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Evidence based testing and outcomes in transplantation

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A thesis submitted in fulfilment of the requirements for the degree of

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DECLARATION

The work in this thesis is the result of original research and has not been submitted for a higher degree at any other university or institution.

Thomas E Rogerson Date: 29/08/2014

AUTHOR’S CONTRIBUTION

The author, Thomas Rogerson, carried out the body of work in this thesis under the supervision of Associate Professor Angela Webster (School of Public Health, The University of Sydney) and co-supervision of Professor Jonathan Craig (School of Public Health, The University of Sydney).

The author planned the research, designed the study protocols, submitted ethics applications, collected, managed and analysed the data, interpreted results, drafted and revised the manuscripts for submission to peer-reviewed journals, and wrote and compiled this thesis.

ETHICAL CLEARANCE

The studies presented in *Chapters 2-3* did not require ethical clearance, as the data within the studies was in the public domain.

The Human Research Ethics Committee of the Western Sydney Local Health District approved the use of patient hospital data for the analyses presented in *Chapter 4 (*HREC reference: LNR/13/WMEAD/120). The interpretation of these data is the responsibility of the author and should in no way be seen as an official policy or interpretation of the Western Sydney Local Health District.

ABSTRACT

Chapter 1: Introduction

In the 1990's a new paradigm in clinical practice emerged; evidence based-medicine (EBM). This new process of systematically finding, appraising, and using research findings in clinical decision making, also required new ways of retrieving relevant studies from a growing body of literature in online medical databases, and synthesising the results of several studies on the same topic.

For interventional medicine, EBM quickly revolutionised the way studies were reported, indexed in online databases, and meta-analysed. New guidelines for reporting controlled trials were developed, and indexers at the US National Library of Medicine began tagging randomised controlled trials with a unique ".rct" publication identifier.

In contrast, the up-take of EBM principles in diagnostic medicine has been slow. Despite the publication of a statement for '*Standards of Reporting Diagnostic Studies*' (STARD) in 2001, the quality of reporting diagnostic studies remains poor. Finding diagnostic studies in MEDLINE is difficult because no dedicated publication identifier exists, and often diagnostic studies on the same test are spread over a range of specialist journals.

Methodologies for the systematic review and meta-analysis of diagnostic studies were also established much later than interventional studies, and rely on studies to provide conventional metrics of diagnostic test performance (sensitivity and specificity). While useful, these methods are of limited use when there is no reference standard test available.

This thesis explores solutions to these problems within the framework of a specific clinical question: *What is the best test for diagnosing latent tuberculosis in people undergoing solid organ transplant?*

Chapter 2: Efficient strategies to find diagnostic test accuracy studies

In clinical practice there is often uncertainty about which tests to use and how to interpret their results. Diagnostic test accuracy studies can be used to answer

diagnostic questions, but finding them quickly in the MEDLINE database can be difficult, particularly in the clinical setting. While it possible to use ‘clinical queries limits’ (inbuilt search strategies in PubMed/ Ovid SP) to limit searches to diagnostic studies, other search strategies are also available and may perform better. The primary aim of the study presented in *Chapter 2* was to evaluate the performance of published search strategies for diagnostic tests in nephrology journals. Nephrology journals were chosen because people with end stage kidney disease represent the majority of candidates for solid organ transplant. Our secondary aim was to determine if test performance had improved since the international publication of the ‘Standards for Reporting Diagnostic Accuracy Studies’ statement. We hypothesised that improved standards of publication may have led to improved reporting and indexing within MEDLINE, and hence improved search strategy performance.

We hand-searched three prominent nephrology journals (the American Journal of Kidney Disease, The Journal of the American society for Nephrology, and Kidney International) between the years 2002-3 and 2009-10 for diagnostic accuracy studies. The studies identified formed a reference set, which were then used to evaluate the performance of fourteen published search strategies in terms of sensitivity, specificity and number needed to search.

Our hand-search identified 103 diagnostic test accuracy studies, which formed 2.1% of the total literature published. The most specific search strategy was the Haynes 2004 Narrow Clinical Queries limit (sensitivity: 0.20, 95%CI 0.13-0.29; specificity: 0.99, 95%CI 0.99-0.99). Using the Haynes 2004 Narrow Clinical Queries limit, a searcher would need to screen 3 (95%CI 2-6) articles from the search results to find one diagnostic study. Bachmann 2002 was the best-balanced search strategy, which was sensitive (0.88, 95%CI 0.81-0.94), but also specific (0.74, 95%CI 0.73-0.75), with a number needed to screen of 15 (95%CI 14-17).

The Haynes 2004 Narrow Clinical Queries Limit (inbuilt into PubMed and Ovid SP) was the most specific search strategy, but identified only 20% of the total literature. To answer clinical questions about diagnostic tests in nephrology, clinicians may wish to consider using the Deville 2000 Balanced search strategy, which had similar specificity to the Haynes 2004 Clinical Queries Limit, but identified 57% of the total literature.

Chapter 3: Tests for latent tuberculosis in candidates for solid organ transplantation: a systematic review

Tuberculosis is common infection in people undergoing solid organ transplantation due to increased risk of exposure to *Mycobacterium tuberculosis* bacteria and immunosuppression. The risk of developing tuberculosis after transplantation can be minimised with prophylactic drugs, but their toxic side-effects necessitate test-directed treatment. Latent tuberculosis infections are difficult diagnose because bacteria cannot be cultured from sputum samples. The tuberculin skin test, and newer interferon gamma release assays measure the immune response to *M. tuberculosis* antigens, but their accuracy cannot be measured in conventional metrics of sensitivity and specificity because no reference standard test is available. Many studies have compared the results of the tuberculin skin test and interferon gamma release assays with clinical risk factors for tuberculosis as proxies for true infection. To synthesis the evidence presented by these studies we conducted a systematic review and meta-analysis of all studies that assessed the performance of a test for latent tuberculosis and reported risk factors for tuberculosis in people undergoing solid organ transplant.

We identified 18 studies with data for meta-analysis. Both a positive tuberculin skin test and ELISA-based IGRA were strongly associated with clinical risk factors for TB (TST: OR 3.87; 95%CI 1.99-7.56, p<0.01, ELISA-based IGRA: OR 2.56; 95%CI 2.56-5.27, p=0.01). A positive tuberculin skin test was also associated with radiological evidence of past TB (OR 3.18; 95%CI 1.76-5.76, p<0.01) and a history of contact with active TB (OR 3.24; 95%CI 1.13-9.29, p=0.03). A positive ELISA-based IGRA was more likely to occur in participants who had received TB treatment (OR 22.31; 95%CI 7.80-63.76, p<0.01) or had diabetes. Few studies compared IGRAs with the tuberculin skin test head-to-head. No evidence of a difference in relative test performance was identified.

Few studies were available to compare the performance of the TST and IGRAs. On best available evidence, either a TST or IGRA, or both can be used to diagnose latent TB in people undergoing solid organ transplant. This finding is congruent with current international guidelines for testing latent TB in immunosuppressed populations.

Chapter 4: Mismatch between risk of tuberculosis and testing practices in people being assessed for kidney transplantation

In Australia, people on dialysis are ten times more likely to develop active tuberculosis than the general population. Current international guidelines recommend that all candidates for kidney transplant are screened for tuberculosis before transplantation. To determine whether testing practices in Australia meet current guidelines, we conducted a cross-sectional descriptive study of all people who underwent assessment for kidney transplant over a two-year period at a regional transplant centre. We searched hospital records, and collected data on the types of tests patients received, the test results, and clinical risk factors for latent tuberculosis.

Two-hundred and one patients were assessed for kidney transplant. Patients had a mean age of 50.8 ±12.6 years, 63.7% were male and 22.9% had been BCG vaccinated. The most frequent cause of kidney failure was diabetic nephropathy (29.4%). At least one risk factor for latent TB, other than chronic kidney disease, was present in 49.8% of patients. The most prevalent risk factors for latent TB were high-risk country of birth (29.4%), diabetes mellitus (27.4%), and prior immunosuppression (20.9%). Forty seven patients (23.4%) were tested for latent TB. Of patients with at least one risk factor for latent TB, only 37.0% were tested. Thirteen (35.1%) of the 37 people with risk factors for TB and were tested, returned a positive result.

Despite a high prevalence of risk factors for tuberculosis in candidates for kidney transplant, few patients were tested. These data indicate an unawareness of current guidelines amongst transplant staff in Australia and demonstrate the need for a nation-wide screening protocol.

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PUBLICATIONS ARISING FROM THIS THESIS

All chapters in this thesis have been submitted for publication in peer reviewed medical journals.

Chapter 2 Under review at The Journal of Nephrology

Chapter 3 Under review at The American Journal of Transplantation

Chapter 4 Not yet under review

Other related publications and reports are presented as appendices

Appendix 1 Rogerson TE, Chen S, Kok J, et al. Tests for latent tuberculosis in people with ESRD: a systematic review. *American Journal of Kidney Diseases.* 2013; 61(1):33-43.

CHAPTER 1: INTRODUCTION

Context of this thesis

My interest in diagnostic medicine developed from my background in immunology and pathology as a basic scientist, and the dilemmas around diagnostic testing that arose during my post-graduate training as a clinical epidemiologist. After finishing my training, I began working at the Centre for Kidney Research (The Children's Hospital at Westmead) on a systematic review examining the evidence of testing for latent tuberculosis in people with end-stage kidney disease (*see Appendix A*). This review compared the performance of new interferon gamma release assays to the old tuberculin skin test using novel methodology for assessing test performance in the absence of a reference standard. Through this project I became interested in how clinicians use diagnostic evidence from the literature and apply it to their clinical practice using evidence-based principles.

Developing the clinical themes of this thesis

The use of diagnostic tests is central to the practice of modern medicine, but knowing which test to use, and when, can be problematic. To make evidence-based diagnoses, clinicians need efficient ways of (1) accessing diagnostic studies, (2) interpreting the results of several studies, and (3) checking the applicability of studies to their own setting. The aim of this thesis is to explore solutions to these problems by addressing a specific clinical question; *What is the best screening test for latent tuberculosis in patients undergoing transplantation?* This chapter presents background information on how I developed my clinical questions, how diagnostic tests are currently used in the Australia, and the epidemiology of latent tuberculosis in transplantation. *Chapter 2* presents evidence on the performance of methodological filters designed to find diagnostic studies in MEDLINE, with a focus on specialist nephrology journals. *Chapter 3* presents the results of a systematic review and meta-analysis of tests for latent tuberculosis in solid organ transplantation. *Chapter 4* presents the results of a cross-sectional study on the prevalence of risk factors for latent tuberculosis, and testing practices used to diagnose latent TB, in candidates for kidney transplant in Australia. There is no formal 'literature review' chapter, as *Chapters 2-3* systematically review key questions around this topic. Thus, this thesis comprises a mixture of methodological and of clinical content research.

Evidence-based testing is underutilised in Australia

Diagnostic tests play an integral role in all aspects of medical decision making, but surprisingly, little is known about their utilisation and cost in the Australian setting. We know that test usage is high, with approximately 9.25 million hospital inpatient diagnoses made in between 1993 to 2012[1]. We also know that a high proportion of testing is probably unnecessary; one study of hospital emergency department found that 40% of testing could be reduced by educating staff and introducing protocols for test ordering[2]. Unnecessary testing is not just a burden on the health budget, it can also be harmful. Over-screening in the cancer setting is well-documented. For some cancers, the psychological effects of labelling patients with disease, and the invasive nature of some diagnostic procedures outweigh any advantage of diagnosis[3]. On the other hand, missed or misdiagnosis can also be harmful. An audit of the United States National Practitioner Data Bank revealed that approximately 40-80,000 deaths occur each year due to misdiagnosis, and that 5% of all autopsies reveal avoidable diagnostic errors[4]. While no data on the potential morbidity and mortality of misdiagnosis in Australia exist, from 2010-11 diagnostic errors made up 26.5% of public sector compensation claims[5]. Over-testing and misdiagnosis can be reduced by using evidence-based practices. For clinical questions about the performance and interpretation of diagnostic tests, the best form of primary research evidence is a diagnostic test accuracy study, and best of all a systematic review and meta-analysis of these studies. In order to make evidence-based decisions with information derived from diagnostic test studies, clinicians require efficient ways to access studies from online medical databases, and to appraise and interpret the results of several studies on the same test and population.

Finding relevant diagnostic studies in online databases is difficult

The MEDLINE database contains of over 21 million references to journal articles published between 1946 and the present day. Finding relevant diagnostic studies is problematic, because studies about the same test are often published in diverse population, methodology and disease specific journals. Each journal article indexed in MEDLINE is allocated a publication type and Medical Subject Headings (MeSH) by staff at the US National Library of Medicine. MeSH is a hierarchal, well-defined vocabulary of terms that provide a consistent way of retrieving studies

from MEDLINE. Similarly, studies may also be retrieved using a specific publication type. Unlike randomised controlled trials, which have a specific ".rct" publication type in MEDLINE, no publication type for diagnostic studies is available. Diagnostic studies must therefore be located using MeSH terms and text words, a method which is often hampered by poor reporting of studies by authors, and suboptimal indexing by the US National Library of Medicine. A recent review of diagnostic studies in nephrology found that the standard of reporting diagnostic accuracy studies had not increased over past 30 years, despite the publication of a *Standards of Reporting Diagnostic Studies* consensus statement in 2001[6]. To find diagnostic studies quickly and efficiently, many medical specialties have developed search strategies designed to reduce the number of search results when searching for diagnostic accuracy studies. By increasing search specificity, these search strategies should allow clinicians to access evidence for diagnostic test performance quickly in the clinical setting. However, little is actually known about the performance of these search strategies in the chronic kidney disease and transplant setting. *Chapter 1* presents the results of an investigation into the performance of search strategies for diagnostic studies in nephrology journals.

The epidemiology of tuberculosis is different in people undergoing solid organ transplantation

In the general population the life-time risk of reactivation of latent tuberculosis infection is 5-10%, but the risk in transplant populations is 20-74 times higher[7]. The reasons for increased risk may be the use of immunosuppressive drugs to prevent rejection, as well as infections acquired from donated organs, underlying chronic kidney disease, and chronic liver disease. Transplant recipients are also more likely to die from active tuberculosis, with a mortality rate of 19-40%, over 10 fold higher than the general population[7]. In Australia, the annual incidence of active tuberculosis in people on dialysis, including potential kidney transplant recipients, was 67 cases per 100,000 people, some 10 times higher than the general population rate. More dialysis patients were also from high risk countries of birth of birth (≥100 cases per 100,000) compared to the general population[8].

The complex pathogenesis of tuberculosis limits our ability to diagnose tuberculosis in immunosuppressed people, including transplant recipients. After

initial colonisation of the host, the causative agent of tuberculosis (*Mycobacterium tuberculosis*) is typically encased in granulomatous tissue by the host immune system, usually within the lungs. Decreased oxygen availability within the granuloma induces dormancy in the bacteria, which corresponds clinically with an asymptomatic period of latency. While granulomatous tissue quarantines the bacteria and prevents pathogenesis, crucially, it also prevents testing by in vitro culture. Tests for latent tuberculosis, including the tuberculin skin test and interferon-γ release assays measure the host immune response to tuberculosis antigens, but without the ability to culture bacteria from latently infected people, it is difficult to evaluate their diagnostic performance.

New methodologies are needed to assess the performance of tests for latent tuberculosis in transplant patients

The conventional method for assessing the diagnostic performance of a new test, is to compare new test results against a reference standard test (also referred to as a gold-standard test) in a defined population. This method assumes the reference standard test is a perfect discriminator of people that have disease, and those that don't. Using this method, test performance is calculated in metrics of sensitivity (the proportion of people with disease that are correctly identified) and specificity (the proportion of people without disease that are correctly identified). Sensitivity and specificity are useful metrics in evidence-based medicine because given a defined pre-test probability of disease, they can be used calculate the absolute risk of disease after testing. But what happens when no reference standard test is available, as is often the case when a new test is introduced to replace an old test? In the diagnosis of latent tuberculosis, the tuberculin skin test (or Mantoux test) has been in used for over 100 years and has well-described deficiencies as a reference standard test. Newer tests, the interferon-gamma release assays, measure the T-cell mediated response of the host immune system to tuberculosis antigens. Interferon-γ release assays are thought to be superior to the tuberculin skin test because they utilise a control for the immune response and do not display cross-reactivity with the Bacille Calmete-Guérin vaccination. Without a reference standard test, the accuracy of interferon-γ release assays cannot be assessed by conventional epidemiological methods. To overcome this challenge, some studies have evaluated tests for latent tuberculosis using clinical risk factors for tuberculosis a proxy reference standard for latent tuberculosis.

Chapter 3 of this thesis systematically reviews these studies and presents a meta-analysis of their results.

Test performance may vary with prevalence of tuberculosis

The utility of latent tuberculosis testing at an institutional level depends on whether the benefits of identifying and treating cases of tuberculosis outweigh the complications of treatment in patients who test falsely positive. If the underlying prevalence of latent tuberculosis is low, then a test may be of limited value for preventing tuberculosis, but could generate high volumes of unnecessary treatment. Anti-tuberculosis drugs can unpredictably affect the bio-availability of immunosuppressive drugs used to prevent rejection after transplant, as well cause neurotoxicity and hepatotoxicity. This particularly problematic for liver transplant candidates, who may have little residual liver function. Data collected by the World Health Organisation suggests that significant variation in the prevalence tuberculosis exists around the world[9]. In *Chapter 4* we sought to determine whether the findings from our systematic review presented in *Chapter 3* were applicable to our own clinical setting; a major regional centre for kidney transplant. *Chapter 4* presents the results of a cross-sectional study on the prevalence of risk factors in candidates for kidney transplantation, and testing practices used to diagnose latent TB, over a two-year period at our centre.

References

1. Principal diagnosis cubes for 1993–94 to 2011–12. *Principal diagnosis data cubes* 2012; http://www.aihw.gov.au/hospitals-data/principal-diagnosis-data-cubes/. Accessed 01/03/2013, 2013.
2. Stuart PJ, Crooks S, Porton M. An interventional program for diagnostic testing in the emergency department. *Medical Journal of Australia.* 2002;177(3):131-134.
3. Zackrisson S, Andersson I, Janzon L, Manjer J, Garne JP. Rate of over-diagnosis of breast cancer 15 years after end of Malmö mammographic screening trial: follow-up study. *BMJ.* 2006;332(7543):689-692.
4. Newman-Toker DE, Pronovost PJ. DIagnostic errors—the next frontier for patient safety. *JAMA.* 2009;301(10):1060-1062.
5. *Australia's Medical Indemnity Claims 2010-11.* Australian Institute of Health and Welfare;2012.
6. McGee RG, Neuen BL, Mitchell RL, Craig JC, Webster AC. Diagnostic test studies in nephrology: quantity, quality, and scope. *The American Journal of Kidney Disease.* 2011;58(6):921-927.
7. Bumbacea D, Arend SM, Eyuboglu F, et al. The risk of tuberculosis in transplant candidates and recipients: a TBNET consensus statement. *The European respiratory journal.* Oct 2012;40(4):990-1013.
8. Dobler CC, McDonald SP, Marks GB. Risk of Tuberculosis in Dialysis Patients: A Nationwide Cohort Study. *PLoS ONE.* 2011;6(12):e29563.
9. *Annex 4: Key indicators for the world, WHO regions and individual countries.* World Health Organisation;2013.

CHAPTER 2: EFFICIENT STRATEGIES TO FIND DIAGNOSTIC TEST ACCURACY STUDIES

Publication details

Currently under review at the Journal of Nephrology (NEP-2014-0374)

Author contributions

TER designed the study, undertook hand searching to establish the reference standard, constructed and carried out search strategies in MEDLINE, conducted all statistical analyses and wrote the manuscript.

ML validated the reference set, assisted with preliminary statistical analyses and reviewed the manuscript.

RM identified relevant search strategies from the literature, assisted with construction of search strategies, provided expert opinion and reviewed the manuscript.

JCC proved expert opinion on study methodology, contributed in the study design process, as well as the analysis and interpretation of results, and drafting and review of the manuscript.

ACW conceived the study idea, helped design the study, participated in the analysis and interpretation of results, and assisted with drafting and reviewing the manuscript.

2.1: Abstract

Background: Nephrologists looking for quick answers to diagnostic clinical questions in MEDLINE can use a range of published search strategies or Clinical Query limits to improve the precision of their searches. We aimed to evaluate existing search strategies for finding diagnostic test accuracy studies in nephrology journals.

Study design: Cross sectional analytic study.

Setting and participants: Diagnostic studies published in the American Journal of Kidney Disease, the Journal of the American Society for Nephrology, and Kidney International (KI) in 2002-3 and 2009-10.

Index tests: Fourteen published search strategies for diagnostic studies were used in MEDLINE with terms to restrict search results to the journals and years of interest.

Reference standard: Two investigators independently hand searched the same journals to create a reference set of diagnostic test accuracy studies.

Results: We identified 103 diagnostic test accuracy studies, accounting for 2.1% of all studies published. The most specific search strategy was the Haynes 2004 Narrow Clinical Queries limit (sensitivity: 0.20, 95%CI 0.13-0.29; specificity: 0.99, 95%CI 0.99-0.99). Using the Haynes 2004 Narrow Clinical Queries limit, a searcher would need to screen 3 (95%CI 2-6) articles from the search results to find one diagnostic study. The most sensitive search strategy was van der Weijden 1999 Extended (sensitivity: 0.95; CI 0.89-0.98; specificity 0.55, 95%CI 0.53-0.56), but required a searcher to screen 24 (95%CI 23-26) articles to find one diagnostic study. Bachmann 2002 was the best-balanced search strategy, which was sensitive (0.88, 95%CI 0.81-0.94), but also specific (0.74, 95%CI 0.73-0.75), with a number needed to screen of 15 (95%CI 14-17).

Limitations: Given the low number of diagnostic studies published in nephrology journals, estimates of sensitivity were imprecise.

Conclusions: Diagnostic studies are infrequently published in nephrology journals. The addition of a strategy for diagnostic studies to a subject search strategy in MEDLINE may reduce the volume of records needed to screen, whilst preserving adequate search sensitivity for routine clinical use.

2.2: Background

The use of diagnostic tests is central to the practice of modern medicine, but knowing which test to use, and when, can be problematic. Each year in the United States an estimated 40-80,000 deaths occur due to misdiagnosis, and 5% of all autopsies reveal avoidable diagnostic errors[1]. Information provided by diagnostic tests must be accurate for clinicians to make evidence based decisions about treatment[2]. Often several diagnostic tests can be used for the same disease, and to determine which test is more accurate for a given clinical situation, clinicians must base their decisions on information from published diagnostic test accuracy (DTA) studies.

