th, 2010.

Attachment 1: Schedule of Events

SCHEDULE OF EVENTS:

ARM A (STANDARD PRACTICE: TST ALONE)

Study procedures	Visit 1 (day -3)	Visit 2 (Day 0)	Visit 3* (1st month)	Visit 4* (3rd month)	Visit 5 (6th month)	Visit 6 (12th month)	Visit 7 (18th month)	Visit 8 (24th month)
Screening (criteria)	X	X						
Informed consent form		X						
Randomization		X						
Clinical evaluation (rule out active TB)	X	X	X	X	X	X	X	X
TST (if negative, repeat it 8 weeks after the last exposure to TB case)	X procedure	X lecture						
Treatment (if positive TST)		X	X	X	X			
Blood chemistries (only if treatment needed)		X	X	X				
Radiologic tests (chest x-ray)		X			X			X

(*): these visits are required only if treatment is given.

ARM B (COMBINED STRATEGY: TST + QFT)

Study procedures	Visit 1 (day -3)	Visit 2 (Day 0)	Visit 3 (1st week) ONLY IS QFT IS PRACTISED	Visit 4* (1st month)	Visit 5* (3rd month)	Visit 6 (6th month)	Visit 7 (12th month)	Visit 8 (18th month)	Visit 9 (24th month)
Screening (criteria)	X	X							
Informed consent form		X							
Randomization		X							
Clinical evaluation (rule out active TB)	X	X		X	X	X	X	X	X
TST (if negative, repeat it 8 weeks after the last exposure to TB case)	X procedure	X lecture							
QFT (IF POSITIVE TST) (if 1st TST was positive and QFT is negative, repeat it 8 weeks after the last exposure to TB case)		X procedure	X result						
Treatment (if positive QFT)			X	X	X	X			
Blood chemistries (only if treatment needed)			X	X	X				
Radiologic tests (chest x-ray)		X				X			X

(*): these visits are required only if treatment is given.

2. Supplementary Material (Clin Infect Dis 2014; 60: 349-356)

Supplementary material

Table 1: Characteristics of the cohort according to the study period

	1st period (n= 230)	2nd period (n= 162)	3rd period (n= 334)	*p-value*
Male gender (%)	88 (38.3)	78 (47.6)	146 (43.7)	0.25
Age years, mean (SD)	51.3 (12.2)	48.9 (12.6)	47.7 (14.5)	<0.01
Born in high-burden TB countries (%)	7 (3.0)	14 (8.6)	37 (11.1)	<0.01
BCG-vaccinated (%)	37 (16.1)	39 (24.1)	125 (37.4)	<0.01
Previous TB infection (%)	11 (4.8)	1 (0.6)	14 (4.2)	0.87
IMID (%)				
Rheumatoid arthritis	129 (56.1)	73 (45.1)	109 (32.6)	<0.01
Ankylosing spondylitis	28 (12.2)	24 (14.8)	60 (18)	0.06
Psoriasis *	70 (30.4)	61 (37.7)	120 (36)	0.21
Inflammatory bowel disease	3 (1.3)	4 (2.5)	23 (6.9)	<0.01
Others	-	-	22 (6.6)	
Immunosuppressive treatment (%)	187 (81.3)	125 (77.2)	239 (71.6)	0.01
Corticosteroid therapy (%)	133 (71.1)	75 (60)	135 (56.2)	<0.01
Anti-TNF-α agent	194 (84.3)	130 (80.2)	218 (65.3)	<0.01
- Infliximab	60 (30.9)	52 (40)	50 (22.9)	<0.01
- Adalimumab	99 (51)	65 (50)	106 (48.6)	0.89
- Etanercept	106 (54.6)	44(33.8)	96 (44)	<0.01
- Certolizumab	7 (3.6)	5 (3.8)	8 (3.7)	0.99
- Golimumab	4 (2.1)	3 (2.3)	17 (7.8)	0.01

* Includes cutaneous psoriasis and psoriatic arthritis.

SD: standard deviation, TB: tuberculosis, BCG: Bacillus Calmette-Guérin, IMID: immune-mediated inflammatory disease, TNF: Tumor necrosis factor.