Finding relevant published studies can be time consuming, and most search strategies identify a mix of useful and less useful papers. A comprehensive literature search is often not a realistic option for nephrologists in the time-pressured clinical setting. Nephrologists conducting searches for diagnostic studies in MEDLINE have the option of adding an automated Clinical Queries limit or a methodological search strategy to their subject search strategy to improve search efficiency. The Clinical Queries limits for diagnosis in MEDLINE use search terms for diagnostic studies developed by Haynes et al in 2004[3]. The Haynes search strategies were developed and validated in a set of MEDLINE records from a diverse range of medical specialties[3]. For ease of use, the Clinical Queries limits for diagnosis have been integrated into the user interfaces of the PubMed and OvidSP search portals for MEDLINE. Searchers may select either a broad (more sensitive) or narrow (more specific) search strategy to suit the purpose of their search. A precise (best balance of sensitivity and specificity) search strategy is also available in OvidSP. The Clinical Queries limits for diagnosis have a reported sensitivity and specificity in MEDLINE of 98% and 78% respectively for the broad option, and 64% and 98% respectively for the narrow option[4].

In addition to the integrated Clinical Queries limits for MEDLINE, several other search strategies have been developed for finding diagnostic studies within particular medical disciplines, as well as the MEDLINE database more generally[3,5-9]. The reported sensitivities and specificities of search strategies for diagnostic studies in MEDLINE range from 31-98.8% and 73-99% respectively, suggesting

they may perform as well, or better, than the current Clinical Queries limits for diagnosis[3,5-9].

The best search strategy for finding diagnostic studies in specialist nephrology journals is currently unknown. We hypothesised that the performance of published search strategies may behave differently in nephrology journals because the prevalence of DTA studies is lower and authors' methodological reporting is poorer, causing unreliable indexing in MEDLINE[10]. The primary aim of this study was to characterise the performance of methodological search strategies for diagnostic studies in nephrology journals. Our secondary aim was to compare the performance of search strategies for diagnostic studies before and after the publication of an international statement of STAndards for Reporting Diagnostic accuracy studies (STARD)[11]. We hypothesised that the STARD reporting guidelines may have led to an improvement in the performance of the search strategies through improvements in the way DTA studies were reported by authors and indexed within MEDLINE.

2.3 Methods

Reference set

Two reviewers independently searched by hand the 2002, 2003, 2009 and 2010 issues (excluding supplements) of three major general nephrology journals for diagnostic accuracy studies; the American Journal of Kidney Diseases (AJKD), the Journal of the American Society of Nephrology (JASN), and Kidney International (KI). We defined a DTA study as any study that assessed the accuracy of one or more index tests against a reference standard, for detecting the presence of a disease or measuring a physiological parameter of a disease. Any disagreements were resolved by discussion, and if necessary, arbitration with a third reviewer. We extracted study data on the target condition, index test, reference standard test and study population using a standardised data extraction form. To locate the included studies in MEDLINE we constructed a search strategy composed of unique article identifier numbers.

Search strategy performance

We assessed the accuracy of fourteen published methodological search strategies for DTA studies in MEDLINE[3,5-9]*.* See *Supplementary Table 2.3* for OvidSP search strategy syntax. Search strategies were combined with terms to limit the search results to the journals of interest, the years 2002-3 and 2009-10, and to exclude articles from supplement issues. Using hand searching as the reference standard, we generated a cross-classification table for each search strategy, recording which DTA studies were identified by the search strategy and which were not.

Statistical methods

For each search strategy we calculated the sensitivity, specificity, precision and number needed to search with 95% confidence intervals from cross-classification tables comparing search strategy results and our hand searched reference set. Sensitivity was defined as the proportion of all DTA studies that were correctly identified by the search strategy. Specificity was defined as the proportion of all non-DTA studies that were correctly identified by the search strategy. Precision was defined as the proportion of all studies correctly identified as DTA or non-DTA by the search strategy. The number needed to search was defined as the average

number of records that needed to be searched to locate one DTA study. The number needed to search was calculated by dividing the total number of records returned by the search strategy by the number of DTA studies found. An exact McNemar's test at the $p<0.05$ significance level was conducted to compare the specificity and sensitivity of search strategies with similar performance characteristics. We also compared the sensitivity, specificity and precision of search strategies between 2002-3 and 2009-10 using the Chi-Square test at a significance level of $p<0.05$.

2.4: Results

The results of our hand search are presented in *Figure 2.1*. We hand searched 4908 journal articles and identified 103 DTA studies; 48 (47%) from AJKD, 17 (17%) from JASN; and 38 (37%) from KI.

Characteristics of DTA studies

The DTA studies identified in our hand search were highly diverse in patient population, the type of test assessed and target condition diagnosed. DTA study characteristics are summarised in *Table 2.1*. Of the 103 DTA studies, most were conducted in dialysis patients (37, 36%), assessed either biochemistry (34, 33%) or clinical algorithm based tests (19, 18%), and tests that assessed kidney function (30, 29%) or dialysis delivery (19, 18%).

Methodological search strategy performance

The performance of all search strategies are summarised in *Figure 2.2 and Table 2.2*. The van der Weijden 1997 Extended search strategy had the highest sensitivity in the 2002-3 period (0.95; 95%CI 0.86-0.99), the 2009-10 period (0.95; 95%CI 0.85-0.99), and overall (0.95; 95%CI 0.89-0.98). The overall precision of the van der Weijden 1997 Exended search strategy was 0.55 (95%CI 0.54-0.57) and the mean number of studies needed to screen to find one DTA study was 24 (95%CI 23-26). The Haynes 2004 Narrow search strategy was the most specific search strategy, with an overall specificity of 0.99 (95%CI 0.99-0.99) and was also the most precise search strategy, with an overall precision of 0.97 (95%CI 0.0.97-0.98). The Haynes 2004 Narrow search strategy reduced the number needed to screen to 3 (95%CI 2-6). Visual inspection of the receiver operator curve (*Figure 2.3*) revealed two best-balanced search strategies; Bachmann 2002 and van der Weijden 1997 Short. Bachmann 2002 had a sensitivity of 0.88 (95%CI 0.81-0.94) and a specificity of 0.74 (95%CI 0.73-0.75), and reduced the number needed to search to 15 (95%CI 14-17). The sensitivity of the Bachmann 2002 and van der Weijden 1997 Extended and search strategies were not statistically different (χ^2 = 2.4, p=0.12). The van der Weijden 1997 Short strategy had a sensitivity of 0.76 (95%CI 0.66-0.84) and a specificity of 0.84 (95%CI 0.83-0.85). Using the van der Weijden Short strategy reduced the number needed to search to 12 (95%CI 10-14).

Change in strategy performance over time

Comparing the sensitivity of search strategies between 2002-3 and 2009-10 we observed significant increases in the sensitivity of two search strategies. The Haynes 2004 Broad search strategy increased in sensitivity by 0.22 (95%CI 0.06-0.38, p=0.01) to 0.86 (95%CI 0.73-0.95), but decreased in specificity by 0.05 (95%CI 0.03-0.07, p<0.001) to 0.78 (95%CI 0.76-0.80). The Vincent 2003 Broad search strategy increased in sensitivity by 0.17 (95%CI 0.03-0.32, p=0.03) to 0.89 (95%CI 0.75-0.96), with no significant decrease in specificity. We observed small but statistically significant increases in the specificity of three search strategies; the Haynes 2004 Balanced strategy increased in specificity by 0.01 (95%CI 0.00-0.03, p=0.03) to 0.92 (95%CI 0.91-0.93), the Haynes 1994 Balanced strategy increased in specificity by 0.03 (95%CI 0.00-0.06, p=0.03) to 0.61 (95%CI 0.59-0.63), and the Haynes 2004 Narrow strategy increased in specificity by 0.01 (95%CI 0.00-0.01, p=0.03) to 0.99 (95%CI 0.99-1.00). The Bachmann 2002 search strategy decreased in specificity by 0.07 (95%CI 0.04-0.09, p<0.001) to 0.70 (95%CI 0.68-0.72). See *Supplementary Tables 2.1-2.2* for detailed performance characteristics of search strategies in the 2002-3 and 2009-10 time periods.

2.5: Discussion

To answer questions about diagnostic tests, nephrologists wishing to access current research are able to search MEDLINE using methodological search strategies or inbuilt Clinical Queries limits. This study found that in the context of specialist nephrology journals, a clinician using a methodological search strategy for DTA studies could reduce the volume of records that need to be screened whilst preserving adequate search sensitivity for routine clinical use.

The ideal methodological search strategy depends on the purpose for which a search is being conducted. A narrow search strategy is best suited to quick point-of-care searches that require immediate answers. Our results revealed that the most specific search strategy in the context of nephrology journals was Haynes 2004 Narrow. The Haynes 2004 Narrow strategy reduced the number needed to search to just three articles. Haynes 2004 Narrow is also a Clinical Queries limit in OvidSP and PubMed, allowing nephrologists to access it quickly and easily in the clinical setting. Problematically however, the Haynes 2004 Narrow strategy missed 80% of total DTA studies. The Deville 2000 Broad, Deville 2000 Balanced, Haynes 2004 Balanced, and Vincent 2003 Narrow search strategies demonstrated high specificities comparable to the Haynes 2004 Narrow strategy, but also had fair sensitivity (0.55-0.59), see *Figures 2.2-2.3*. Clinicians may also wish to consider these search strategies for narrow searches.

For more comprehensive searches, a broad (highly sensitive) search strategy is required. Broad search strategies allow the searcher to capture most of the available relevant studies, but may also include many irrelevant studies. A broad search strategy would be most useful to a clinician worried about missing relevant studies and with sufficient time to screen search results. Overall, the van der Weijden 1997 Extended search strategy was the most sensitive, capturing 95% of all relevant literature while offering a modest (50%) reduction in the number needed to search. Using the van der Weijden 1997 Extended strategy, an investigator would need to screen an average of 24 studies to find one DTA study. We found no significant difference between the sensitivity of the van der Weijden Extended and Bachmann 2002 search strategy, and therefore clinicians may also wish to us the Bachmann 2002 strategy in sensitive searches.

A best-balanced search strategy provides the best trade-off between reduction in sensitivity and gain in specificity. We identified two comparable best-balanced search strategies; Bachmann 2002, which captured 88% of all DTA studies and reduced the number needed to search by 70% to an average of 15, and van der Weijden 1997 Short strategy, which captured 76% of DTA studies and reduced the number needed to search to 12. A best-balanced search strategy may be useful when both sensitivity and specificity of the search are important, but a compromise must be made between capturing all DTA studies and reducing the workload of the search.

Due to the small number of diagnostic studies identified in our journal set, this study lacked the statistical power to detect increases in the sensitivity of search strategies between 2002-3 and 2009-10. Overall we observed a general trend toward increasing sensitivity of search strategies, but only two increases (Haynes 2004 broad and Vincent 2003) were statistically significant. We also observed small but statistically significant increases in the specificity of three search strategies (Haynes 2004 balanced, Haynes 1994 balanced, and Haynes 2004 narrow). The observed increases in the performance of search strategies between 2002-3 and 2009-10 may represent better standards of reporting diagnostic studies by authors, following the publication of the STARD statement.

In addition to nephrology journals, DTA studies in people with kidney disease may also be published in test or target condition-specific journals. Garg et al showed that nephrology-related information is spread across a wide range of journals, including general medicine, transplantation and urology journals[12]. For example, a study assessing the performance of computed tomography for detecting renal carcinoma in dialysis patients may be published in a nephrology journal, a nuclear medicine journal or a cancer journal. The search strategy performance estimates derived in this study may not be applicable to these journals. Also cause for concern was the underrepresentation of DTA studies conducted in transplant patients in our reference set. Kidney transplant recipients are a major subgroup of kidney disease patients, however in our study only 8 (7.8%) of DTA studies were conducted in this population. It is likely that DTA studies conducted in kidney transplant patients are preferentially published in transplant journals rather than nephrology journals.

The application of methodological search strategies to clinical searches has the potential to substantially reduce the burden of manual record screening, whilst maintaining search sensitivity adequate for routine clinical use. However, care must be taken to ensure the performance characteristics of the search strategy chosen are appropriate for the purpose of the search. Future research in this area should focus on assessing the performance of diagnostic search strategies in record sets derived from relevant systematic reviews. Taking advantage of quality assessments performed in existing systematic reviews, a future study could compare the quality of DTA studies captured and missed by search strategies. If studies missed by search strategies are consistently poor quality, the trade-off in reduced sensitivity for reduced number needed to search is advantageous.

2.6: References

1. Newman-Toker DE, Pronovost PJ. Diagnostic errors--the next frontier for patient safety. *Jama.* Mar 11 2009;301(10):1060-1062.
2. Price CP. Evidence-based laboratory medicine: supporting decision-making. *Clinical chemistry.* Aug 2000;46(8 Pt 1):1041-1050.
3. Haynes RB, Wilczynski NL. Optimal search strategies for retrieving scientifically strong studies of diagnosis from Medline: analytical survey. *Bmj.* May 1 2004;328(7447):1040.
4. Lokker C, Haynes RB, Wilczynski NL, McKibbon KA, Walter SD. Retrieval of diagnostic and treatment studies for clinical use through PubMed and PubMed's Clinical Queries filters. *J Am Med Inform Assoc.* Sep-Oct 2011;18(5):652-659.
5. Bachmann LM, Coray R, Estermann P, Ter Riet G. Identifying diagnostic studies in MEDLINE: reducing the number needed to read. *J Am Med Inform Assoc.* Nov-Dec 2002;9(6):653-658.
6. Deville WL, Bezemer PD, Bouter LM. Publications on diagnostic test evaluation in family medicine journals: an optimal search strategy. *J Clin Epidemiol.* Jan 2000;53(1):65-69.
7. Haynes RB, Wilczynski N, McKibbon KA, Walker CJ, Sinclair JC. Developing optimal search strategies for detecting clinically sound studies in MEDLINE. *J Am Med Inform Assoc.* Nov-Dec 1994;1(6):447-458.
8. van der Weijden T, Ijzermans CJ, Dinant GJ, van Duijn NP, de Vet R, Buntinx F. Identifying relevant diagnostic studies in MEDLINE. The diagnostic value of the erythrocyte sedimentation rate (ESR) and dipstick as an example. *Fam Pract.* Jun 1997;14(3):204-208.
9. Vincent S, Greenley S, Beaven O. Clinical Evidence diagnosis: Developing a sensitive search strategy to retrieve diagnostic studies on deep vein thrombosis: a pragmatic approach. *Health Info Libr J.* Sep 2003;20(3):150-159.
10. McGee RG, Neuen BL, Mitchell RL, Craig JC, Webster AC. Diagnostic test studies in nephrology: quantity, quality, and scope. *American journal of kidney diseases : the official journal of the National Kidney Foundation.* Dec 2011;58(6):921-927.

11. Bossuyt PM, Reitsma JB, Bruns DE, et al. Towards complete and accurate reporting of studies of diagnostic accuracy: The STARD Initiative. *Radiology.* Jan 2003;226(1):24-28.

12. Garg AX, Iansavichus AV, Wilczynski NL, et al. Filtering Medline for a clinical discipline: diagnostic test assessment framework. *Bmj.* 2009;339:b3435.

Figure 2.1 Process for identification of diagnostic test accuracy studies (reference set)

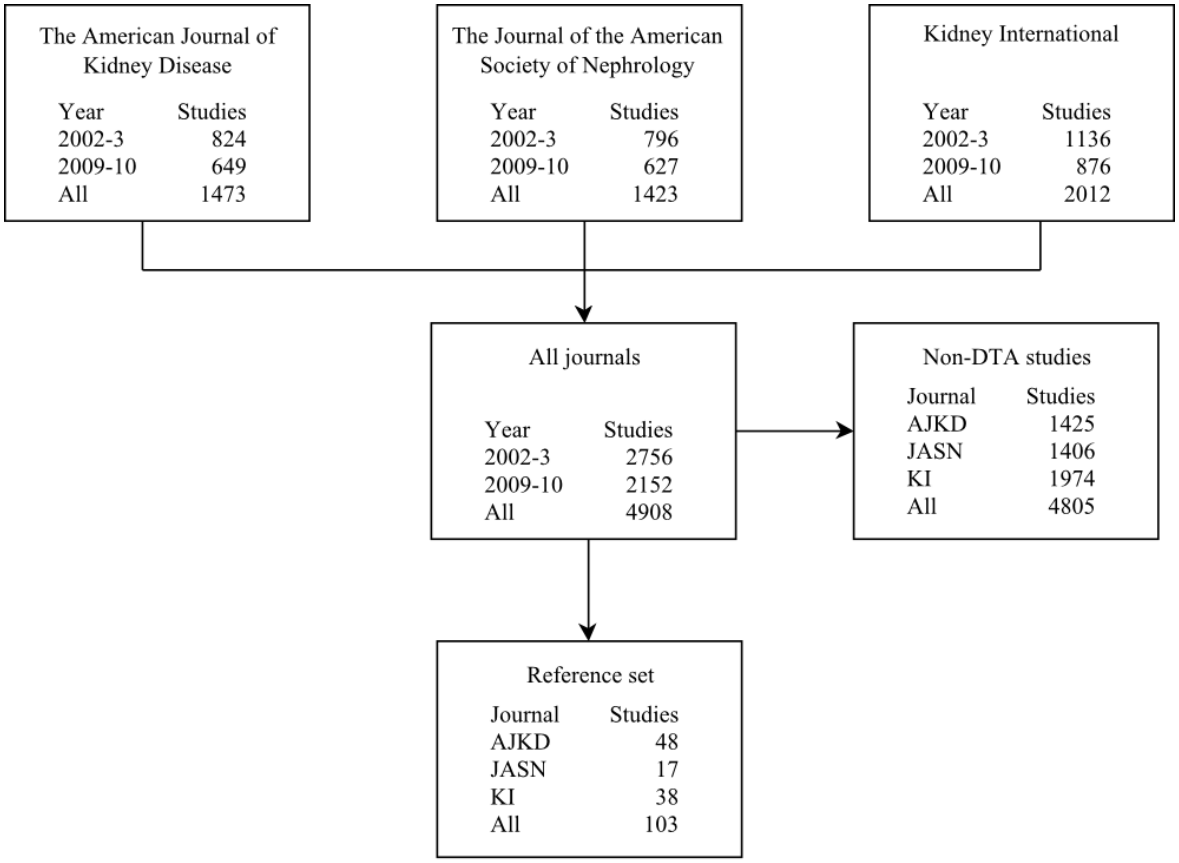
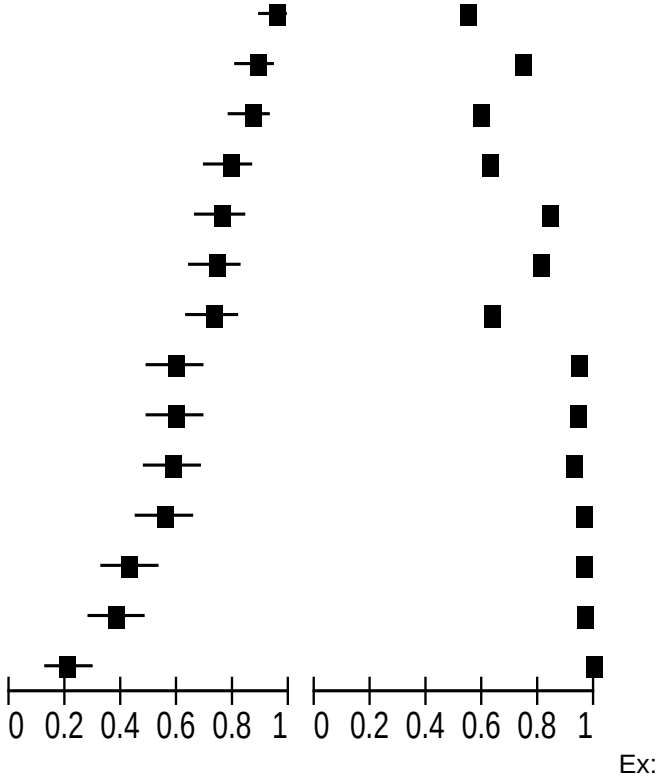


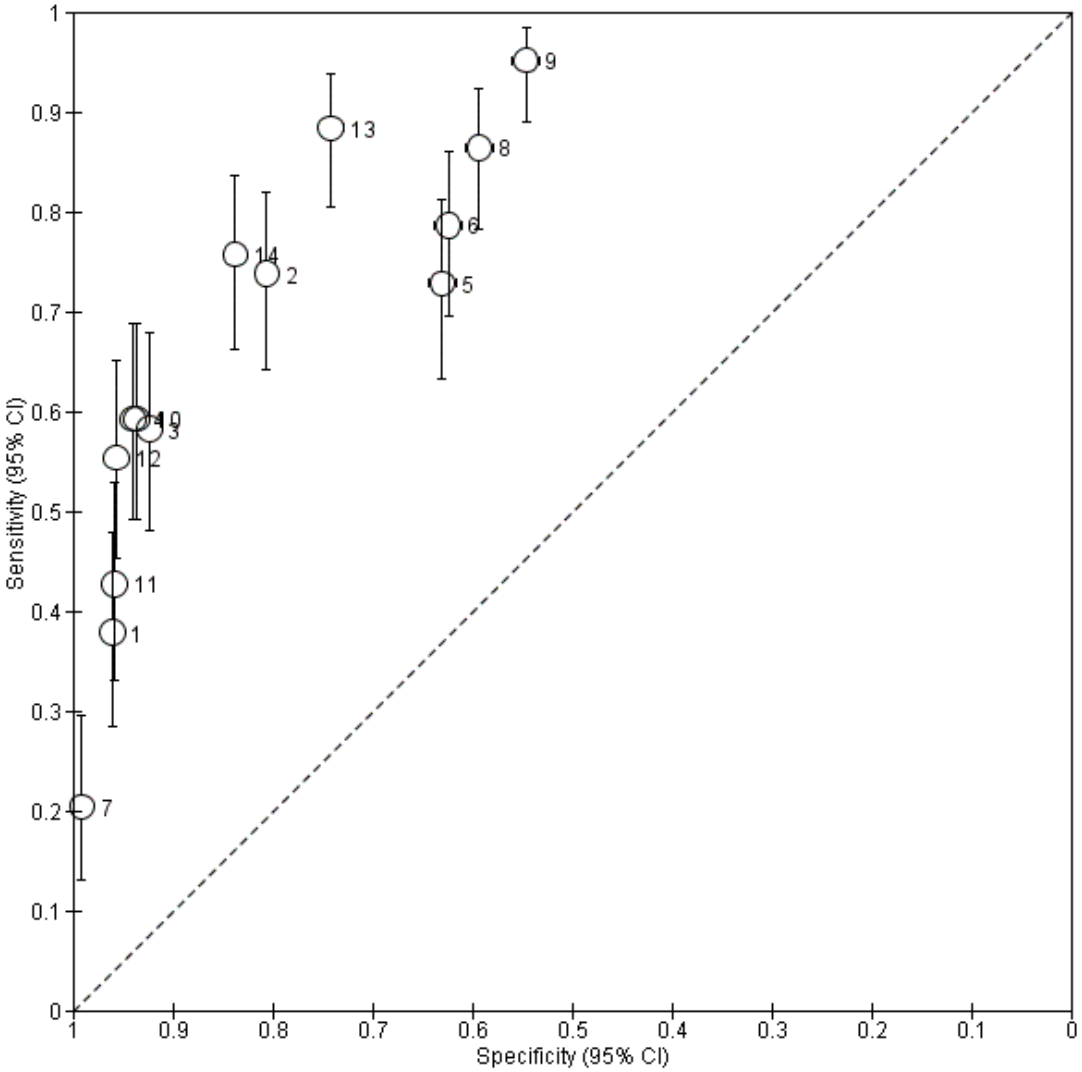
Figure 2.2: Sensitivity and specificity of search strategies for locating DTA studies

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
van der Weijden 1997 (Ex)	98	2182	5	2623	0.95 [0.89, 0.98]	0.55 [0.53, 0.56]
Bachmann 2002	91	1242	12	3563	0.88 [0.81, 0.94]	0.74 [0.73, 0.75]
Haynes 1994 (Balanced)	89	1957	14	2848	0.86 [0.78, 0.92]	0.59 [0.58, 0.61]
Vincent 2003 (Broad)	81	1808	22	2997	0.79 [0.69, 0.86]	0.62 [0.61, 0.64]
van der Weijden 1997 (Sh)	78	781	25	4024	0.76 [0.66, 0.84]	0.84 [0.83, 0.85]
Haynes 2004 (Broad)	76	934	27	3871	0.74 [0.64, 0.82]	0.81 [0.79, 0.82]
Haynes 1994 (Broad)	75	1778	28	3027	0.73 [0.63, 0.81]	0.63 [0.62, 0.64]
Deville 2000 (Broad)	61	291	42	4514	0.59 [0.49, 0.69]	0.94 [0.93, 0.95]
Vincent 2003 (Narrow)	61	308	42	4497	0.59 [0.49, 0.69]	0.94 [0.93, 0.94]
Haynes 2004 (Balanced)	60	368	43	4437	0.58 [0.48, 0.68]	0.92 [0.92, 0.93]
Deville 2000 (Balanced)	57	211	46	4594	0.55 [0.45, 0.65]	0.96 [0.95, 0.96]
Deville 2000 (Narrow)	44	201	59	4604	0.43 [0.33, 0.53]	0.96 [0.95, 0.96]
Haynes 1994 (Narrow)	39	189	64	4616	0.38 [0.28, 0.48]	0.96 [0.95, 0.97]
Haynes 2004 (Narrow)	21	43	82	4762	0.20 [0.13, 0.29]	0.99 [0.99, 0.99]



Extended, Sh: Short, TP: True Positive, FP: False Positive, FN: False Negative, TN: True Negative

Figure 2.3: Receiver operator curve of performance characteristics of search strategies



Legend

1 Haynes 1994 (Narrow)	6 Vincent 2003 (Broad)	11 Deville 2000 (Narrow)
2 Haynes 2004 (Broad)	7 Haynes 2004 (Narrow)	12 Deville 2000 (Balanced)
3 Haynes 2004 (Balanced)	8 Haynes 1994 (Balanced)	13 Bachmann 2002
4 Deville 2000 (Broad)	9 van der Weijden 1997 (Ex)	14 van der Weijden 1997 (Sh)
5 Haynes 1994 (Broad)	10 Vincent 2003 (Narrow)	

Table 2.1: Characteristics of diagnostic test accuracy studies

Characteristic	2002-3 n (%)	2009-10 n (%)	Overall n (%)
Journal			
Total	59 (100)	44 (100)	103 (100)
American Journal of Kidney Disease	31 (53)	17 (39)	48 (47)
Journal of the American Society of Nephrology	8 (14)	9 (21)	17 (17)
Kidney International	20 (34)	18 (41)	38 (37)
Type of test (based on index test)			
Biochemistry	18 (31)	16 (36)	34 (33)
Clinical	8 (14)	6 (14)	14 (14)
Imaging	11 (19)	2 (5)	13 (13)
Diagnostic algorithm	8 (14)	11 (25)	19 (18)
Dialysis related	6 (10)	6 (14)	12 (12)
Other‡	8 (14)	3 (7)	11 (11)
Population (excluding healthy controls)			
Dialysis	30 (51)	7 (19.0)	37 (35.9)
Transplant	2 (3)	6 (75.0)	8 (7.8)
Chronic kidney disease	4 (7)	5 (55.6)	9 (8.7)
Acute kidney injury	3 (5)	5 (62.5)	8 (7.8)
General population	3 (5)	4 (57.1)	7 (6.8)
Diabetics	6 (10)	3 (33.3)	9 (8.7)
Other†	11 (19)	14 (56.0)	25 (24.3)
Target Condition			
Kidney function	14 (46.7)	16 (53.3)	30 (29.1)
Dialysis delivery	16 (84.2)	3 (15.8)	19 (18.4)
Cardiovascular diseases	10 (83.3)	2 (16.7)	12 (11.7)
Kidney injury	2 (20.0)	8 (80.0)	10 (9.7)

	Metabolic bone diseases	6 (85.7)	1 (14.3)	7 (6.8)
	CKD symptoms	4 (66.7)	2 (33.3)	6 (5.8)
	Inherited kidney diseases	2(33.3)	4 (66.7)	6 (5.8)
	Other*	5 (38.5)	8 (61.5)	13 (12.6)

*Other: Allograft rejection, psychological disorders, infectious diseases, diabetes, and kidney diseases

‡Other: Genetic, microbiology, haematology, endocrinology, histopathology,

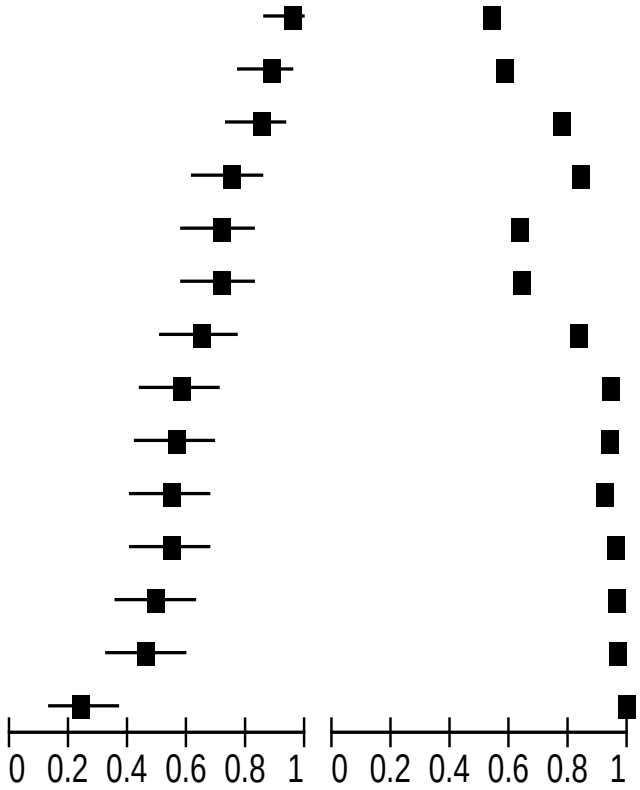
†Other: Renal clinic patients, hospitalised patients, cardiothoracic surgery, polycystic kidney disease, unidentified renal disease, proteinuria, liver transplant candidates

Table 2.2: Search strategy performance in a hypothetical set of 5000 publications with a 2% prevalence of diagnostic test accuracy studies

Search strategy	DTA study prevalence = 2%, N=5000		
	DTA studies (N=100)		Records to search to identify one DTA study (95%CI)
	Captured	Missed	
van der Weijden 1997 (Extended)	95	5	24 (23-26)
Bachmann 2002	88	12	15 (14-17)
Haynes 1994 (Best balance)	86	14	24 (22-27)
Vincent 2003 (Broad)	79	21	24 (22-28)
van der Weijden 1997 (Short)	76	24	12 (10-14)
Haynes 2004 (Broad)	74	26	14 (12-16)
Haynes 1994 (Broad)	73	27	26 (22-30)
Deville 2000 (Broad)	59	41	6 (5-8)
Vincent 2003 (Narrow)	59	41	6 (5-8)
Haynes 2004 (Best balance)	43	57	7 (6-9)
Deville 2000 (Best balance)	55	45	5 (4-6)
Deville 2000 (Narrow)	43	57	6 (4-8)
Haynes 1994 (Narrow)	38	62	6 (5-9)
Haynes 2004 (Narrow)	20	80	3 (2-6)

Supplementary figure 2.1: Sensitivity and specificity of search strategies for the years 2002-3

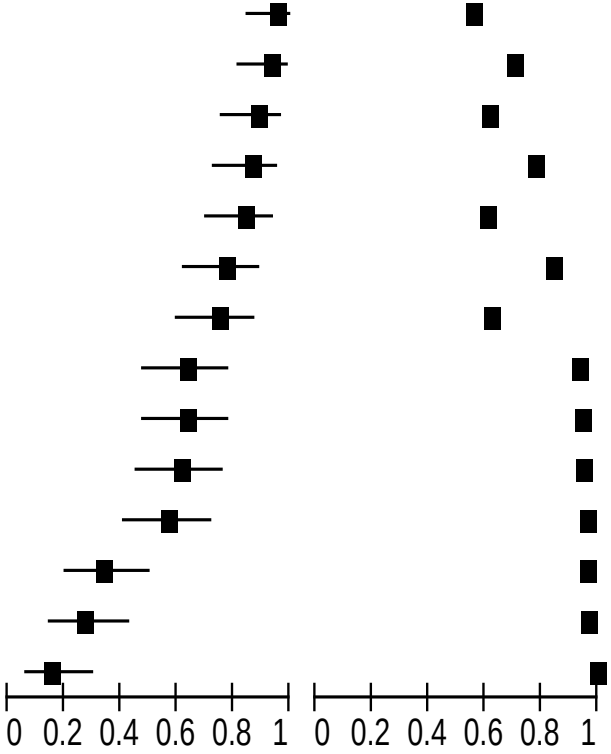
Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
van der Weijden 1997 (Ex)	56	1256	3	1441	0.95 [0.86, 0.99]	0.53 [0.52, 0.55]
Haynes 1994 (Balanced)	52	1135	7	1562	0.88 [0.77, 0.95]	0.58 [0.56, 0.60]
Bachmann 2002	50	619	9	2078	0.85 [0.73, 0.93]	0.77 [0.75, 0.79]
van der Weijden 1997 (Sh)	44	447	15	2250	0.75 [0.62, 0.85]	0.83 [0.82, 0.85]
Vincent 2003 (Broad)	42	1001	17	1696	0.71 [0.58, 0.82]	0.63 [0.61, 0.65]
Haynes 1994 (Broad)	42	982	17	1715	0.71 [0.58, 0.82]	0.64 [0.62, 0.65]
Haynes 2004 (Broad)	38	465	21	2232	0.64 [0.51, 0.76]	0.83 [0.81, 0.84]
Deville 2000 (Broad)	34	176	25	2521	0.58 [0.44, 0.70]	0.93 [0.92, 0.94]
Vincent 2003 (Narrow)	33	185	26	2512	0.56 [0.42, 0.69]	0.93 [0.92, 0.94]
Haynes 2004 (Balanced)	32	226	27	2471	0.54 [0.41, 0.67]	0.92 [0.91, 0.93]
Deville 2000 (Balanced)	32	128	27	2569	0.54 [0.41, 0.67]	0.95 [0.94, 0.96]
Deville 2000 (Narrow)	29	121	30	2576	0.49 [0.36, 0.63]	0.96 [0.95, 0.96]
Haynes 1994 (Narrow)	27	110	32	2587	0.46 [0.33, 0.59]	0.96 [0.95, 0.97]
Haynes 2004 (Narrow)	14	31	45	2666	0.24 [0.14, 0.37]	0.99 [0.98, 0.99]



Ex: Extended, Sh: Short, TP: True Positive, FP: False Positive, FN: False Negative, TN: True Negative

Supplementary figure 2.2: Sensitivity and specificity of search strategies for the years 2009-10

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
van der Weijden 1997 (Ex)	42	926	2	1182	0.95 [0.85, 0.99]	0.56 [0.54, 0.58]
Bachmann 2002	41	623	3	1485	0.93 [0.81, 0.99]	0.70 [0.68, 0.72]
Vincent 2003 (Broad)	39	807	5	1301	0.89 [0.75, 0.96]	0.62 [0.60, 0.64]
Haynes 2004 (Broad)	38	469	6	1639	0.86 [0.73, 0.95]	0.78 [0.76, 0.80]
Haynes 1994 (Balanced)	37	822	7	1286	0.84 [0.70, 0.93]	0.61 [0.59, 0.63]
van der Weijden 1997 (Sh)	34	334	10	1774	0.77 [0.62, 0.89]	0.84 [0.83, 0.86]
Haynes 1994 (Broad)	33	796	11	1312	0.75 [0.60, 0.87]	0.62 [0.60, 0.64]
Haynes 2004 (Balanced)	28	142	16	1966	0.64 [0.48, 0.78]	0.93 [0.92, 0.94]
Vincent 2003 (Narrow)	28	123	16	1985	0.64 [0.48, 0.78]	0.94 [0.93, 0.95]
Deville 2000 (Broad)	27	115	17	1993	0.61 [0.45, 0.76]	0.95 [0.93, 0.95]
Deville 2000 (Balanced)	25	83	19	2025	0.57 [0.41, 0.72]	0.96 [0.95, 0.97]
Deville 2000 (Narrow)	15	80	29	2028	0.34 [0.20, 0.50]	0.96 [0.95, 0.97]
Haynes 1994 (Narrow)	12	79	32	2029	0.27 [0.15, 0.43]	0.96 [0.95, 0.97]
Haynes 2004 (Narrow)	7	12	37	2096	0.16 [0.07, 0.30]	0.99 [0.99, 1.00]



Ex: Extended, Sh: Short, TP: True Positive, FP: False Positive, FN: False Negative, TN: True Negative

Supplementary Table 2.3: OvidSP search syntax

Search strategy	OvidSP syntax
Bachmann 2002	1. exp "Sensitivity and Specificity"/ 2. predict$.tw. 3. diagnos$.tw. 4. di.fs. 5. du.fs. 6. accura$.tw. 7. or/1-6
Deville 2000 (Broad)	1. exp "Sensitivity and Specificity"/ 2. specificity.tw. 3. false negative.tw. 4. accuracy.tw. 5. screening.tw. 6. or/1-5
Deville 2000 (Best balance)	1. exp "Sensitivity and Specificity"/ 2. specificity.tw. 3. false negative.tw. 4. accuracy.tw. 5. or/1-4
Deville 2000 (Narrow)	1. exp "Sensitivity and Specificity"/ 2. specificity.tw. 3. false negative.tw. 4. or/1-3
Haynes 2004 (Broad)	1. sensitiv$.mp. 2. diagnos$.mp. 3. di.fs. 4. or/1-3
Haynes 2004 (Best balance)	1. sensitiv$.mp. 2. predictive value$.mp. 3. accurac$.tw. 4. or/1-3
Haynes 2004 (Narrow)	1. specificity.tw.
Haynes 1994 (Broad)	1. exp "Sensitivity and Specificity"/ 2. di.xs. 3. du.fs. 4. sensitivity.tw. 5. specificity.tw. 6. or/1-5
Haynes 1994 (Best balance)	1. exp "Sensitivity and Specificity"/ 2. exp Diagnosis/ 3. du.fs. 4. specificity.tw. 5. (predictive and value$).tw.

	6. or/1-5
Haynes 1994 (Narrow)	1. exp "Sensitivity and Specificity"/ 2. (predictive and value$).tw. 3. or/1-2
Vincent 2003 (Broad)	1. exp "sensitivity and specificity"/ 2. (sensitivity or specificity or accuracy).tw. 3. ((predictive adj3 value$) or (roc adj curve$)).tw. 4. ((false adj positiv$) or false negativ$).tw. 5. ((observer adj variation$) or (likelihood adj3 ratio$)).tw. 6. likelihood function/ 7. exp mass screening/ 8. diagnosis, differential/ or exp Diagnostic errors/ 9. di.xs. or du.fs. 10. or/1-9
Vincent 2003 (Narrow)	1. exp "Sensitivity and Specificity"/ 2. (sensitivity or specificity or accuracy).tw. 3. ((predictive adj3 value$) or (roc adj curve$)).tw. 4. ((false adj positiv$) or (false adj negativ$)).tw. 5. (observer adj variation$).tw. 6. likelihood function/ 7. exp Diagnostic Errors/ 8. (likelihood adj3 ratio$).tw. 9. or/1-8
van der Weijden 1997 (Extended)	1. exp Diagnosis/di [Diagnosis] 2. Diagnosis Differential/ 3. exp "Sensitivity and Specificity"/ 4. Reference Values/ 5. False Negative Reactions/ 6. False Positive Reactions/ 7. exp Mass Screening/ 8. or/1-7 9. diagnos$.tw. 10. (sensitivity or specificity).tw. 12. predictive value$.tw. 13. reference value$.tw. 14. ROC.tw. 15. likelihood ratio$.tw. 16. monitoring.tw. 17. or/9-16 18. or/8,17
van der Weijden 1997 (Short)	1. exp Diagnosis/ 2. exp "Sensitivity and Specificity"/ 3. Reference Values/ 4. False Negative Reactions/ 5. False Positive Reactions/ 6. exp Mass Screening/

	7. or/1-7 8. diagnos$.tw. 9. (sensitivity or specificity).tw. 10. predictive value$.tw. 11. reference value$.tw. 12. ROC.tw. 13. likelihood ratio$.tw. 14. monitoring.tw. 15. or/8-14 16. or/7,15

CHAPTER 3: TESTS FOR LATENT TUBERCULOSIS IN SOLID ORGAN TRANSPLANTATION: A SYSTEMATIC REVIEW AND META-ANALYSIS

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Contribution of authors

TR conceived, designed and developed the protocol and search strategy for the review, contacted authors, identified and extracted data from included studies, analysed and interpreted the results and wrote the manuscript.

KN contributed to protocol development, and identified and extracted data from the included studies.

AW and **JC** contributed to the conception, design and development of the protocol, the analysis and interpretation of the results and to the drafting and revision of the manuscript.

3.1: Abstract

Transplant recipients with latent tuberculosis (TB) are at high risk of developing active TB. Active TB can be prevented with chemoprophylaxis, but latent TB must first be identified. The most accurate test for diagnosing latent TB in candidates for organ transplantation is uncertain. We conducted a systematic review and meta-analysis to assess the relative test performance of interferon gamma release assays (IGRAs) and the tuberculin skin test (TST) in people undergoing solid organ transplantation. Test performance was measured as a diagnostic odds ratio (OR), with a positive odds defined as the odds of a positive test in the presence of a clinical risk factor (used as a proxy for the presence of TB). We identified 18 studies with data, including eight studies that compared an IGRA with the TST directly, and 10 studies that evaluated only the TST. Both a positive tuberculin skin test and IGRA were strongly associated with clinical risk factors for TB (TST: OR 3.87; 95%CI 1.99-7.56, p<0.01, IGRA: OR 2.56; 95%CI 1.24-5.27, p=0.01), and radiological evidence of past TB (TST: OR 3.18; 95%CI 1.76-5.76, p<0.01, IGRA: OR 2.56; 95%CI 1.69-3.88, p<0.01). Due to limited data, estimates of relative performance of IGRAs and TST were imprecise. Test superiority was uncertain for clinical risk factors (relative OR: 0.65; 95%CI 0.36-1.18, p=0.15), radiological evidence of TB (relative OR: 1.09; 95%CI 0.22-5.35, p=0.92), diabetes (relative OR: 0.76; 95%CI 0.22-2.60, p=0.67) and BCG vaccination (relative OR: 1.12; 95%CI 0.63-2.00, p=0.69). On current evidence it is unclear whether IGRAs perform better, worse or the same as the TST because of substantial uncertainty in their absolute and relative test performance.

3.2: Background

Immunosuppressive therapy after transplantation is necessary to prevent graft rejection, but increases risk of bacterial infections, including *Mycobacterium tuberculosis*[1]. Transplant recipients are up to 74-times more likely to develop active tuberculosis (TB) than the general population, and have a substantially increased mortality rate (17-40%)[1]. Due to the non-specific presentation of symptoms and atypical pathogenesis of TB in transplant recipients, the diagnosis of active TB may be initially missed in up to 33% of cases[2]. Once diagnosed, effective anti-TB drugs are available, but their interactions with immunosuppressive agents are complex and may increase or decrease immunosuppression levels or give added toxicity[1,3,4]. It is therefore preferable to treat patients before they receive a transplant, and before reactivation of latent infection. Anti-TB prophylaxis should be test-directed to ensure that patients at most risk of TB receive treatment, whilst avoiding the complications of treatment in low risk patients. Current international guidelines recommend that potential transplant recipients can be screened with either the tuberculin skin test (TST) or an interferon gamma release assay (IGRA), or both[1].

The TST measures the immune response of a subject to an intradermal injection of purified tuberculin protein. After placement of tuberculin protein, the induration of swelling around the injection site is measured 24-48 hours later. Current guidelines recommend an induration of 5mm or greater as the cut-off for a positive TST in immunosuppressed patients, and 10mm or greater in immunocompetent patients. Several attributes of the TST that may affect its accuracy in transplant candidates. The tuberculin antigen used in the TST shares immunogenic epitopes with the Bacille Calmette Guerin (BCG) vaccination. As a result, BCG vaccinated patients may return false positive TST results[5]. To operate correctly, the TST relies on the immunocompetency of the test subject. The presence of no induration after 48 hours may indicate the absence of latent TB, but it may also indicate immunosuppression[6].

Interferon-γ release assays are designed to detect the TB-specific cell mediated immune response of people previously exposed to TB. IGRAs are available in two formats; the enzyme-linked immunosorbent assay (ELISA), which that measures

the titre of interferon-γ produced by T cells, and the enzyme-linked immunospot assays (ELISPOT), which measures the number of T cells producing interferon-γ. IGRAs may be advantageous in the diagnosis of latent TB because they are not confounded by BCG vaccination and have a negative mitogen control.