Table 2: Characteristics of four patients who developed Tuberculosis

	1st period (n= 230)	2nd period (n= 162)	3rd period (n=334)	
No. of cases	1	1	2	
Sex, Age (years)	Man, 59	Man, 55	Woman, 77	Man, 46
Underlying disease	Rheumatoid Arthritis	Cutaneous Psoriasis	Rheumatoid Arthritis	Rheumatoid Arthritis
TNF-α antagonist	Adalimumab	Infliximab	Adalimumab	Not given
LTBI diagnosis	No Two-step TST (-)	Yes Two-step TST(+)/QFT(-)	No Single-step TST(-)/QFT (-)	No Single-step TST(-)/QFT(-)
Treatment for LTBI	Not given	Rifampicin (4 m)	Not given	Not given
Type of TB	Disseminated	Pleural	Pulmonary	Pleural
Diagnosis	Sputum culture	Lymphocytic exudative pleural effusion with high ADA* activity. Response to treatment.	Sputum culture	Lymphocytic exudative pleural effusion with high ADA* activity. Response to treatment
Days from 1st dose of anti-TNF	173	209	356	1 year after screening

*ADA: Adenosine Deaminase, TNF: Tumor necrosis factor TNF, LTBI: latent tuberculosis infection, TST: tuberculin skin test, QFT: QuantiFERON®-TB Gold In-Tube.

3. Supplementary Material (J Inf 2013; 66: 381-387)

Table 1. Characteristics of the 11 studies included

Reference (year)	Design	Country (income status)	Population (age group)	HIV+ve individuals in cohort	IGRA	TST cut-off (mm)	PT offered	Individuals in the original study/in the analysis (n/n)
Song[16] (2007)	Retrospective	South Korea (High)	TB case-contacts (Adolescents)	Unknown*	QFT-GIT	≥10	Yes, QFT-GIT (+)	1826/1826
Hill[24] (2008)	Prospective	The Gambia (Low)	TB case-contacts (Adults and children)	Yes (2%)	ELISPOT (in house)	≥10	No	2381/1648
Bakir[22] (2008)	Prospective	Turkey (Middle)	TB case-contacts (Children)	Unknown*	ELISPOT (in house)	≥5 and ≥15	Yes, <6 years and ≥6 years and TST (+)[†]	1024/287
Kik[17]	Prospective	Netherlands	TB case-contacts	No,	T-SPOT	≥5, ≥10,	No	433/433

(2009)		(High)	(Adults)	exclusion criterion		and ≥15		
Kik[17] (2009)	Prospective	Netherlands (High)	TB case-contacts (Adults)	No, exclusion criterion	QFT-GIT	≥5, ≥10, and ≥15	No	433/433
Laffite[19] (2009)	Retrospective	Switzerland (High)	Anti-TNF candidates (Adults)	Unknown*	T-SPOT	≥10	Yes, T-SPOT (+)	50/46
Diel[18] (2010)	Retrospective	Germany (High)	TB case-contacts (Adults and children)	No, exclusion criterion	QFT-GIT	≥5, ≥10, and ≥15	Yes, QFT-GIT (+)	1417/901
Leung[25] (2010)	Retrospective	China (Middle)	Patients with silicosis (Adults)	Unknown*	T-SPOT	≥5, ≥10, and ≥15	Yes, TST (+)	331/241
Harstad[21] (2010)	Prospective	Norway (High)	Asylum seekers (Adults)	Unknown	QFT-GIT	≥6, ≥15	N.R.‡	823/810
Mahomed[26] (2011)	Prospective	South Africa (Middle)	Cohort study (Adolescents)	Unknown	QFT-GIT	≥5	No	6363/5244

Garcovich[20] (2011)	Prospective	Italy (High)	Anti-TNF candidates (Adults)	Unknown*	QFT-GIT	≥5	Yes, QFT-GIT (+)	50/44
Chang[23] (2011)	Not stated	South Korea (High)	Anti-TNF candidates (Adults)	Unknown*	QFT-GIT	≥10	Yes, QFT-GIT (+)	107/83

*Prevalence of HIV infection unknown, but probably low.

†In accordance with Turkish Ministry of Health guidelines, PT was offered to all children younger than 6 years, regardless of TST results; children 6 years or older with a positive result in the first TST (≥10 mm in unvaccinated children and ≥15 mm in vaccinated children); and children 6 years or older with conversion from negative to positive TST. ‡3% of those with QFT-GIT (+) were treated.

TB= tuberculosis; HIV= human immunodeficiency virus; IGRA= interferon-gamma release assays; TST= tuberculin skin test; PT= preventive treatment; T-SPOT= T-SPOT.TB; QFT-GIT= QuantiFERON-TB Gold In-tube; TNF= tumor necrosis factor; N.R.= not reported.