The relative diagnostic accuracy of IGRAs and the TST in transplant candidates is unknown and may not be transferable from the end-stage kidney disease setting because transplant recipients have reduced cell-mediated immunity. In this systematic review we aimed to determine whether ELISA- or ELISPOT based IGRAs are more accurate than the TST for diagnosing latent TB in candidates for solid organ transplant.

3.3: Methods

Study inclusion/ exclusion criteria

We included all studies in any language that reported the results of a test for latent TB, and at least one risk factor for tuberculosis in solid organ transplant candidates or recipients. Studies including cases of active TB were excluded when data for active cases could not be separated from the rest of the study population. Case studies and case series were also excluded.

Search strategy

We searched MEDLINE and EMBASE on 1 July 2014 for studies that met our inclusion criteria. The search strategy we used was developed in collaboration with a medical librarian and combined terms for transplant candidates/ recipients, with terms for tests, and terms for TB (see *Supplementary Table 1* for detail). Relevant studies were also identified through searches of reference lists from key studies and systematic reviews, and by correspondence with experts in field.

Data abstraction

Two authors (TR and KN) independently reviewed search results and extracted data from included studies using standardised data extraction forms. Data was extracted on study methodology, patient characteristics, test results and risk factors for TB. Where additional data was required, corresponding authors were contacted by email and requested to provide data. Discrepancies were resolved by consensus with a third author (AW).

Quality assessment

We assessed study quality with an adapted Quality Assessment of Diagnostic Accuracy Studies tool (QUADAS-2). (*Supplementary Table 2).* QUADAS-2 appraises four domains of potential bias; patient selection, index test conduct, reference standard conduct and flow and timing of tests. The domains for reference standard bias and applicability were replaced with domains to assess the quality of the latent TB risk factor assessment, as this was the comparator in our review.

Statistical analysis

All statistical analyses were performed in STATA 11 and Review Manger 5.2.

Where sufficient data was available, we constructed two by two tables to compare risk factors and test results. Indeterminate and negative test results were pooled. We calculated Odds Ratios (OR) with 95% confidence intervals (CI) for odds of positive test in participants with risk factor, against the odds of a positive test in participants without the risk factor. If by chance all study participants had the same risk factor or the same test result, resulting in a zero cell, we added 0.5 to all cells to allow calculation of an OR[7]. To compare tests, we calculated the Ratio of Odds Ratios (RORs) with 95% CI. Variance was assumed to be 0.5 in calculations of standard error for RORs, as this method has been shown to generate conservative estimates of 95% CI for RORs[8]. Meta-analysis summary ORs and RORs were calculated using a random effects model weighted by inverse variance. Summary RORs were calculated for studies that assessed two or more tests in the same population which enabled a direct comparison of test performance to be made.

3.4: Results

Studies identified

We identified 31 reports of 36 relevant studies from our search of online medical databases, and a further 15 studies from previous work and correspondence with experts. In total 46 reports of 41 studies were included in the review, but only 24 reports of 18 studies contained sufficient data for meta-analysis **(***Figure 3.1***).** Characteristics of included studies that contributed to meta-analysis are shown in *Table 3.1*. Eight studies compared an IGRA with the TST directly and 10 studies evaluated only the TST. Eight studies were conducted in kidney transplantation, 6 in liver transplantation, 2 in lung transplantation and 2 in mixed solid organ transplantation. Most studies (13/18, 72%) were conducted in countries with a low prevalence of TB (<50 cases/ 100,000 persons) and had more males than females (16/18, 89%). Mean age of study participants ranged from 34.2-56.4 years. BCG vaccination status was not commonly reported.

Risk of bias assessment was hampered by poor reporting of study methodology. The poorest domains for incomplete reporting were patient selection bias (6/18, 33%), index test bias (16/18, 89%) and risk factor assessment bias (17/18, 94%). The risk of bias in flow and timing of study was generally considered low (11/18, 61%), as was the applicability of the patient population (13/18, 72%), index tests (16/18, 89%) and risk factor assessment (18/18, 100%) to the review question. A high risk of bias was observed in the method of patient selection in 4/18 (22%) of studies. We also observed a high risk of bias in the conduct of the index tests in 2/18 (11%) of studies, flow and timing in (3/18, 17%) of studies, and applicability of the patient population in (5/18, 28%) of studies. Refer to *Table 3.2* for a summary of the risk of bias of included studies.

Association between a positive test result and risk factor

Similar magnitudes of association between test positivity and risk factors were observed across ELISA- and ELISPOT IGRAs, and the TST (see *Figure 3.2-3.3*). For both the IGRAs and the TST, patients were more likely to return a positive test if they had a study-defined clinical risk factor for TB (TST: OR 3.87; 95%CI 1.99-7.56*, p*<0.01, IGRA: OR 2.56; 95%CI 1.24-5.27, *p*=0.01), or a radiological evidence of past TB (TST: OR 3.18; 95%CI 1.76-5.76, *p*<0.01, IGRA: OR 2.56; 95%CI 1.69-3.88, *p*<0.01). A positive TST result was also associated with patients

who had a history of contact with active TB (OR 3.24; 95%CI 1.13-9.29, *p*=0.03). A positive IGRA more likely to occur in participants who had received TB treatment (OR 22.31; 95%CI 7.80-63.76, *p*<0.01), but less likely in immunosuppressed patients (OR 0.45; 95%CI 0.27-0.74, p<0.01).

Only two studies assessed the ELISPOT-based IGRA test for detecting latent TB in transplant candidates. These studies did not provide sufficient data to determine the direction of association with risk factors (see *Supplementary figure 3.1*). Removing only ELISPOT-based IGRA data from our analysis of IGRAs did not change the direction of association of positive tests with risk factors, but did widen confidence intervals (see *Supplementary figure 3.2).*

We also identified two studies that assessed the performance of the TST with an antigen control panel in potential lung transplant recipients, but insufficient data was available to determine the direction of association with risk factors.
Our subgroup analyses did not identify any significant differences in the association of TST positivity with risk factors between studies that assessed kidney, liver, or mixed populations of transplant candidates (clinical risk factors, Chi^2=0.02, p=0.89; radiological evidence of past TB, Chi^2=0.03, p=0.87; contact history, Chi^2=2.05, p=0.36; previous TB, Chi^2=0.00, p=0.96; BCG, Chi^2=3.37, p=0.06). Similarly, no difference in odds of IGRA positivity was found between kidney, liver or mixed transplant candidates (clinical risk factors, Chi^2=0.55, p=0.46; radiological evidence of past TB, Chi^2=0.34, p=0.56; contact history, Chi^2=0.83, p=0.36; previous TB, Chi^2=0.00, p=0.96; diabetes, Chi^2=2.34, p=0.31; BCG, Chi^2=1.20, p=0.55).

Interferon gamma release assays versus Tuberculin skin test

Data available for head-to-head comparison of tests was sparse. Five studies compared an IGRA with the TST, including three studies of ELISA-based IGRA and two studies of ELISPOT-based IGRAs, see *Figure 3.4*. We found no evidence of a difference in odds of a positive test between IGRAs and the TST for transplant candidates with clinical risk factors (ROR 0.65; 95%CI 0.36-1.18, p=0.15), radiological evidence of past TB (ROR 1.09; 95%CI CI 0.22-5.35, p=0.92), history of contact with active TB (ROR 1.75; 95%CI 0.15-20.01, p=0.65), diabetes (ROR 0.76; 95%CI CI 0.22-2.60; p=0.67) or BCG vaccination (ROR 1.12; 95%CI 0.63-

2.00, p=0.69). One study found that people a previous TB infection were more likely to test positive with an ELISA-based IGRA than the TST (ROR 22.15 (95%CI 1.05-466.73, p=0.05).

Our sensitivity analysis (see *Supplementary figures 3.3-3.4*) found that ELISPOT-based IGRAs were more likely to return a positive result than the TST in diabetic transplant recipients (ROR 0.45; 95% CI 0.21-0.97, p=0.04). No data was available to compare the relative performance of ELISA- and ELISPOT-based IGRAs.

3.5: Discussion

This is the first systematic review to examine the performance of tests for latent TB in solid organ transplant recipients and candidates. The absence of a reference standard test precludes the generation of sensitivity and specificity and precision as metrics of relative diagnostic performance. In this review we synthesized data from 18 studies that assessed diagnostic accuracy by comparing test results against risk factors as proxy reference standards. We found that positive TST and IGRA test results were closely associated with risk factors for tuberculosis. People who had clinical risk factors for tuberculosis, radiological evidence of past TB, or had contact with active TB were 3-4 times more likely to return a positive TST result. Similarly, people who had clinical risk factors for tuberculosis or radiological evidence of past TB were three times more likely to return a positive IGRA. We also found that people with a history of previous TB were 22 times more likely to have a positive IGRA result.

To determine whether IGRAs perform better, worse or the same as the TST, we also examined the relative association of tests with risk factors in studies that reported the results of at least two of these tests, head-to-head. We found no significant difference in the odds of testing positive in the presence of risk factors between the TST and IGRAs, except in people who had previous TB, where IGRA was better. A person previously treated for TB was found to be 22 times more likely to return a positive IGRA than a positive TST, however confidence intervals were wide. No data were available to compare the performance of ELISA- and ELISPOT-based IGRAs. Given these data, it remains uncertain whether IGRAs or the TST are superior for detecting latent tuberculosis in people undergoing solid organ transplant. Clinically important differences may exist but data are too sparse to be confident about the true relative test performance of TST and IGRA for latent TB.

Our results are consistent with our earlier findings about these tests for latent tuberculosis in people with end-stage kidney disease[9]. Like transplant recipients, people with end-stage kidney disease were also more likely to have a positive ELISA-based IGRA if they had radiological evidence of past TB or had contact with actives cases of TB. While this review was unable to show any difference in test performance between IGRAs and the TST, in the end-stage kidney disease

population a positive ELISA-based IGRA was four times more likely in people with radiological evidence of past TB than a positive TST, and three times more likely in people who had a contact history with TB. Another systematic review in the general population, also found that IGRAs were approximately four times more likely to return a positive result than the TST in people who had high TB exposure[8].

Current international guidelines for screening solid organ transplant candidates for latent TB do not make specific recommendations about which test should be used[1], concluding that either test may be used, singly or in combination. Additionally, no guidance is offered on which modality of IGRA, ELISA- or ELISPOT-based, should be used. Our review shows there are currently insufficient data to differentiate one IGRA from another, and so current guidelines are still appropriate for the transplant setting. A recent systematic review of TB screening strategies found that IGRA plus TST screening, and IGRA screening alone were the most cost-effective strategies in high risk groups.[10] In resource poor settings, clinicians may wish to consider using IGRAs over the TST as they are cheaper, and only require a single patient visit to collect blood, while the TST requires two clinical visits.

An ideal reference standard is able to perfectly discriminate between the presence and absence of disease, but risk factors often correlate imperfectly with disease status. For this reason, indicators of test accuracy generated by the comparison of tests with proxy reference standards may not reflect true test performance. This may be an inevitable limitation of this review, given a reference standard is not available. Previous reviews on latent TB tests have used studies in different populations, composed of either patients with active TB or healthy individuals to estimate test sensitivity and specificity respectively[11-13]. Reviews based on these studies have limited applicability because the host immune response to active TB infection is different to latent TB and healthy individuals are immunocompetent[14,15].

In summary, we found there is no evidence of a difference in test performance between IGRAs and the TST in people undergoing solid organ transplantation, except in patients who had previous TB, when IGRA was superior. We also found no data on the relative test performance of ELISA- and ELISPOT-based IGRAs. At

this time no specific recommendations on test usage are appropriate. Future studies should aim to assess the relative diagnostic performance of IGRAs and TST using well-designed large trials with risk factors for TB as proxy references standards. Additionally, a randomized controlled trial of test-directed treatment may also elucidate whether any difference in diagnostic performance between IGRAs and TST is associated with improved clinical outcomes for patients

3.6: References

1. Bumbacea D, Arend SM, Eyuboglu F, et al. The risk of tuberculosis in transplant candidates and recipients: A TBNET consensus statement. *European Respiratory Journal.* 2012;40(4):990-1013.
2. Aguado JM, Herrero JA, Gavaldá J, et al. Clinical presentation and outcome of tuberculosis in kidney, liver, and heart transplant recipients from Spain. *Transplantation.* 1997;63(9):1278-1286.
3. Niemi M, Backman JT, Fromm MF, Neuvonen PJ, Kivisto KT. Pharmacokinetic interactions with rifampicin: clinical relevance. *Clinical Pharmacokinetics.* 2003;42(9):819-850.
4. Ngo BT, Pascoe M, Khan D. Drug interaction between rifampicin and sirolimus in transplant patients. *Saudi Journal of Kidney Diseases & Transplantation.* 2011;22(1):112-115.
5. Sester U, Junker H, Hodapp T, et al. Improved efficiency in detecting cellular immunity towards M. tuberculosis in patients receiving immunosuppressive drug therapy. *Nephrology Dialysis Transplantation.* 2006;21(11):3258-3268.
6. Sester M, Sester U, Clauer P, et al. Tuberculin skin testing underestimates a high prevalence of latent tuberculosis infection in hemodialysis patients. *Kidney International.* 2004;65(5):1826-1834.
7. Agresti A. Contingency Tables. *An Introduction to Categorical Data Analysis*: John Wiley & Sons, Inc.; 2006:21-64.
8. Dinnes J, Deeks J, Kunst H, et al. A systematic review of rapid diagnostic tests for the detection of tuberculosis infection. *Health Technology Assessment (Winchester, England).* 2007;11(3):1-196.
9. Rogerson TE, Chen S, Kok J, et al. Tests for latent tuberculosis in people with ESRD: a systematic review. *American journal of kidney diseases : the official journal of the National Kidney Foundation.* 2013;61(1):33-43.
10. Nienhaus A, Schablon A, Costa JT, Diel R. Systematic review of cost and cost-effectiveness of different TB-screening strategies. *BMC Health Services Research.* 2011;11:247.
11. Chang KC, Leung CC. Systematic review of interferon-gamma release assays in tuberculosis: focus on likelihood ratios. *Thorax.* 2010;65(3):271-276.

12. Pai M, Riley LW, Colford JM, Jr. Interferon-gamma assays in the immunodiagnosis of tuberculosis: a systematic review. *The Lancet Infectious Diseases.* 2004;4(12):761-776.
13. Pai M, Zwerling A, Menzies D. Systematic review: T-cell-based assays for the diagnosis of latent tuberculosis infection: an update. *Annals of Internal Medicine.* 2008;149(3):177-184.
14. Rothel JS, Radford AJ. Comparison of tuberculosis tests: finding truth or confirming prejudice? *Clinical Infectious Diseases.* 2003;36(9):1206-1207; author reply 1209-1210.
15. Hirsch CS, Toossi Z, Othieno C, et al. Depressed T-cell interferon-gamma responses in pulmonary tuberculosis: analysis of underlying mechanisms and modulation with therapy. *Journal of Infectious Diseases.* 1999;180(6):2069-2073.
16. Agarwal SK, Gupta S, Bhowmik D, Mahajan S. Tuberculin skin test for the diagnosis of latent tuberculosis during renal replacement therapy in an endemic area: A single centre study. *Indian Journal of Nephrology.* 2010;20(3):132-136.
17. Aydogan O, Gurgun A, Basoglu OK, et al. Tuberculin skin test reactivity in patients with chronic renal failure. *Tuberkuloz ve Toraks.* 2009;57(3):268-276.
18. Basoglu OK, Atasever A, Gunduz Telli C, et al. T-lymphocyte subgroups and tuberculin skin test reactivity in patients with chronic renal failure. *Tuberkuloz ve Toraks.* 2006;54(1):5-10.
19. Merino E. Evaluation of Infectious Diseases Assessment in Kidney Transplant Candidates. The 50th Interscience Conference on Antimicrobial Chemotherapy; 2010, Sept 12-15; Boston, MA.
20. Shankar MSR, Aravindan AN, Sohal PM, et al. The prevalence of tuberculin sensitivity and anergy in chronic renal failure in an endemic area: tuberculin test and the risk of post-transplant tuberculosis. *Nephrology Dialysis Transplantation.* 2005;20(12):2720-2724.
21. Ahmadinejad Z, Azmoudeh Ardalan F, Razzaqi M, Davoudi S, Jafarian A. QuantiFERON-TB Gold In-Tube test for diagnosis of latent tuberculosis (TB) infection in solid organ transplant candidates: a single-center study in an area endemic for TB. *Transplant Infectious Disease.* 2013;15(1):90-95.

22. Kim JS, Cho JH, Park GY, et al. Comparison of QuantiFERON-TB Gold with tuberculin skin test for detection of latent tuberculosis infection before kidney transplantation. *Transplantation Proceedings.* 2013;45(8):2899-2902.
23. Kim SH, Lee SO, Park JB, et al. A prospective longitudinal study evaluating the usefulness of a T-cell-based assay for latent tuberculosis infection in kidney transplant recipients. *American Journal of Transplantation.* Sep 2011;11(9):1927-1935.
24. Kim SH, Lee SO, Park IA, et al. Diagnostic usefulness of a T cell-based assay for latent tuberculosis infection in kidney transplant candidates before transplantation. *Transplant Infectious Disease.* 2010;12(2):113-119.
25. Benito N, Sued O, Moreno A, et al. Diagnosis and treatment of latent tuberculosis infection in liver transplant recipients in an endemic area. *Transplantation.* 2002;74(10):1381-1386.
26. Fabrega E, Sampedro B, Cabezas J, et al. Chemoprophylaxis with isoniazid in liver transplant recipients. *Liver Transplantation.* 2012;18(9):1110-1117.
27. Singh N, Wagener MM, Gayowski T. Safety and efficacy of isoniazid chemoprophylaxis administered during liver transplant candidacy for the prevention of posttransplant tuberculosis. *Transplantation.* 2002;74(6):892-895.
28. Casas S, Munoz L, Moure R, et al. Comparison of the 2-step tuberculin skin test and the quantiFERON-TB Gold In-Tube Test for the screening of tuberculosis infection before liver transplantation. *Liver Transplantation.* 2011;17(10):1205-1211.
29. Manuel O, Humar A, Preiksaitis J, et al. Comparison of quantiferon-TB gold with tuberculin skin test for detecting latent tuberculosis infection prior to liver transplantation. *American Journal of Transplantation.* 2007;7(12):2797-2801.
30. Lindemann M, Dioury Y, Beckebaum S, et al. Diagnosis of tuberculosis infection in patients awaiting liver transplantation. *Hum Immunol.* 2009;70(1):24-28.
31. Caruso K, Gutta R, Pien L. Clinical utility of anergy panel testing in conjunction with purified protein derivative (PPD) tuberculin skin testing (TST) for detection of latent tuberculosis infection (LTBI) in pre-lung

transplant patients. *Journal of Allergy and Clinical Immunology.* 2012;1:AB58.

32. Roman A, Bravo C, Levy G, et al. Isoniazid prophylaxis in lung transplantation. *J Heart Lung Transplant.* 2000;19(9):903-906.
33. Povitz M, Fisher D. Quantiferon In Practice - Experience From a Canadian Centre. *American Journal of Respiratory and Critical Care Medicine.* 2010;181:A4775.
34. Theodoropoulos N, Lanternier F, Rassiwala J, et al. Use of the QuantiFERON-TB Gold interferon-gamma release assay for screening transplant candidates: A single-center retrospective study. *Transplant Infectious Disease.* 2012;14(1):1-8.

Figure 3.1: Identification of studies included in review

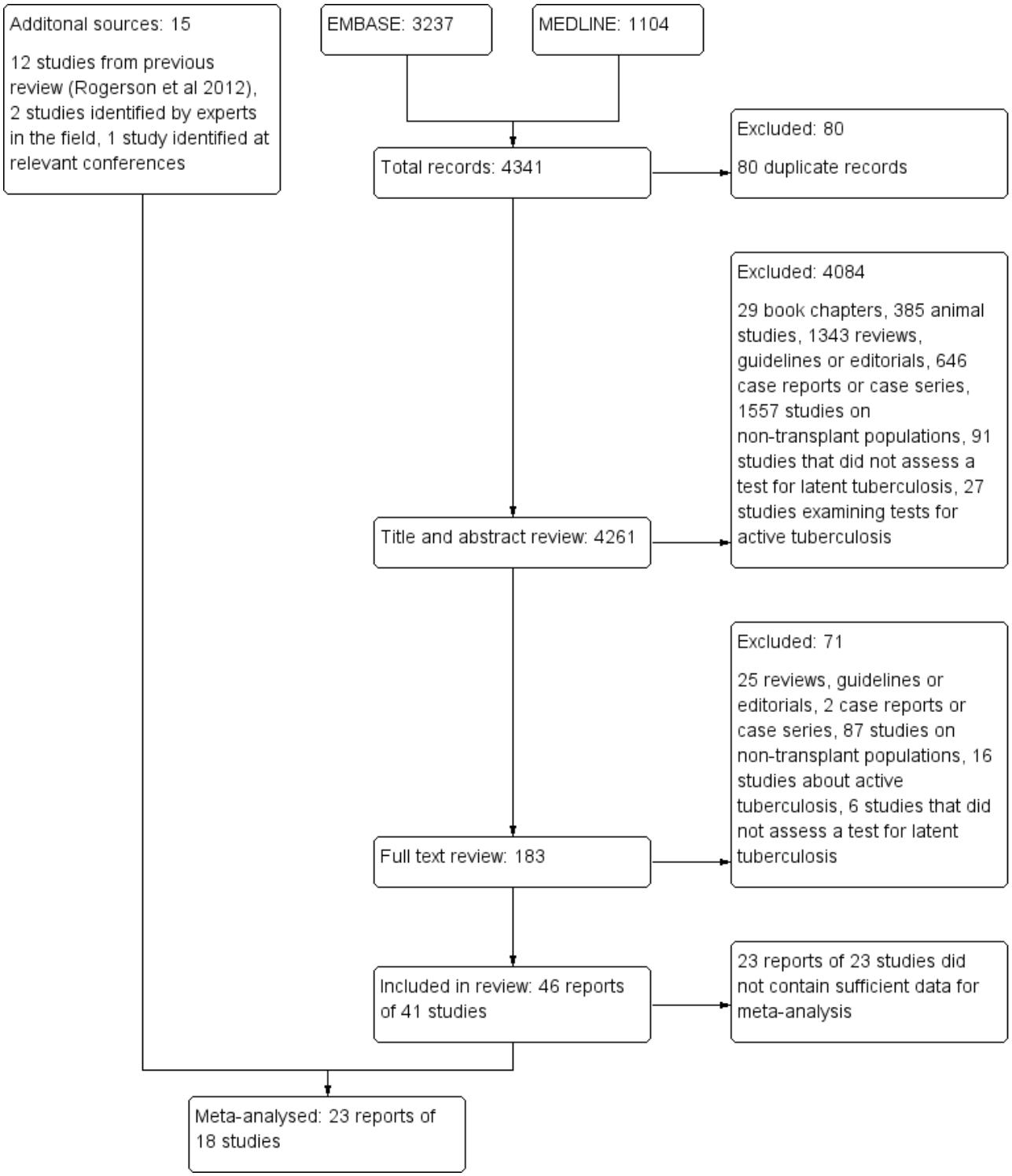
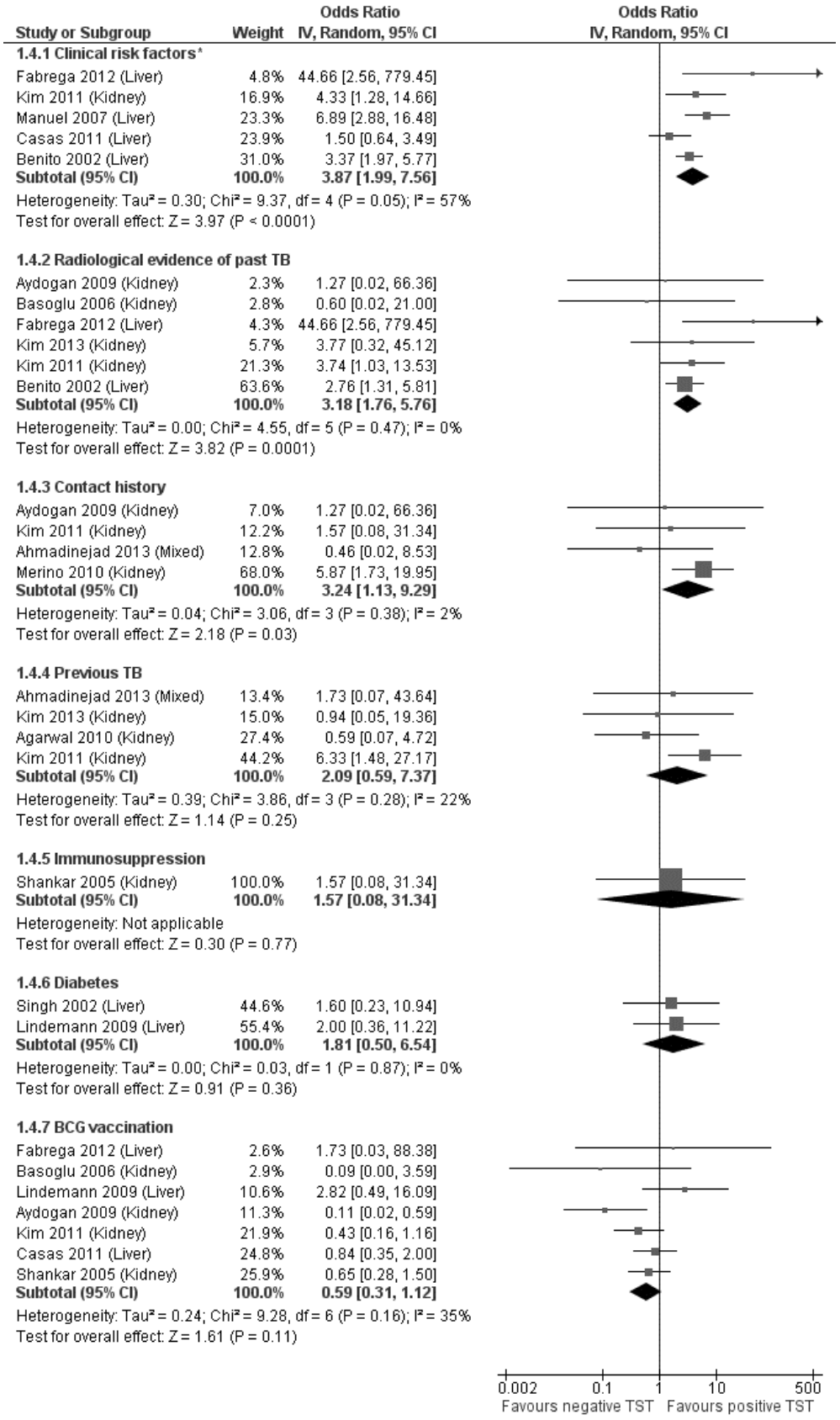
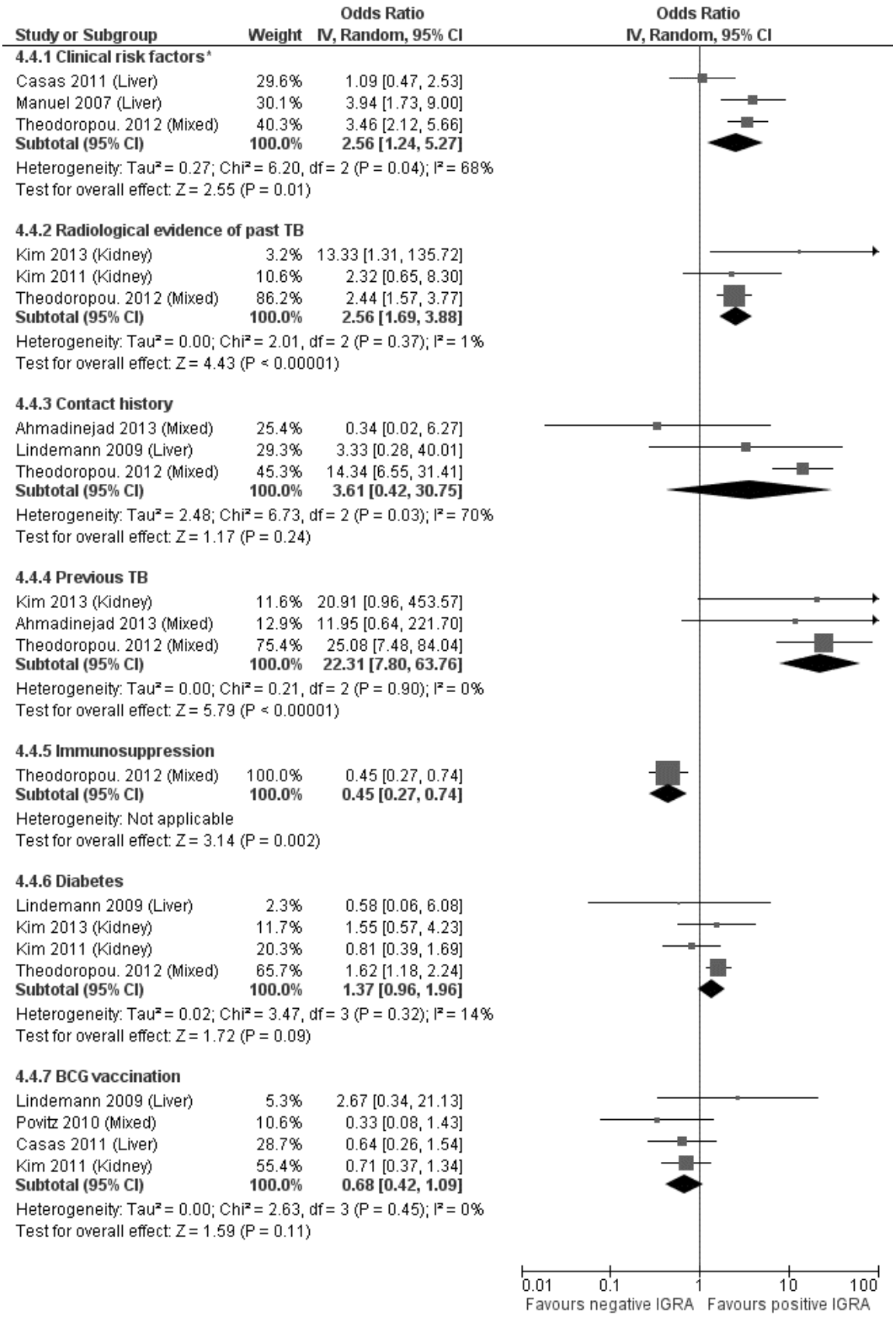


Figure 3.2: Odds of positive TST in transplant candidates with risk factors for TB



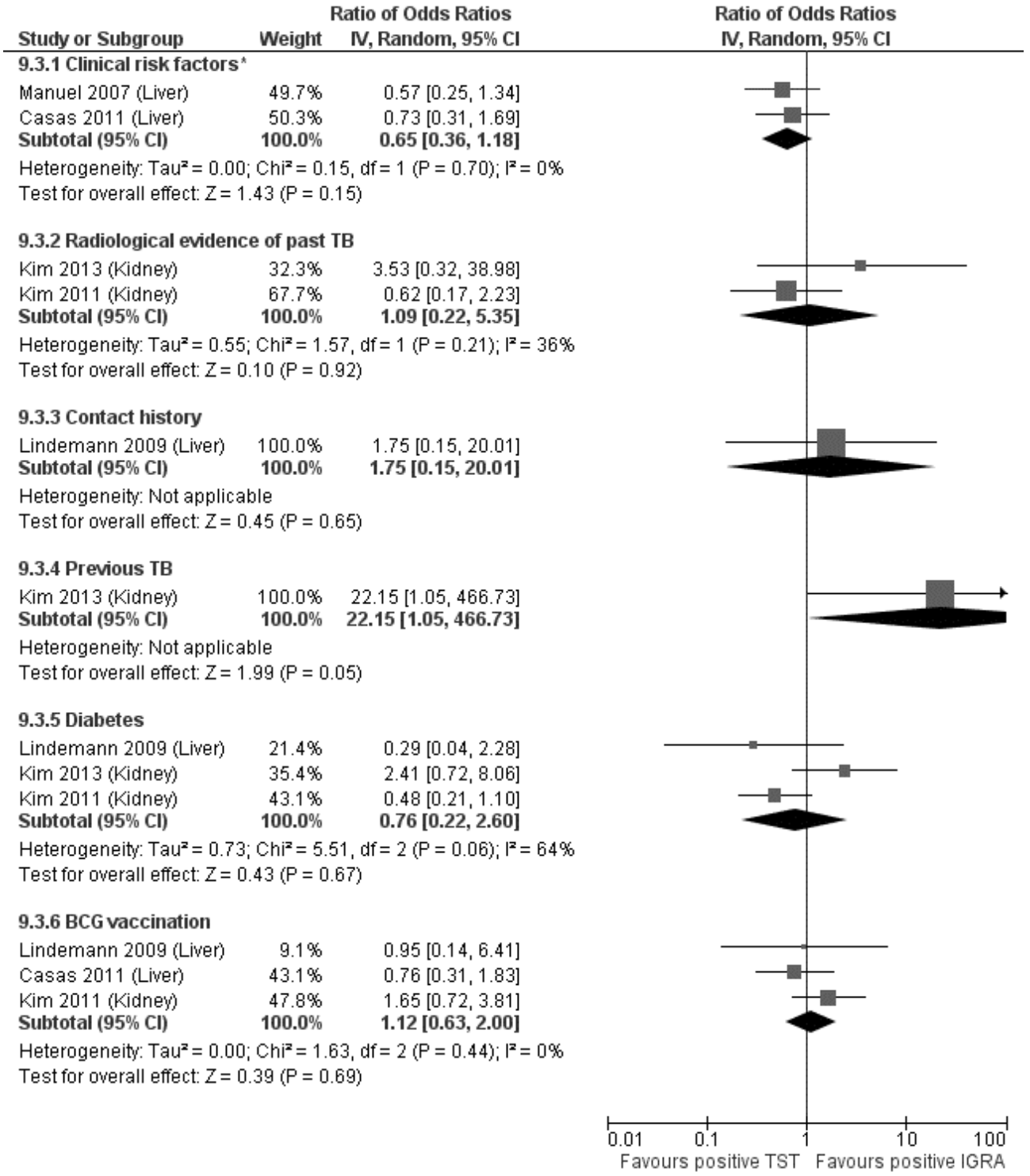
*Clinical risk factors as defined by study, IV, Random: inverse variance, random effects meta-regression, 95% CI: 95% confidence interval

Figure 3.3: Odds of positive IGRA in transplant candidates with risk factors for TB



*Clinical risk factors as defined by study, IV, Random: inverse variance, random effects meta-regression, 95% CI: 95% confidence interval

Figure 3.4: Relative performance of the TST and IGRAs



*Clinical risk factors as defined by study, IV, Random: inverse variance, random effects meta-regression, 95% CI: 95% confidence interval

Table 3.1: Characteristics of included studies

Study	Comparison	N	Country	Male (n, %)	Age (mean ± SD*)	Prevalence of TB (cases/10^5 [95% CI])§†
Kidney transplant						
Agarwal 2010[16]	TST	200	India	169 (84.5)	34.7 ± 11.1	438 (382-498)
Aydogan 2009[17]	TST	50	Turkey	24 (48.0)	34.2 ± 12.7	25 (12-43)‡
Basolgu 2006[18]	TST	7	Turkey	*nr*§	*nr*	28 (13-48)‡
Merino 2010[19]	TST	992	Brazil	575 (58.0)	42	61 (26-112)
Shankar 2005[20]	TSTa	108	India	78 (72.2)	37.8 ± 11.8	365 (295-443)‡
Ahmadinejad 2013[21]	TST vs QFT	187	Iran	38 (59.4)	38.5 ± 12.1	29 (11-55)
Kim 2013[22]	TST vs QFT	109	South Korea	68 (62.4)	44.7 ± 11.5	431 (156-842)
Kim 2011[23,24]	TST vs TSPOT	312	South Korea	176 (56.4)	42.5 ± 10.2	431 (156-842)
Liver transplant						
Benito 2002[25]	TST	529	Spain	333 (62.9)	50 (12-66)‖	30 (12-55)
Fabrega 2012[26]	TST	145	Spain	111 (76.6)	54 (20-66)‖	27 (11-50)
Singh 2002[27]	TST	36	Germany	36 (100.0)	nr	27 (10-50)
Casas 2011[28]	TST vs QFT	110	Spain	72 (65.5)	56.4 ± 7.6	20 (8.2-37)
Manuel 2007[29]	TST vs QFT	153	USA	122 (79.7)	54.6 ± 8.2	6.4 (2.6-12)
Lindemann 2009[30]	TST vs TSPOT vs LTT	48	Germany	21 (43.8)	54 (22-69)‖	9.4 (3.7-18)
Lung transplant						
Caruso 2012[31]	TSTa	100	USA	56 (56.0)	56.1	5.1 (2.1-9.4)
Roman 2000[32]	TSTa	61	Spain	38 (62.3)	42 (16-67)‖	30 (12-55)
Mixed solid organ transplant						
Povitz 2010[33]	TST vs QFT	43	Canada	*nr*	*nr*	6.7 (2.7-13)
Theodoropoulos 2012[34]	TST vs QFT	2394	USA	564 (59.9)	54.2 ± 11.4	5.3 (2.2-9.9)

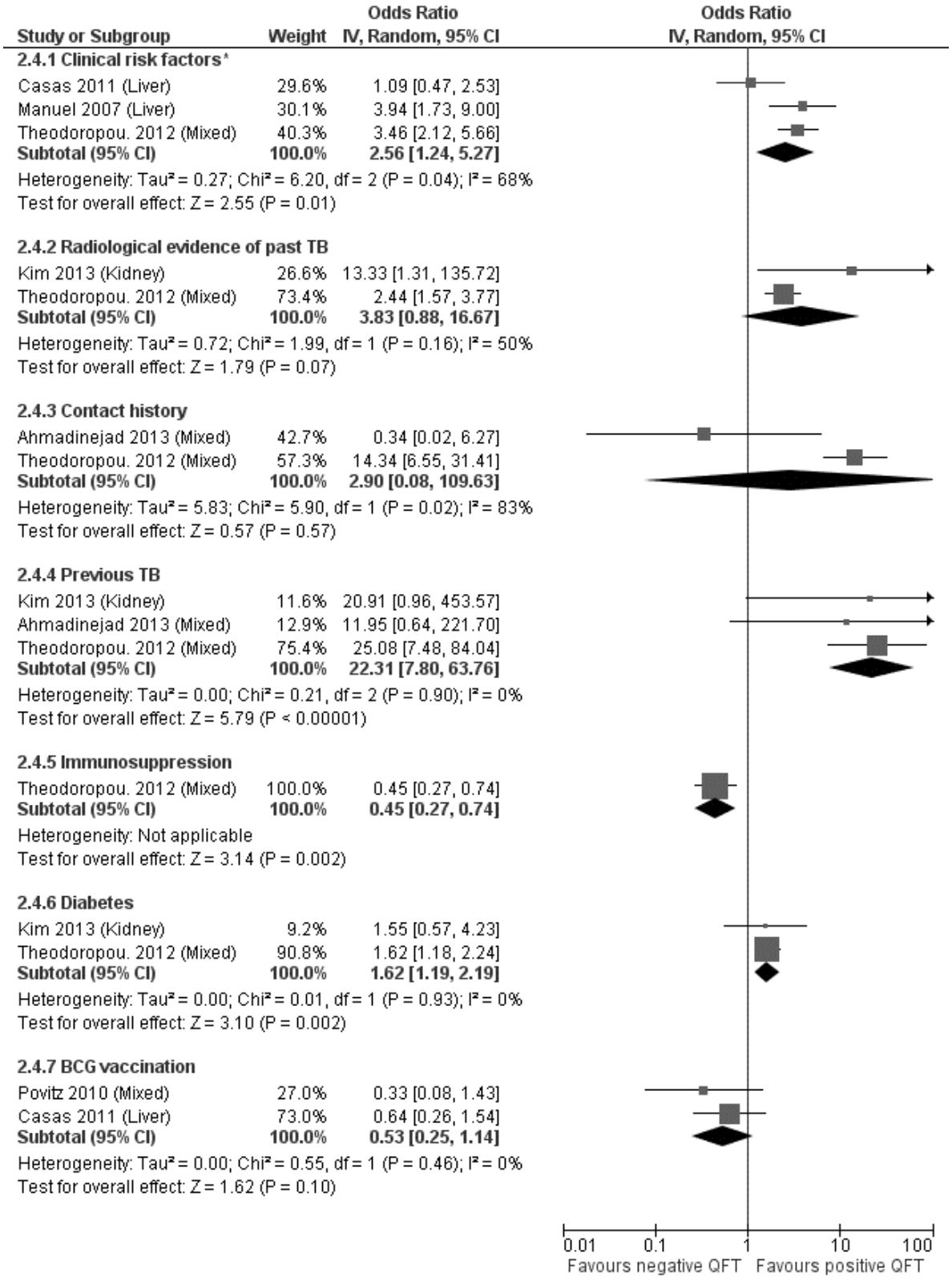
*Standard Deviation, † WHO data for first year of study, ‡ Data for year of publication, § Not reported, ‖ Median (range)

Table 3.2: Risk of bias of included studies

	BIAS				APPLICABILITY		
STUDY	**PATIENT SELECTION**	**INDEX TEST(S)**	**RISK FACTORS**	**FLOW AND TIMING**	**PATIENT SELECTION**	**INDEX TEST(S)**	**RISK FACTORS**
KIDNEY							
Agarwal 2010[16]	✖	?	?	✓	✖	✓	✓
Ahmadinejad 2013[21]	✓	?	?	✓	✓	✓	✓
Aydogan 2009[17]	?	?	?	✓	✓	✓	✓
Basoglu 2006[18]	?	?	?	✓	✓	✓	✓
Kim 2011[23,24]	✓	?	?	✓	✓	✓	✓
Kim 2013[22]	✓	?	?	✓	✓	✓	✓
Merino 2010[19]	?	?	?	✖	✖	?	✓
Shankar 2005[20]	✓	?	?	✓	✓	✓	✓
LIVER							
Benito 2002[25]	✓	?	?	?	✓	✓	✓
Casas 2011[28]	✖	?	?	✖	✖	✓	✓
Fabrega 2012[26]	✓	?	?	✖	✓	✓	✓
Lindemann 2009[30]	?	?	?	✓	✓	✓	✓
Manuel 2007[29]	✓	?	✓	✓	✓	✓	✓
Singh 2002[27]	✖	?	?	✓	✖	✓	✓
LUNG							
Caruso 2012[31]	?	?	?	?	✓	?	✓
Roman 2000[32]	?	?	?	✓	✓	✓	✓
MIXED							
Povitz 2010[33]	✖	✖	?	?	✖	✓	✓
Theodoropoulos 2012[34]	✓	✖	?	?	✓	✓	✓

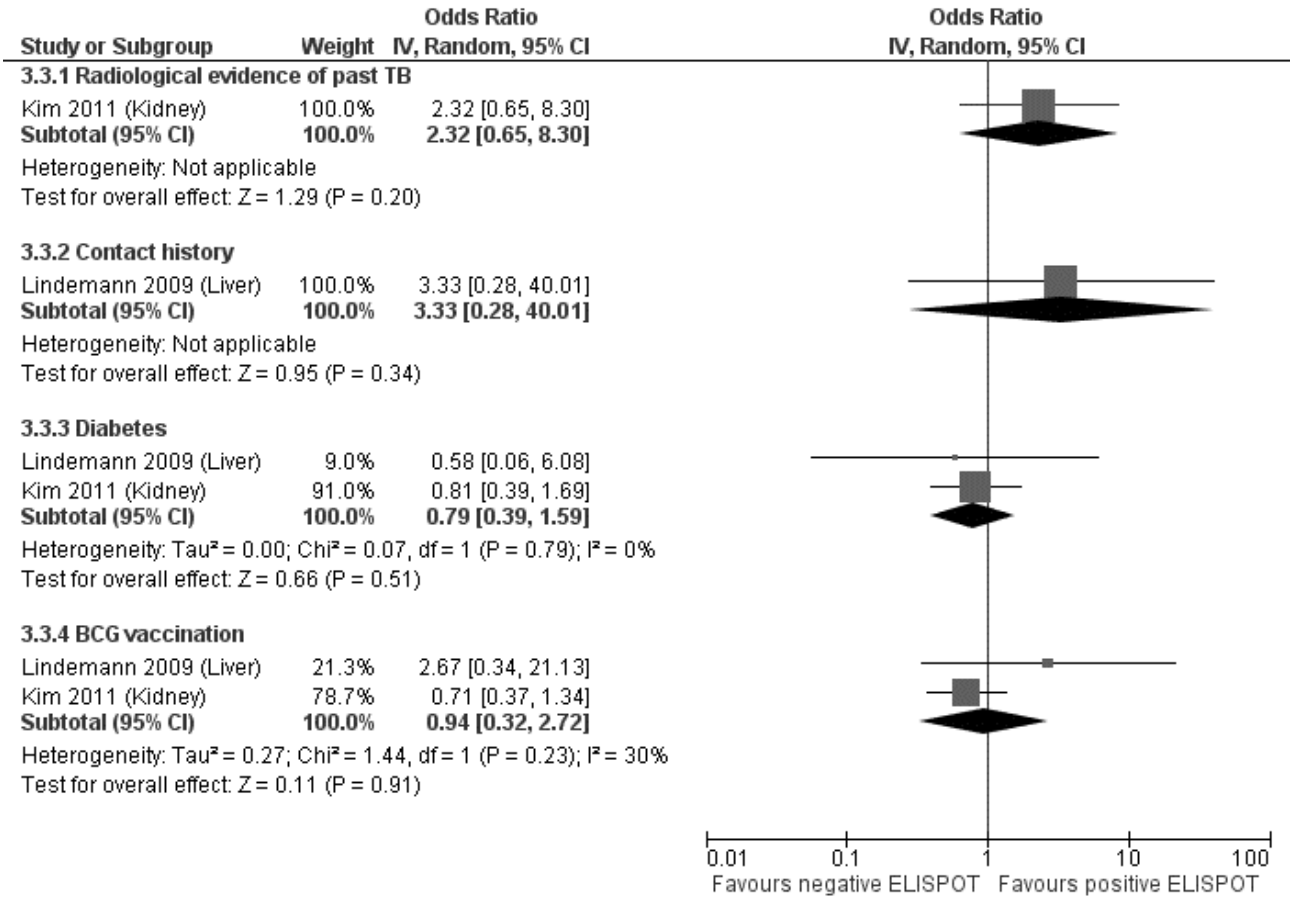
✓ Low risk of bias, ✖ High risk of bias,**?** Unclear risk of bias