Table 2. Summary of the modified Newcastle-Ottawa scale assessment of studies included

	TB contact study (n= 5)	**Anti-TNF candidates screening** (n= 3)	**Other cohorts at risk for TB** (n= 3)	**Overall** (n= 11)
Selection				
Representative sample	5	3	3	11
IGRA and TST from same source population	5	3	3	11
Active TB excluded at baseline				
yes	4	3	2	9
not reported	1*	--	1*	2
Comparability				
Adjustment of confounders	3	0	1	4
Outcome				
Assessment of outcome				
active follow-up (ascertainment of symptoms)	3†	3	2	8
record linkage	2	--	1	3

>50% of incident cases culture-confirmed				
yes	2	N.A.	2	4
no	2	N.A.	--	2
not reported	1	N.A.	1	2
IGRA/TST incorporated into reference standard				
yes	2	--	2	4
no (or not reported)	3	3	1	7
Follow-up ≥2 years	5	1	2	8
≥80% of cohort followed up				
yes	4	3	2	9
not reported	--	--	1	1
Outcome report				
cumulative incidence	3	NA	1	4
incidence rate	1	NA	--	1
cumulative incidence and incidence rate	1	NA	2	3

*Not explicitly reported, but probably performed.

[†]TST (+) were regularly followed at the clinic, and TB was investigated by ascertaining health complaints potentially due to TB. TST (-) were not followed up, and TB assessment was record-linked.

TB= tuberculosis; NA= not applicable; TST= tuberculin skin test; IGRA= interferon-gamma release assays; TNF= tumor necrosis factor.

Table 3. Results of TST and IGRAs in the 11 studies included

Reference (year)	IGRA test	N	TST (+) n (%)	IGRA (+) n (%)	*P* Value*	TST (+)/ IGRA (+) n (%)	*P* value†	TST (-)/ IGRA (-) n (%)	TST (+)/ IGRA (-) n (%)	TST (-)/ IGRA (+) n (%)
Song[16] (2007)	QFT-GIT	1826	≥10 mm: 270 (14.8)	203‡	--	203‡	<0.001	--	67	--
Hill[24] (2008)	ELISPOT (in house)	1648	≥10 mm: 658 (40.0)	605 (36.7)	0.06	428 (26.0)	<0.001	813 (49.3)	230 (14.0)	177 (10.7)
Bakir[22] (2008)	ELISPOT (in house)	287	≥5 mm: 93 (32.4)	46 (16.0)	<0.001	20 (7.0)	<0.001	168 (58.5)	73 (25.4)	26 (9.1)
Kik[17] (2009)	T-SPOT	433	≥5 mm: 299 (69.1)†	181‡	--	181‡	<0.001	--	118	--
Kik[17] (2009)	QFT-GIT	433	≥5 mm: 327 (75.5)†	160‡	--	160‡	<0.001	--	167	--

Laffite[19] (2009)	T-SPOT	46	≥10 mm: 18 (39.1)	8 (17.4)	0.02	8 (17.4)	0.02	28 (60.9)	10 (21.7)	0
Diel[18] (2010)	QFT-GIT	901	≥5 mm: 555 (61.6)†	145 (16.3)	<0.001	143 (15.9)	<0.001	346 (38.4)	410 (45.5)	2 (0.2)
Leung[25] (2010)	T-SPOT	241	≥5 mm: 161 (66.8) ≥10 mm: 136 (56.4) ≥15 mm: 89 (36.9)	151 (62.7)	0.35 0.16 <0.001	124 (51.5)	<0.001 .. <0.001	53 (22.0)	37 (15.4)	27 (11.2)
Harstad[21] (2010)	QFT-GIT	810	≥6 mm: 415 (51.2) ≥15 mm: 121 (14.9)	237 (29.3)	<0.001 <0.002	207 (25.6)	<0.001 <0.002	255 (31.5)	208 (25.7)	30 (3.7)
Mahomed[26] (2011)	QFT-GIT	5244	≥5 mm: 2894 (55.2)	2669 (50.9)	<0.001	2383 (45.4)	<0.001	2064 (39.4)	511 (9.7)	286 (5.5)
Garcovich[20] (2011)	QFT-GIT	44	≥5 mm: 5 (11.4)	2 (4.5)	0.4	2 (4.5)	0.4	39 (88.6)	3 (6.8)	0
Chang[23] (2011)	QFT-GIT	83	≥10 mm: 35 (42.2)	36 (43.4)	0.9	19 (22.2)	0.008	48 (57.8)	16 (19.3)	0

*p value for the difference in prevalence of positive results between TST and IGRA.

†p value for the difference in prevalence of positive results between TST and TST/IGRA.

‡IGRA performed only in individuals with positive TST

TST= tuberculin skin test; IGRA= interferon-gamma release assays; T-SPOT= T-SPOT.TB