Supplementary figure 3.1: Odds of positive ELISA-based IGRA in transplant candidates with risk factors for TB

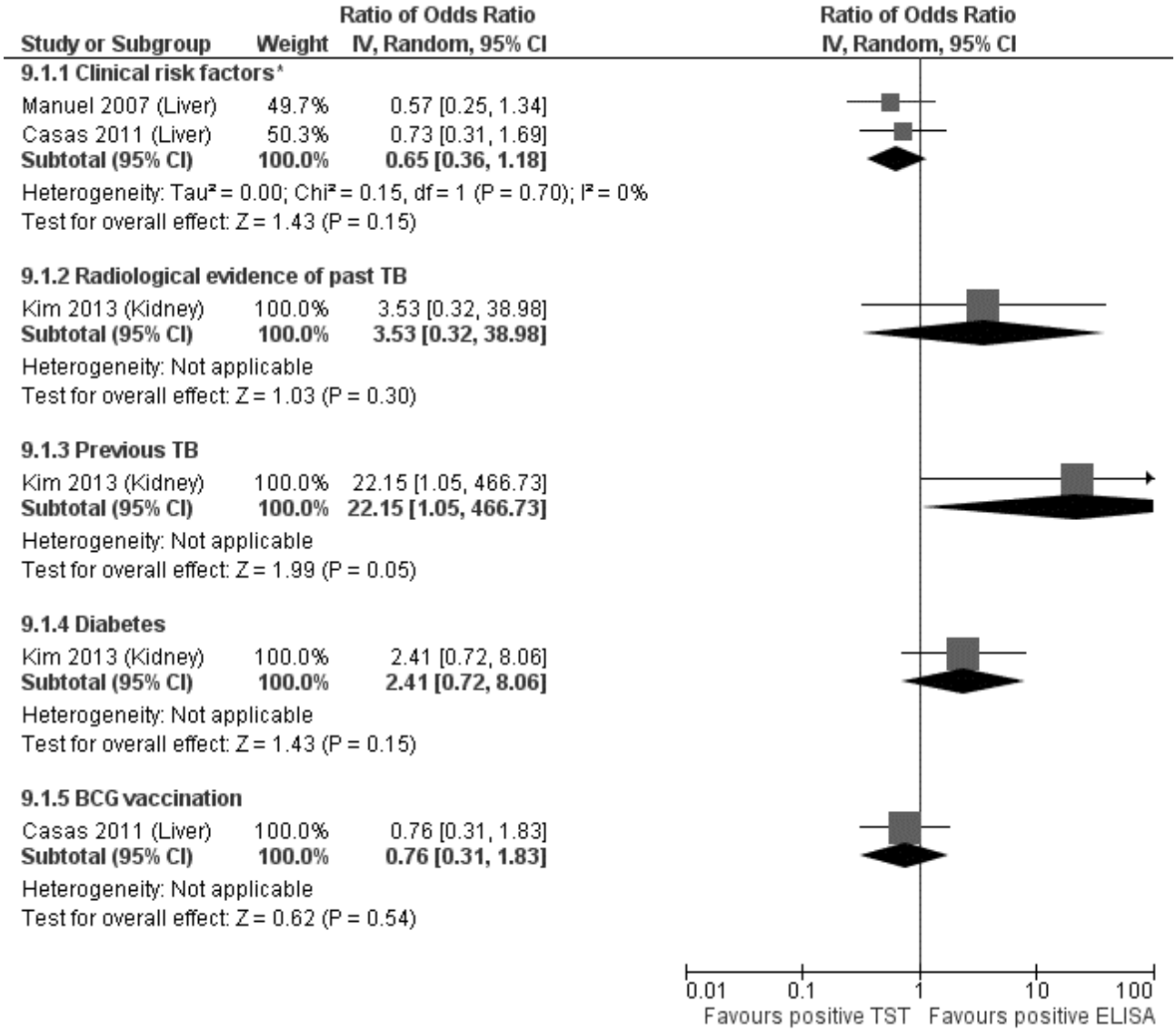


*Clinical risk factors as defined by study, IV, Random: inverse variance, random effects meta-regression, 95% CI: 95% confidence interval

Supplementary figure 3.2: Odds of positive ELISPOT-based IGRA in transplant candidates with risk factors for TB

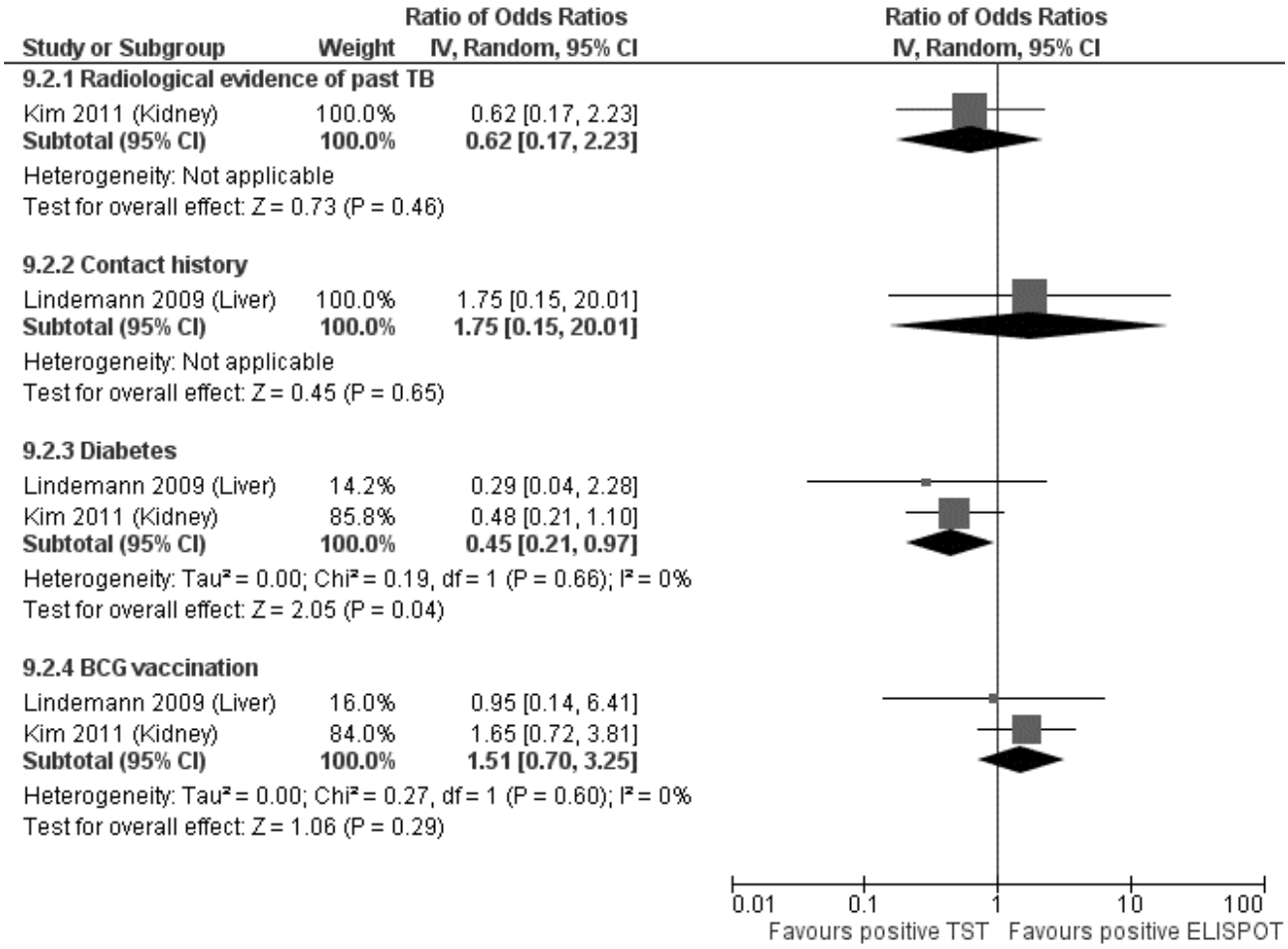


Supplementary figure 3.3: Relative performance of the TST and ELISA-based IGRA test



*Clinical risk factors as defined by study, IV, Random: inverse variance, random effects meta-regression, 95% CI: 95% confidence interval

Supplementary figure 3.4: Relative performance of the TST and ELISPOT-based IGRA test



CHAPTER 4: MISMATCH BETWEEN RISK OF TUBERCULOSIS AND TESTING PRACTICES IN PEOPLE BEING ASSESSED FOR KIDNEY TRANSPLANTATION

Author contributions

TER designed the study, obtained ethics approval, collected, managed and analysed the data, interpreted results, conducted all statistical analyses and wrote the manuscript.

RG assisted with locating eligible patients and provided access to hospital data systems, and resolved data discrepancies.

GR assisted with searching hospital databases.

JCC proved expert opinion on study methodology, contributed in the study design process, as well as the analysis and interpretation of results, and drafting and review of the manuscript.

ACW conceived the study idea, helped design the study and in obtaining ethics approval, participated in the analysis and interpretation of results, and assisted with drafting and reviewing the manuscript.

4.1: Abstract

Active tuberculosis (TB) is a dangerous complication of kidney transplantation. International guidelines recommend pre-transplant screening for latent TB, but little is known about the frequency of testing, or prevalence of risk factors among potential transplant recipients in Australia. We conducted a cross-sectional study of all patients who underwent kidney transplant assessment from 2011-2012 in a regional transplant centre in Australia. We assessed the prevalence of risk factors, as well as the frequency of testing, and the frequency of positive test results when tested. We collected data on patient characteristics, risk factors for TB, and tests from hospital records. Two-hundred and one patients underwent kidney transplant assessment over the 2 year study period. Patients had a mean age of 50.8 ± 12.6 years, 63.7% were male and 22.9% had been BCG vaccinated. The most frequent cause of kidney failure was diabetic nephropathy (29.4%). At least one risk factor for latent TB, other than chronic kidney disease, was present in 49.8% of patients. The most prevalent risk factors for latent TB were high-risk country of birth (29.4%), diabetes mellitus (27.4%), and prior immunosuppression (20.9%). Forty seven patients (23.4%) were tested for latent TB. Of patients with at least one risk factor for latent TB, only 37.0% were tested. Thirteen (35.1%) of the 37 people with risk factors for TB and were tested, returned a positive result. Despite a high prevalence of risk factors for TB in candidates for kidney transplantation, latent infection may be missed in transplant work-up due to under use of available tests.

4.2: Background

Active tuberculosis (TB) is a dangerous complication of kidney transplantation with a high mortality rate (17-40%)[1]. For the majority of transplant patients, active TB infection occurs from the reactivation of a latent infection acquired many years earlier. In Australia, candidates for kidney transplantation who are receiving dialysis are 10 times more likely to develop active TB than their general population counter parts[2]. After receiving a transplant, the risk of developing active TB increases further because of immunosuppression induced by anti-rejection drugs[3]. Chemoprophylaxis before transplant is effective in preventing active TB, but is also known to cause hepatoxicity, neurotoxicity and unpredictable drug-drug interactions with immunosuppressant therapy[4-7]. Using risk factor assessment and tests for latent TB, chemoprophylaxis can be targeted to transplant candidates at most risk of developing active disease, while sparing candidates at low risk from unnecessary complications of treatment[3,8].

In the absence of a gold-standard test for latent TB, clinical risk factors are a useful way of estimating the likelihood of TB infection and can be used in conjunction with test information to guide treatment. A recent review found that people with end-stage kidney disease who had clinical risk factors for latent TB were more likely to return positive interferon gamma release assays (IGRAs) and tuberculin skin tests (TST) than people without risk factors[9]. Specifically, patients were more likely to have a positive IGRA if they had radiological evidence of past TB or previous contact with active TB cases TB. Identifying groups of patients with risk factors for latent TB that are not identified under current routine clinical practices may decrease the morbidity and mortality associated with tuberculosis in kidney transplantation.

Current international guidelines recommend all transplant recipients are screened with the tuberculin skin test (TST) and/or an interferon gamma release assay (IGRA) prior to transplant[1]. In Australia, the actual frequency of pre-transplant screening, the types of tests used and the characteristics of patients who receive screening is unknown. With this study, we aimed to describe the risk profile of latent TB in patients undergoing kidney transplant assessment and the testing practices used by clinicians to investigate this disease.

4.3 Methods

Patient population

Ethics approval to conduct this study was granted by Western Sydney Local Health District Human Research Committee (LNR/13/WMEAD/120). We retrospectively enrolled all patients who attended our transplant clinic between January 2011 and December 2012. Our transplant clinic is a major regional centre for kidney transplant in Australia. Patients on our active transplant waiting list return to the clinic every two years for review. A two-year sample was chosen to capture all patients currently active on the waiting list as well as new patients undergoing transplant assessment for the first time.

Medical records search

Two investigators searched electronic and paper hospital records for relevant data. We collected data on patient characteristics, including age, sex, aetiology of renal disease, time on dialysis and number of previous transplants. We also collected data on risk and protective factors for latent TB, including HIV status, country of birth, diabetes, malignancy, previous TB disease, radiological evidence of TB, contact with active TB, previous treatment with immunosuppression, smoking and BCG vaccination. Refer to *Supplementary Table 4.1* for definitions of risk factors. Additionally, we recorded the results of tests for latent TB, including the tuberculin skin test, interferon gamma release assays and chest X-ray. Patients were not asked to complete any tasks or provide additional information. A full list of extracted data is available in *Supplementary Table 4.2.*

Statistical analysis

All statistical analyses were conducted in STATA 11.0 (Texas, USA). We calculated the prevalence of risk factors and frequency of testing for latent TB as simple proportions. All continuous variables are reported with mean, standard deviation and range.

4.4: Results

Population characteristics

Two hundred and forty-two participants were enrolled in the study, but complete records were only available for 201 patients. Characteristics of the study participants are summarised in *Table 4.1*. Study participants were mostly male (128, 63.7%), had mean age of 50.5 ± 12.6 years (range 21.0-73.2) and had been on dialysis for a mean of 3.9 ± 3.0 years. Candidates who had at least one prior kidney transplant accounted for 13.9% of the total study population. Less than half of all participants smoked (91, 45.3%) and approximately one quarter (46, 22.9%) were BCG vaccinated. The most common aetiology of end stage kidney disease was diabetic nephropathy (59, 29.4%), followed by IgA nephropathy (33, 16.4%) and polycystic kidney disease (19, 9.5%).

Prevalence of risk factors for tuberculosis

We observed a high prevalence of risk factors for latent TB in the study group (see *Table 4.2*). Overall, 101 (49.8%) of participants had at least one risk factor for latent TB. Of the 101 patients with risk factors for TB, 68 (67.3%) had one risk factor, 22 (21.7%) and two risk factors, and 10 (10.9%) had three or more risk factors in combination. The most common risk factors for latent TB were a high-risk country of birth 59 (29.4%), insulin-dependent diabetes mellitus (55, 27.4%) and previous immunosuppressive drug use (42, 20.9%). We also identified 16 patients who had a history of cancer, 12 patients with radiological evidence of past TB, nine patients who had been treated for TB in the past, and six patients who had a history of contact with active cases of TB. In two patients who were previously transplanted, the donors had a history of TB. One patient had occupational exposure to TB.

Frequency of testing

Forty-seven patients (23.4%) were tested for latent TB. Forty patients were tested using an interferon-gamma release assay (QuantiFERON Gold In-Tube), seven patients had a TST, and three patients had both an IGRA and a TST. Of the 47 patients tested, 14 were positive for latent TB, 26 were negative, and for seven patients the test was either indeterminate, ordered but never completed, or the test

result was not recorded. There was no disagreement between the IGRA and TST in patients that received both tests.

Of the 100 candidates with at least one risk factor for TB, only 36 (36%) were tested for latent TB. The frequency of testing across risk factors was inconsistent. Despite being the most common risk factor for TB, people who were from a high-risk country of birth, had diabetes, or been taking immunosuppressive drugs were the least likely to be tested (see *Table 4.2)*. The highest rates of testing were observed in people with a history of TB treatment (100%), people who had contact with cases of active TB (83%) and people with radiological evidence of past TB (75%).

Frequency of positive tests

Positive test results were more common in kidney transplant candidates with risk factors for TB. Of the 14 positive test results, 13 occurred in candidates with one or more risk factors. We observed a trend of increasing likelihood of test positivity with increasing number of risk factors (see *Figure 4.1*). Of the 37 participants who both received a test for latent TB and had at least one risk factor for TB, 13 (36.1%) tested positive. The highest rate of test positivity were observed in participants with a history of TB treatment (77.8%), see *Table 4.2*.

4.5: Discussion

Despite a high prevalence of risk factors for TB amongst kidney transplant candidates, we observed a low frequency of testing for latent TB in this transplant centre. A recent Australian linking transplant and mandatory TB reporting databases identified 37 cases of active TB in 14,506 dialysis patients between 2001 and 2006, corresponding to an incidence of 66.8 cases per 100,000 persons[2]. This rate was over 10 times higher than the general Australian population, and mortality was also higher. Given that only a small fraction (5-10%) of people with latent TB go on to develop active TB, this data suggests a significant reservoir of latent TB may exist in candidates for kidney transplant in Australia. Current international guidelines recommend that all transplant recipients should be screened for latent TB, and preferably prior to transplant. At our centre however, only 23.4% of all candidates for transplant were tested, and only 37% of candidates with risk factors for TB were tested. The low frequency of testing for latent TB we observed at this centre highlights missed opportunities for interventions and prevention of active disease, and an unawareness of international guidelines among staff.

Interestingly, the likelihood of testing varied across risk factors for TB. All patients with a history of prior TB underwent testing, but only 52.5% of candidates with a high-risk country of birth were tested. This suggests that some clinicians may not recognise certain risk factors for TB as readily as others, or place different magnitudes of importance on risk factors in their decision to order a test. Clinicians were also more likely to order a test when a patient presented with two or more risk factors for TB, indicating that a clinicians' decision to test was influenced by not only the type of risk factors, but also how many occurred in combination. Evaluating the risk of TB in potential transplant recipients is a complex task. Clinicians may benefit from a diagnostic algorithm for combining risk factor information into a finite measure of risk.

Whilst not always practical, pre-transplant screening and treatment is advantageous as the risk of TB reactivation increases after transplant and symptoms of active TB can often be nonspecific and difficult diagnose. This study found a high rate of test positivity amongst patients with who had prior TB therapy (80%), indicating that tests for latent TB could be useful tools for identifying

individuals at high risk of TB. This finding is congruent with a recent systematic review of tests for latent TB in people with end stage kidney disease, which found that patients with a medical history of TB were six times more likely to test positive on an interferon gamma release assay and two times more likely to test positive on a tuberculin skin test[9].

While the generalisability of our results are limited by our small sample size and single centre setting, the data presented here reflect the experiences of a large kidney transplant centre and may indicate a larger problem in TB management across other transplant centres in Australia. This study highlights the need for nation-wide evidence-based TB screening protocols, as well as programs for education and training in the management of TB. We recommend that all kidney transplant clinics evaluate their current TB screening practices and where necessary incorporate current international guidelines on latent TB screening into their pre-transplant assessment protocols.

4.6: References

1. Aguado J, Torre-Cisneros J, Fortun J, et al. Tuberculosis in solid-organ transplant recipients: Consensus statement of the Group for the Study of Infection in transplant recipients (GESITRA) of the Spanish society of infectious diseases and clinical microbiology. *Clinical Infectious Diseases.* 2009;48(9):1276-1284.
2. Dobler CC, McDonald SP, Marks GB. Risk of tuberculosis in dialysis patients: a nationwide cohort study. *PLoS ONE.* 2011;6(12):e29563.
3. Bumbacea D, Arend SM, Eyuboglu F, et al. The risk of tuberculosis in transplant candidates and recipients: A TBNET consensus statement. *European Respiratory Journal.* 2012;40(4):990-1013.
4. Blumberg HM, Burman WJ, Chaisson RE, et al. American Thoracic Society/Centers for Disease Control and Prevention/Infectious Diseases Society of America: treatment of tuberculosis. *Am J Respir Crit Care Med.* 2003;167(4):603-662.
5. Thompson NP, Caplin ME, Hamilton MI, et al. Anti-tuberculosis medication and the liver: dangers and recommendations in management. *European Respiratory Journal.* 1995;8(8):1384-1388.
6. Niemi M, Backman JT, Fromm MF, Neuvonen PJ, Kivisto KT. Pharmacokinetic interactions with rifampicin : clinical relevance. *Clin Pharmacokinet.* 2003;42(9):819-850.
7. Sousa M, Pozniak A, Boffito M. Pharmacokinetics and pharmacodynamics of drug interactions involving rifampicin, rifabutin and antimalarial drugs. *J Antimicrob Chemother.* 2008;62(5):872-878.
8. Gupta KB. Challenges in diagnosis and treatment of latent tuberculosis infection. *Indian Journal of Tuberculosis.* 2012;59(1):1-5.
9. Rogerson TE, Chen S, Kok J, et al. Tests for latent tuberculosis in people with ESRD: a systematic review. *American Journal of Kidney Diseases.* 2013;61(1):33-43.

Figure 4.1: Likelihood of positive latent TB test increases with number of risk factors

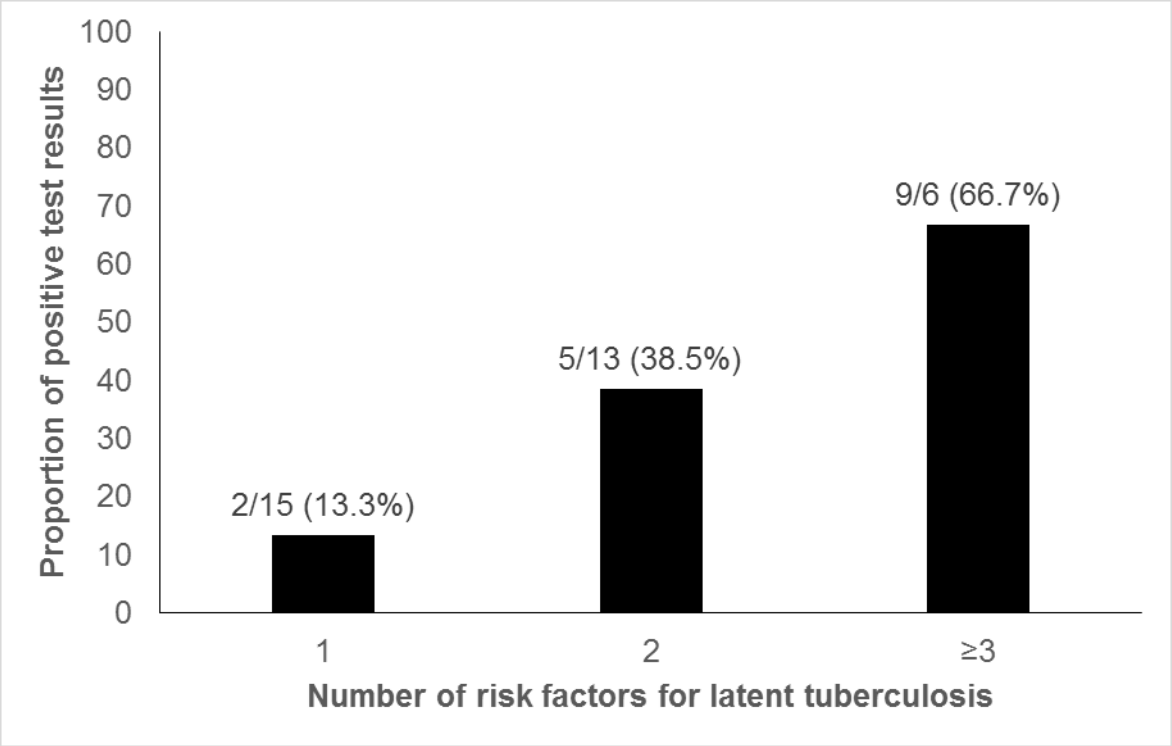


Table 4.1: Characteristics of kidney transplant candidates

Characteristic	**N (%)**
Total	201 (100.0)
Males	128 (63.7)
Age (mean ± SD, years)	50.5 ± 12.6
≤ 30	15 (7.5)
31-40	35 (17.4)
41-50	39 (19.4)
51-60	53 (26.7)
≥ 61	59 (29.4)
BCG vaccinated	46 (22.9)
BMI (mean ± SD)	27.8 ± 5.1
Ever smoked	91 (45.3)
Diabetes mellitus	68 (33.8)
Insulin dependent	55 (27.4)
Non-insulin dependent	13 (6.5)
Prior kidney transplant	28 (13.9)
Time on dialysis (mean ± SD, years)	3.9 ± 3.0
Infectious diseases	
Human immunodeficiency virus	0 (0.0)
Hepatitis B (Chronic)*	1 (<1.0)
Hepatitis C†	3 (1.0)
Aetiology of ESKD	
Diabetic nephropathy	59 (29.4)
IgA nephropathy	33 (16.4)
Polycystic kidney disease	19 (9.5)
Focal segmental glomerulosclerosis	15 (7.5)
Reflux nephropathy	9 (4.5)
Other‡	49 (24.4)
Unknown	17 (8.5)

***** HBV surface Ag detected, HBV core Ab reactive

†HCV antibody reactive, HCV RNA detected

‡Posterior urethral valves disease, trauma, amyloidosis, urosepsis, congenital defects, hypertension, glomerulonephritis, medullary cystic disease, drug-induced nephropathy, membranous glomerulonephritis, Wegener's granulomatosis, obstructive uropathy

Table 4.2: Prevalence of risk factors for TB in kidney transplant candidates

Number of risk factors	**N (%)** **Total = 201**	**Tested (%)** **Total = 47**	**Test positive (%)** **Total = 14**
0	101 (50.2)	10 (21.3)	1 (7.1)
1	68 (33.8)	15 (32.0)	2 (14.3)
2	22 (10.9)	13 (27.6)	5 (35.7)
≥3	10 (5.0)	9 (19.1)	6 (42.9)
Risk factors history			
Any risk factor	100 (49.8)	37 (78.7)	13 (93.0)
High risk country of birth	59 (29.4)	31 (66.0)	13 (93.0)
Insulin dependent diabetes	55 (27.4)	13 (27.6)	2 (14.3)
Immunosuppressive drugs	42 (20.9)	13 (27.6)	5 (35.7)
Malignancy	16 (8.0)	4 (8.5)	2 (14.3)
Old TB on CXR	12 (6.0)	8 (17.0)	4 (28.6)
Prior TB therapy	9 (4.5)	9 (19.1)	7 (50.0)
Contact history	6 (3.0)	5 (10.6)	2 (14.3)
Other*	4 (2.0)	3 (6.4)	1 (7.1)

Supplementary table 4.1: Definitions of risk factors for latent tuberculosis

Risk factor	Definition
High risk country of birth	A participant born in a country with 50 or more cases of tuberculosis per 100,000 persons per year. Prevalence of tuberculosis was estimated from World Health Organisation data.
Contact history	A participant or clinician report of contact with an individual with confirmed or suspected active tuberculosis.
Radiological evidence of past tuberculosis	Radiological evidence of old tuberculosis on chest x-ray or computed tomography scan, including pulmonary nodules in the hilar or upper lobes of the lung, pleural scarring or volume loss.
Prior anti-tuberculosis therapy	A participant or clinician report of prior anti-tuberculosis therapy, including ethambutol, isoniazid, rifampicin or pyrazinamide, or a combination thereof.
Diabetes mellitus	A participant with either insulin or non-insulin dependent diabetes mellitus. We excluded participants with gestational diabetes mellitus.
Prior immunosuppression therapy	A participant who had previously received any immunosuppressive therapy prior to transplantation, including glucocorticoids, cytostatics, antibodies, immunophilins (ciclosporin, tacrolimus, sirolimus), interferons, tumour necrosis factor binding agents and mycophenolate.
Malignancy	A participant with a history of any malignant cancer except basal and squamous cell carcinomas.

Supplementary table 4.2: Summary of data extracted from patient records

Variable	Data collected
Patient characteristics	
Date of last transplant review	Day/ month/ year
Transplant type	Deceased donor/ living related donor/ living unrelated
Suitability for transplant	Yes/ no/ marginal
Etiology of renal failure	Disease
Age	Years
Sex	Male/ female
Height	Meters
Weight	Kilograms
Dialysis and kidney transplant history	
Time on dialysis	Months
Number of previous kidney transplants	1/ 2/ ≥3
Type of previous transplant	Deceased donor/ living related donor/ living unrelated
Time since previous transplant	Months
Time since previous transplant failure	Months
Risk factors for tuberculosis	
Diabetes	Non diabetic, insulin dependent diabetic, non-insulin dependent diabetic
Smoking	Current, previous, never
Human immunodeficiency virus	Positive/ negative
Hepatitis C	Positive/ negative
Hepatitis B profile	Surface antigen/ surface antibody/ core antibody/ e-antigen/ e-antibody)
Cancer profile	Type of malignancy/ time since last malignancy
Country of birth	Country, prevalence of tuberculosis
Bacillus Calmette-Guerin vaccination	Vaccinated/ non vaccinated
Tuberculosis treatment history	Time since treatment/ drugs prescribed
Tuberculosis contact history	Time since last tuberculosis contact/

	proximity/ time
Prior immunosuppressant therapy	Yes/ no/ drugs used
Tests for tuberculosis	
Tuberculin Skin Test (date, result)	Date/ result
QuantiFERON (date, result)	Date/ result
TSPOT.TB (date, result)	Date/ result
Chest X-ray (date, result)	Date/ result
Chest computed tomography scan (date, result)	Date/ result
Sputum culture (date, result)	Date/ result
Mycobacterium nucleic acid test (date, result)	Date/ result
Ziehl-Neelsen stain (date, result)	Date/ result

CHAPTER 5: DISCUSSION

The cross-sectional analytic study presented in *Chapter 2* examined the performance of fourteen methodological filters for diagnostic studies. We found that while methodological filters generally lacked the sensitivity for systematic review purposes, some filters could be useful in clinical setting to reduce the volume of search results. The surprise finding of this study was that the current 'specific' clinical queries limit for diagnosis (used in PubMed and Ovid SP) missed up to 80% of studies in nephrology journals. Other filters (Deville 2000 Broad, Deville 2000 Balanced, Haynes 2004 Balanced, and Vincent 2003 Narrow) had similar specificity to the 'specific' clinical queries limit, but identified a greater proportion of the total evidence. When systematic reviews are not available, clinicians need fast access to primary studies from the literature. Our findings in *Chapter 2* will help clinicians in the kidney transplant specialty to access information about diagnostic tests more efficiently, improving their utilisation of evidence-based medicine, and ultimately improving outcomes for patients. A limitation of this study was the imprecision around our estimates of filter sensitivity, which arose because of the low prevalence of diagnostic test accuracy studies in the literature. In the future we plan to extend this study to additional nephrology and transplant journals, as this will allow us to both validate our findings, and derive more precise estimates of filter sensitivity. Additionally, we also wish to extend this research to assess the quality of diagnostic studies identified by filters.

In *Chapter 3* we presented the results of a systematic review of test for latent tuberculosis in the solid organ transplant setting. The primary challenge of this review was to use novel methodologies for the meta-analysis of data from studies of tests with no reference standards. The conventional approach to the assessment of diagnostic test accuracy is to compare the results of a new test against a reference standard, and to use this data to derive estimates of test sensitivity, specificity and precision. For latent TB however, there is no reference standard test, and therefore standard methodologies fail.

In our earlier work on a systematic review of tests for latent TB in people with end-stage kidney disease, we identified studies in the literature that reported the results of tests for latent TB stratified by risk factors for TB. We were able to use

this data to generate estimates of test performance, as the odds of a positive test in the presence of clinical risk factors for TB, and conduct a meta-analysis.

Applying these same methods to the solid organ transplant population, the systematic review presented in *Chapter 3* found that people undergoing solid organ transplant, were more likely to have a positive interferon gamma release assays (IGRAs) and tuberculin skin tests (TST) if they had radiological evidence of past TB, a history of contact with past TB, or previous TB treatment. Interestingly, when we compared our results to our earlier work in people with end-stage kidney disease, we found that the odds of test positivity with risk factors were very similar in magnitude. Contrary to our original hypothesis, this suggests IGRAs and the TST may perform similarly in both the solid organ transplantation and end stage kidney disease populations.

Another finding of our systematic review was little evidence exists on the relative performance of IGRAs and the TST. Current available data was inadequate to determine whether IGRAs perform better, worse or the same as the TST. The current international guidelines for screening solid organ transplant recipients for latent TB recommend using either the TST or IGRA, or both in combination. Our findings support these guidelines and until new evidence emerges, no further clinical recommendations can be made. To address this uncertainty, we are planning a large study that will compare the performance of IGRAs and the TST against a comprehensive risk factor assessment in people undergoing kidney transplantation. This study will be conducted across two major centres for transplant in Australia and will include both ELISA- and ELISPOT-based IGRAs. Another future line of investigation will be to determine whether the testing for latent TB actually improves outcomes for people undergoing transplant. It is unclear at present whether cases of latent TB identified by tests would have ever progressed to active TB without prophylactic treatment. A randomised controlled trial of test-directed treatment would be of value for assessing the overall impact of testing programs on patient outcomes.

The cross-sectional descriptive study presented in *Chapter 4 was* conducted to evaluate the risk of latent tuberculosis in the Australia transplant setting, and to assess the adequacy of current screening practices. We searched the hospital records of 201 candidates for kidney transplant at our centre and found that

approximately half of all patients had at least one risk factor for latent TB. The most common risk factors for latent TB were a high-risk country of birth, diabetes and prior immunosuppression therapy. Surprisingly, despite the high prevalence of risk factors, less than a quarter of candidates were screened for latent TB before transplant. More concerning, was that only 36% of the 101 patient with risk factors for TB were tested. We also found that clinicians were more likely to order a test for latent TB when candidates had two or more risk factors for TB in combination, and when candidates had a history of contact with active TB or prior chemoprophylaxis. This study demonstrates that that candidates for kidney transplant are at increased risk of tuberculosis and highlights the need for a nation-wide TB screening protocol in work-up for transplant.

The ultimate aim of this body of work was to (1) improve the availability of diagnostic evidence to clinicians, (2) provide an unbiased synthesis of the evidence on tests for latent TB in transplantation, and (3) to increase our understanding of the risk latent tuberculosis in the Australian transplant setting. Our findings will enable clinicians to search MEDLINE quickly and efficiently for diagnostic accuracy studies, make evidence based decisions about the results of tests for latent TB, and have a greater understanding of the epidemiology of latent TB in candidates for kidney transplant. Combined, we hope that this information can be used to reduce the morbidity and mortality associated with TB in transplantation.

APPENDIX 1: TESTS FOR LATENT TUBERCULOSIS IN PEOPLE WITH END STAGE KIDNEY DISEASE: A SYSTEMATIC REVIEW AND META-ANALYSIS

AJKD
Original Investigation

Tests for Latent Tuberculosis in People With ESRD: A Systematic Review

Thomas E. Rogerson, BSc,[1,2] Sharon Chen, MBBS, PhD,[3] Jen Kok, MBBS,[3] Andrew Hayen, BA, MM(Biostat), PhD,[2] Jonathan C. Craig, MBBS, PhD,[1,2] Kamal Sud, MBBS,[3] Kathy Kable, BASci, MN,[3] and Angela C. Webster, MBBS, MM(Clin Epid), PhD[1,2,3]

Background: The relative diagnostic accuracy of interferon γ release assays (IGRAs; based on ELISA [enzyme-linked immunosorbent assay] or ELISPOT [enzyme-linked immunosorbent spot], ie, the QuantiFERON and T-SPOT.*TB* tests, respectively) and the tuberculin skin test (TST) for latent tuberculosis (TB) infection in people with end-stage kidney disease is uncertain and national guidelines for their use are inconsistent.

Study Design: Systematic review.

Selection Criteria for Studies: Evaluated performance of tests for latent TB with clinical risk-factor assessment.

Setting & Population: People with end-stage kidney disease (chronic kidney disease stage 5 [eGFR <15] or kidney transplant recipients). No limits on setting.

Index Tests: ELISA- or ELISPOT-based IGRAs, TST, assays to detect antimycobacterial antibodies, and flow cytometry–based tests.

Outcomes: Odds of test positivity with clinical risk factor for latent TB, expressed as ORs and relative ORs (RORs).

Results: 47 studies (6,828 participants) were included, but only 30 studies (4,546 participants) contained sufficient data to contribute to meta-analysis. Studies were predominately in the dialysis population (23/30; 3,700 participants) in countries with low to moderate TB prevalence (0.0-50.0 cases/10^5 persons). BCG vaccination rate was variable (2.7%-100.0%). 9 studies compared IGRAs with the TST directly, 17 studies evaluated the TST only, and the other 4 studies evaluated other tests. Compared to a positive TST result, a positive ELISA-based IGRA result was associated more strongly with radiologic evidence of past TB (ROR, 4.29; 95% CI, 1.83-10.3; $P = 0.001$) and contact with active TB (ROR, 3.36; 95% CI, 1.61-7.01; $P = 0.001$). Compared to a negative TST result, a negative ELISA-based IGRA result was associated more strongly with BCG vaccination (ROR, 0.30; 95% CI, 0.14-0.63; $P = 0.002$). There were insufficient data to compare performance of the ELISPOT-based IGRA with the TST or ELISA-based IGRA.

Limitations: 17 of 47 included studies (36.2%) did not contain sufficient data to contribute to meta-analysis.

Conclusions: Compared to the TST, the ELISA-based IGRA was associated more strongly with risk factors for latent TB in end-stage kidney disease.

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INDEX WORDS: Latent tuberculosis; tuberculin skin test; QuantiFERON; T-SPOT.*TB*; end-stage kidney disease; systematic review; dialysis; transplantation.

Uremia in end-stage kidney disease contributes to generalized immune dysfunction that results in increased susceptibility to infectious diseases, including tuberculosis (TB).[1,2] Individuals with end-stage kidney disease are up to 50 times more likely to develop active TB than the general population, and mortality is high, between 17% and 75%.[3] A recent study in Australia reported an incidence of active TB in people on dialysis therapy of 66.8 cases/100,000 persons per year and an adjusted relative risk of 7.87 compared with the general population.[4] Accurate and timely diagnosis and treatment of latent TB is key to preventing active disease, but is hampered by limitations in gold-standard diagnostic tests for determining true latent TB status.[5] Although prophylaxis with anti-TB medications is effective in preventing active disease, anti-TB medications also are associated with hepatitis, neurotoxicity, and significant drug-drug interactions.[6-9] Screening patients for latent TB can be used to target prophylaxis to patients at the highest risk of developing active TB while avoiding unnecessary complications of treatment in low-risk individuals.

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Until recently, the detection of latent TB has relied on the tuberculin skin test (TST). Utility of the TST in clinical practice is limited by poor sensitivity in immunocompromised people and poor specificity in BCG-vaccinated people.[10] An alternative to the TST are interferon γ release assays (IGRAs), in which interferon γ (an indicator of antimycobacterial effector T cells) can be assayed by ELISA (enzyme-linked immunosorbent assay; eg, the QuantiFERON tests [Cellestis] or ELISPOT (enzyme-linked immunosorbent spot; eg, the T-SPOT.*TB* [Oxford Immunotec]). These in vitro assays measure the response of sensitized T cells to mycobacterial antigens (early secretory antigenic target 6 [ESAT-6] and culture filtrate protein 10) in whole blood.

National guidelines for the diagnosis of latent TB are inconsistent.[11,12] Some guidelines offer no specific recommendation for test use,[13] some propose that IGRAs should be used after a negative TST result,[14,15] and others recommend that IGRAs replace the TST**.** No specific guidelines exist for the end-stage kidney disease population; however, current recommendations from the United Kingdom indicate the use of IGRAs with or without a TST in people with chronic kidney disease.[16] The few guidelines that exist for immunosuppressed populations (excluding human immunodeficiency virus [HIV] populations) also are conflicting. Canadian guidelines for immunosuppressed persons recommend using the TST with or without a supplementary IGRA,[14] whereas the United Kingdom and Switzerland recommend replacing the TST with an IGRA.[17,18]

Determining the diagnostic accuracy of IGRAs in end-stage kidney disease using epidemiologic first principles is problematic because the existing standard test (TST) performs poorly and therefore makes direct comparison invalid. Previous systematic reviews of IGRA test performance are limited to the general population and most use 2 separate populations of people to estimate sensitivity (a population including only active TB cases) and specificity (a population of healthy low-risk people).[19-21] An alternative approach is to measure the association of test positivity with medical evidence of TB infection and epidemiologic risk factors.[22] A pretest clinical risk assessment encompassing a person's risk of exposure, other comorbid conditions, and radiologic imaging may help interpret the validity of a positive or negative result. A test that is both sensitive and specific for latent TB should have test positivity closely associated with risk factors such as old TB on chest radiograph, previous treatment for active TB, contact with an active case of TB, high-risk nationality, and immunosuppression (other than uremic related).

Given the paucity of evidence-based guidance for clinical decision making, we aimed to systematically review all studies that assessed the association of TST or IGRA results with clinical risk factors for latent TB in people with end-stage kidney disease.

METHODS

Inclusion/Exclusion Criteria

We included all studies in any language that reported the performance of any diagnostic test for latent TB in conjunction with either medical evidence or clinical risk factors in adults or children with end-stage kidney disease. We excluded studies that included patients with end-stage kidney disease with active TB at the time of testing when data could not be separated from patients without active TB.

Search Strategy

We searched MEDLINE and EMBASE from inception to October 2010. Articles were located using a search strategy composed of 3 filters, the first for test terms, the second for people with end-stage kidney disease, and the third for TB terms. The full search strategy is shown in Table S1 (available as online supplementary material). We also searched conference proceedings, including Australian Society for Microbiology 2005-2010, Australian Society for Infectious Diseases 2007-2010, Infectious Diseases Society of America 2007-2010, Interscience Conference on Antimicrobial Agents and Chemotherapy 2005-2010, European Congress of Clinical Microbiology and Infectious Diseases 2005-2010, American Society for Microbiology 2010, and International Congress on Infectious Diseases 2008-2010. The search was conducted by hand, or when electronic copies were available, we searched on the following text terms: tuberculosis, interferon, QuantiFERON, QFT, TSPOT.*TB*, ELISPOT, tuberculin skin test, TST, haemodialysis, and hemodialysis.

Data Abstraction

Data were abstracted from studies by 3 investigators working independently, using standardized data abstraction forms. We collected data for study setting and design, participant characteristics, risk factors for latent TB, test details, and test results. We investigated both medical and epidemiologic risk factors for latent TB (Table S2). Medical risk factors included a positive chest radiograph for past TB, previous active TB or prophylactic treatment for TB, and any iatrogenic or disease-related immunosuppression (other than uremia related). Epidemiologic risk factors included nationality and contact with a person with active TB (documented by a health care professional or self-reported). We also investigated BCG vaccination status as a protective factor. Study quality was assessed with an adapted version of the QUADAS (Quality Assessment of Diagnostic Accuracy Studies) tool (Table S3).[23] This tool consists of 14 methodological items that assess study validity in terms of appropriateness of patient spectrum and reference standard, bias, test execution, loss to follow-up, and indeterminate results.

Statistical Analysis

Study setting, patient characteristics, and QUADAS tool data were summarized descriptively. When sufficient data were available, we constructed 2×2 tables and calculated odds ratios (ORs; with 95% confidence intervals [CIs]) for test positivity with each risk factor. When all patients in a study had a risk

factor or all patients with a risk factor had the same result, we added 0.5 to each cell of the 2×2 table to calculate the OR.[24] In studies that assessed 2 or more tests in the same population, we compared the association of test positivity with risk factor between tests as a relative diagnostic OR (relative OR [ROR] with 95% CIs). Variance was calculated using a previously published method that assumes a correlation between tests of 0.5, producing conservative estimates.[22] Statistical significance was tested with a Wald test and reported as a *P* value. Forest plots and summary estimates for ORs and RORs were generated in STATA 11.2 (StataCorp, www.stata.com) using a random-effects model weighted by inverse variance. Between-study heterogeneity was assessed using the I^2 statistic, which reports the percentage of variation across studies that is due to true heterogeneity rather than chance.

Sensitivity Analysis

To test the robustness of our results against interstudy heterogeneity, we conducted sensitivity analyses using random-effects meta-regression. Specifically, we compared studies of dialysis patients alone versus transplantation/mixed populations of dialysis and transplantation patients, studies that used blinding of test interpretation to other test results versus those that did not use blinded interpretation, studies that used a TST cutoff of 5 versus 10 mm, and studies that used second- versus third-generation QuantiFERON tests.

RESULTS

Studies Identified

Our search identified 949 potential citations: 937 citations were identified in electronic databases, 9 citations were identified in conference proceedings, 1 citation came from reference list searches, and 1 citation came from an expert in the field (Fig 1). In total, 47 studies (6,828 participants) were included; however, only 30 studies (4,546 participants) contained sufficient data to contribute to meta-analysis.

Characteristics of included studies that contributed to meta-analysis are listed in Table 1. In general, studies were conducted mostly in dialysis patients (23/30 [76.7%]) and in countries with low (≤5 cases/100,000) to moderate (≤50 cases/100,000) TB prevalence.[25] The study setting was primarily outpatient dialysis clinics and all studies were prospective. Two studies (306 participants) were conducted as contact investigations in response to possible TB exposure.[26,27] Seventeen studies (2,903 participants) evaluated the TST only, 9 studies (1,126 participants)

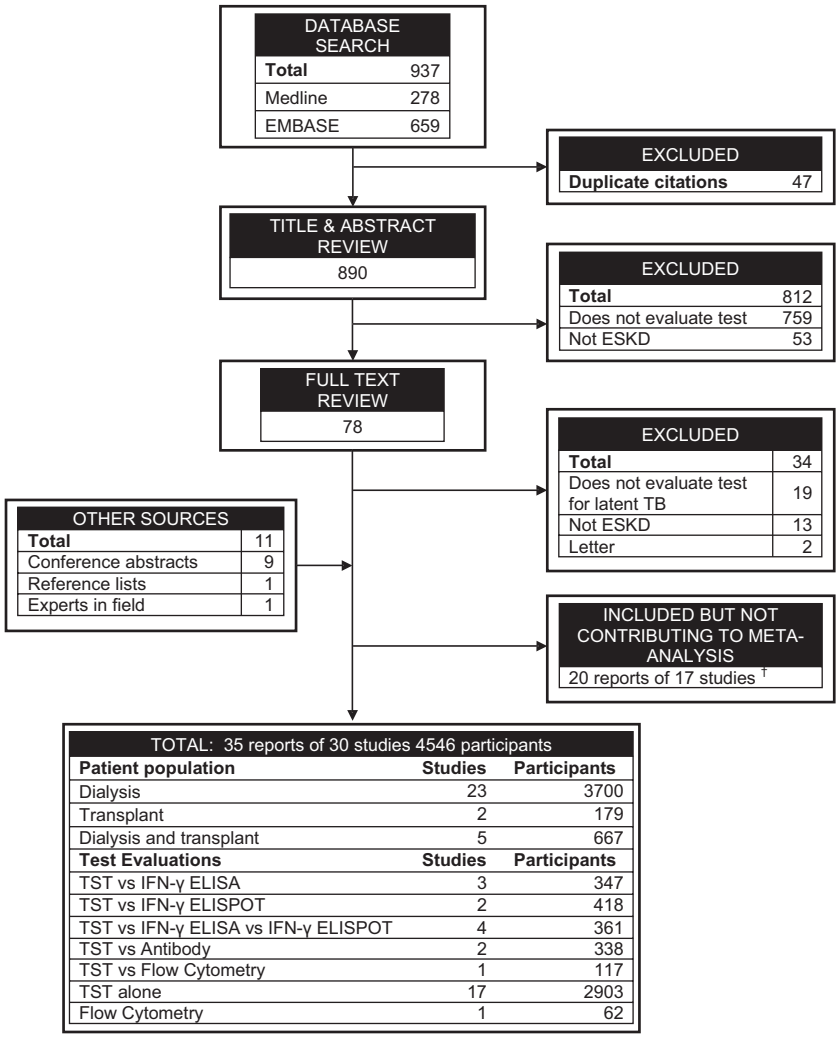

Figure 1. Results of the literature search for studies reporting test performance and risk-factor assessment for latent tuberculosis (TB) in people with end stage kidney disease (ESKD). Abbreviations: ELISA, enzyme-linked immunosorbent assay; ELISPOT, enzyme-linked immunosorbent spot; IFN-γ, interferon γ; TST, tuberculin skin test. †See Table S4 for study details.

Table 1. Characteristics of Included Studies

Study	Test Evaluation	Country	No.	Male Sex	Age (y)	ESKD Treatment (mo)	BCG Vaccinated (%)	TB Prevalence[a]
Dialysis Population								
Inoue et al[28] (2009)	TST vs QFT	JP	154	97 (59.9)	65.4 ± 65	42.4 ± NR	NR[b]	74.6 (8.3-46.0)
Lee et al[29,30] (2010)	TST vs QFT	TW	93	35 (37.6)	58.3 ± 14.9	72.0	61.3	137.0 (56.0-225.0)[c]
Seyhan et al[31] (2010)	TST vs QFT	TR	100	47 (47.0)	56.2 ± 15.3	NR	67.0	41.0 (15.0-71.0)
Kim et al[32,33] (2010)	TST vs T-SPOT.*TB*	KR	209	78 (72.2)	NR	NR	NR	115.0 (38.0-197.0)
Chung et al[34] (2010)	TST vs QFT vs T-SPOT.*TB*	KR	167	71 (42.5)	54.1 ± 14.4	60.8 ± 57.5	66.5	115.0 (38.0-197.0)
Triverio et al[35] (2009)	TST vs QFT vs T-SPOT.*TB*	CH	62	46 (74.2)	65.0 ± 15.0	NR	22.6	5.7 (1.9-9.8)
Lee et al[36,37] (2009)	TST vs QFT vs T-SPOT.*TB*	TW	32	34 (54.8)	54.9 ± 10.1	NR	71.9	137.0 (56.0-225.0)[c]
Eleftheriadis et al[38] (2005)	TST vs Ab detection	GR	95	53 (55.8)	NR	NR	100.0	8.3 (2.8-14.0)
Yanai et al[39] (2006)	TST vs Ab detection	JP	243	148 (60.9)	60.0 ± 11.0	86.0 ± 84.0	NR	29.0 (9.2-49.0)
Wauters et al[40] (2004)	TST	BE	224	130 (58.0)	NR	NR	2.7	15.0 (5.0-25.0)
Shankar et al[41] (2005)	TST	IN	108	78 (72.2)	37.75 ± 11.8	51.6 ± 31.2	70.4	258.0 (114.0-431.0)
Fang et al[42] (2002)	TST	TW	177	78 (44.1)	54.7 ± 17.3	40.0 ± 28.9	48.0	214.0 (99.0-315.0)[c]
Yildiz et al[43,44] (1998)	TST	TR	29	17 (58.6)	30.9 ± 9.5	20.5 ± 17.4	NR	75.0 (32.0-125.0)
Ates et al[45,46] (2010)	TST	TR	779	381 (48.9)	51.2 ± 15.9	35.1 ± 33.4	53.9	41.0 (15.0-71.0)
Habesoglu et al[47] (2007)	TST	TR	187	97 (51.9)	50.0 ± 15.9	53.1 ± 54.9	55.1	42.0 (14.0-73.0)
Taskapan et al[48] (2000)	TST	TR	30	17 (56.7)	42.0 ± 12.0	27.8 ± 15.9	60.0	75.0 (32.0-125.0)
Dogan et al[49] (2005)	TST	TR	124	56 (45.2)	45.3 ± 16.2	30.0 ± 17.0	90.3	46.0 (16.0-80.0)
Cengiz & Seker[50] (2006)	TST	TR	106	47 (44.3)	49.9 ± 14.4	107.0 ± 54.8	100.0	42.0 (14.0-74.0)
Woeltje et al[51] (1998)	TST	US	307	129 (42.0)	58	3.7	0.0	7.9 (2.6-13.0)
Smirnoff et al[52] (1998)	TST	US	50	28 (56.0)	55	44.4	16.0	7.9 (2.6-13.0)
Hickstein[27] (2007)	TST	US	212	NR	NR	NR	NR	6.0 (2.1-10.0)
Linquist et al[26] (2002)	TST	US	94	NR	NR	NR	NR	7.9 (2.6-13.0)
Poduval & Hammes[53] (2003)	TST	US	118	59 (50.0)	NR	NR	NR	6.5 (2.2-11.0)
Transplant Population								
Sester et al[54] (2006)	TST vs Flow cytometry	DE	117	NR	53.1 ± 14.8	NR	NR	8.8 (2.9-15.0)
Sester et al[55] (2009)	Flow cytometry	DE	62	34 (54.8)	NR	NR	NR	6.2 (2.0-11.0)
Mixed Dialysis and Transplant Population								
Passalent et al[56] (2007)	TST vs T-SPOT.*TB*	CA	209	78 (44.1)	NR	NR	78.0	6.1 (2.2-11.0)
Winthrop et al[57] (2008)	TST vs QFT vs T-SPOT.*TB*	US	100	130 (58.0)	NR	NR	NR	4.7 (1.3-8.0)
Kantarci et al[58] (2006)	TST	TR	164	86 (52.4)	35.2 ± 10	43.0 ± 32	14.6	
Aydogan et al[59] (2009)	TST	TR	150	72 (48.0)	48.1 ± 16.7	NR	62.0	41.0 (15.0-71.0)
Basoglu et al[60] (2006)	TST	TR	44	25 (56.8)	46.6 ± 15.6	NR	90.9	42.0 (14.0-71.0)

Note: Unless otherwise indicated, values shown as number (percentage) or mean ± standard deviation.

Abbreviations and definitions: Ab, antibody; BCG, bacille Calmette-Guerin, Canada; BE, Belgium; CH, Switzerland; DE, Germany; ESKD, end-stage kidney disease; GR, Greece; IN, India; JP, Japan; KR, South Korea; NR, not reported; QFT, QuantiFERON (ELISA-based test); TR, Turkey; TSPOT.*TB*, ELISPOT-based TB test; TST, tuberculin skin test; TW, Taiwan; US, United States.

[a]National prevalence of TB in year of study publication, given as cases per 10^5 persons; values in parentheses are 95% confidence intervals.

[b]Not specified.

[c]TB prevalence in Taiwan estimated from data for China.

directly compared an IGRA to the TST, and the other 4 studies (517 participants) evaluated flow cytometry or antibody detection (eg, MycoDot, Determiner TBGL Antibody) kits.

Characteristics of included studies that did not provide sufficient data to contribute to meta-analysis are listed in Table S4. Six studies directly compared the TST and an IGRA, 7 studies evaluated the TST only, 3 studies evaluated an ELISA-based IGRA only, and 1 study evaluated flow cytometry. These studies included 2,282 participants, of whom 1,258 were on dialysis therapy, 820 had undergone transplantation, and the other 204 were not specified. Age and time spent on treatment of participants were similar between studies that contributed to meta-analysis and those that did not. Data for BCG vaccination rate were limited.

Results of the study quality assessment are shown in Fig 2. Overall, the quality of studies included in the review was suboptimal and often insufficient detail was available to make a judgment about potential bias. The method of patient recruitment was unclear in most studies (46/47 [97.8%]). Blinding of test interpretation to other test results and to knowledge of

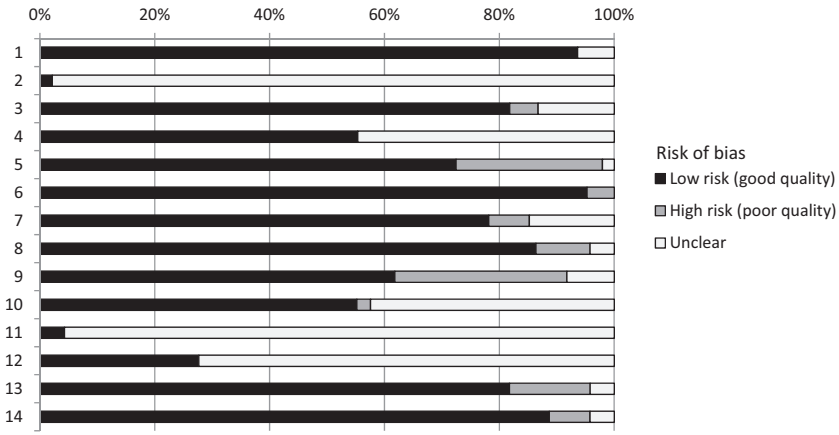

Figure 2. Methodological quality of studies included in review. Numbers indicate QUADAS (Quality Assessment of Diagnostic Accuracy Studies) elements. (1) Generalizability of test results to end-stage kidney disease population (spectrum bias); (2) participant selection method adequate (selection bias); (3) comprehensive risk-factor assessment (verification bias); (4) acceptable delay between testing and risk-factor assessment (disease progression bias); (5) all patients had all tests (partial verification bias); (6) all patients had the same tests (differential verification bias); (7) risk-factor assessment independent from tests (incorporation bias); (8) tests described in sufficient detail to repeat (repeatability); (9) risk-factor assessment described in sufficient detail to repeat (repeatability); (10) test interpretation conducted without knowledge of other test results (blinding); (11) risk-factor assessment conducted without knowledge of test results (blinding); (12) normal clinical information available during risk-factor assessment and test interpretation (information bias); (13) reasons for indeterminate results provided; and (14) reasons for withdrawals provided (intention-to-treat bias). See Fig S1 for quality of studies contributing to meta-analysis.

clinical risk factors also was predominately unclear (20/47 [42.6%] and 45/48 [95.7%], respectively). Risk-factor assessment and test procedures generally were described in sufficient detail to repeat the studies (30/47 [63.8%] and 41/47 [87.2%], respectively). Clinical risk assessment was considered comprehensive in 37 of 47 studies (78.7%). Few studies reported unexplainable indeterminate results or participant withdrawals. Study quality was poor but not different across studies that contributed to meta-analysis and those that did not; see Fig S1 for quality assessment of only studies that contributed to meta-analysis.

Association of Test Positivity With Clinical Risk Factors

Overall, positive test results for latent TB as measured by ELISA-based IGRA, ELISPOT-based IGRA, and TST were associated significantly with a medical history of TB infection (ELISA IGRA: OR, 6.01 [95% CI, 2.66-13.56; $P = 0.001$]; ELISPOT IGRA: OR, 5.02 [95% CI, 2.13-11.87; $P = 0.001$]; TST: OR, 1.95 [95% CI, 1.17-3.23; $P = 0.01$]). A positive ELISA IGRA was associated strongly with radiologic evidence of TB infection (OR, 2.97; 95% CI, 1.30-6.82; $P = 0.01$) and contact with a case of active TB (OR, 3.52; 95% CI, 1.69-7.31; $P = 0.001$). In contrast, there was insufficient evidence to determine the direction of association of a positive TST result with radiologic evidence of TB infection (OR, 0.79; 95% CI, 0.90-3.25; $P = 0.7$) or contact with a case of active TB (OR, 0.88; 95% CI, 0.43-1.82; $P = 0.7$). The direction of association of a positive ELISPOT IGRA result with radiologic evidence of TB infection and contact with a case of active TB also was unclear (ORs of 1.88 [95% CI, 0.43-8.22; $P = 0.4$] and 1.42 [95% CI, 0.80-2.52; $P = 0.2$], respectively). There was insufficient evidence to determine the direction of association of test positivity with high-risk nationality, immunosuppression, and BCG vaccination for all tests (Figs S2-S7). Comparing studies that blinded test interpretation to information about clinical risk factors with those that did not or were unclear, we found no significant differences in association of test positivity for radiologic evidence of TB, medical evidence of TB, contact with active TB, immunosuppression, or nationality ($P > 0.5$). The OR for the association of TST positivity with BCG vaccination was significantly higher in unblinded studies (2.04; 95% CI, 1.18-3.53) compared with blinded studies (0.63; 95% CI, 0.27-1.46; $P = 0.05$). A significant difference also was found in the association of a positive TST result with immunosuppression when studies were stratified by modality of end-stage kidney disease treatment. The summary OR was higher in studies of dialysis patients (OR, 1.38; 95% CI, 0.98-1.94) than transplantation and mixed populations (OR, 0.38; 95% CI, 0.22-0.67; $P = 0.004$). There were no significant differences for other risk factors when studies were stratified by modality of end-stage kidney disease treatment ($P > 0.1$). Our findings also were unchanged when we compared studies using a TST cutoff of 5 versus 10 mm ($P > 0.1$) and those that used second- versus third-generation ELISA IGRAs ($P > 0.4$).

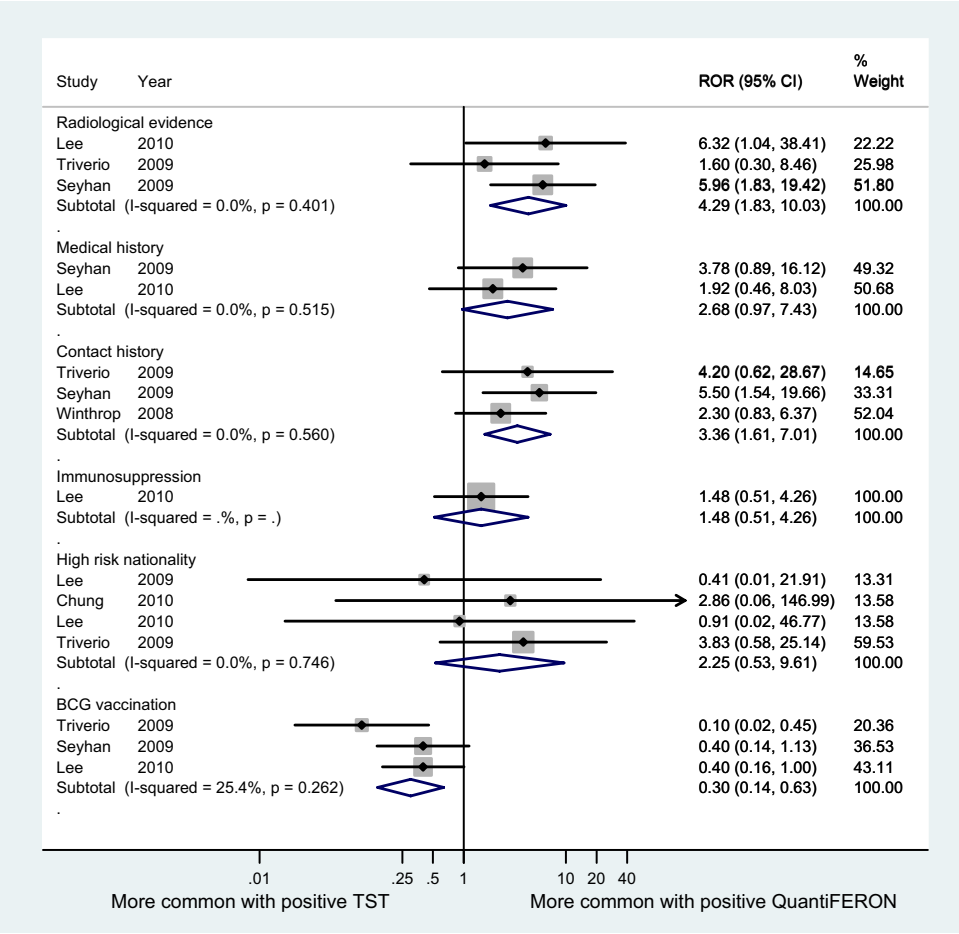

Figure 3. Tuberculin skin test (TST) versus enzyme-linked immunosorbent assay interferon γ release assay (QuantiFERON): relative association of a positive test with risk factors for latent tuberculosis. Abbreviations: CI, confidence interval; ROR, relative odds ratio.

Relative Association of IGRA and TST Positivity With Clinical Risk Factors

Figures 3-5 show the direct comparison between IGRAs and TST results for association of test positivity with clinical risk factors. From the findings of 6 studies, compared to a positive TST result, a positive ELISA IGRA result was associated more strongly with radiologic evidence of past TB (ROR, 4.29; 95% CI, 1.83-10.03; $P = 0.001$) and contact history with active TB (ROR, 3.36; 95% CI, 1.61-7.01; $P = 0.001$; Fig 3). Conversely, a positive ELISA IGRA result was associated less strongly with BCG vaccination compared to a positive TST result (ROR, 0.30; 95% CI, 0.14-0.63; $P = 0.002$; Fig 3). There was no evidence of a difference in association of test positivity with high-risk nationality (ROR, 2.25; 95% CI, 0.53-9.61; $P = 0.3$) or medical history of TB (ROR, 2.68; 95% CI, 0.97-7.43; $P = 0.06$) between the ELISA IGRA and TST results. Data from 6 studies comparing performance of the ELISPOT IGRA and TST showed there was no evidence of a difference between the association of a positive ELISPOT IGRA or TST result for any of the risk factors (Fig 4).

Four studies compared the ELISA and ELISPOT IGRAs directly. One study reported a positive ELISPOT IGRA result to be associated more strongly with radiologic evidence of past TB than a positive ELISA IGRA result (ROR, 0.11; 95% CI, 0.02-0.77; $P = 0.03$). No evidence of a difference was found for any of the other risk factors (Fig 5). These results were robust to heterogeneity in TST cutoff ($P > 0.3$), ELISA IGRA generation ($P > 0.5$), and modality of end-stage kidney disease treatment ($P > 0.3$).

Comparisons of Other Tests for Latent TB

Two studies evaluated flow cytometry; one evaluated both ESAT-6 and Tuberkulin GT 100 as stimulating antigens, and the other evaluated only ESAT-6.[38,39] All transplant recipients were assumed to be immunosuppressed. There was no significant association between flow cytometry positivity (using Tuberkulin GT 100 or ESAT-6 as the stimulating agent) and immunosuppression (ORs of 1.36 [95% CI, 0.08-22.13; $P = 0.8$] and 0.43 [95% CI, 0.01-22.46; $P = 0.7$], respectively; Fig S6). In one study, the TST also was evaluated; however, no difference in association with immunosuppression was found between flow

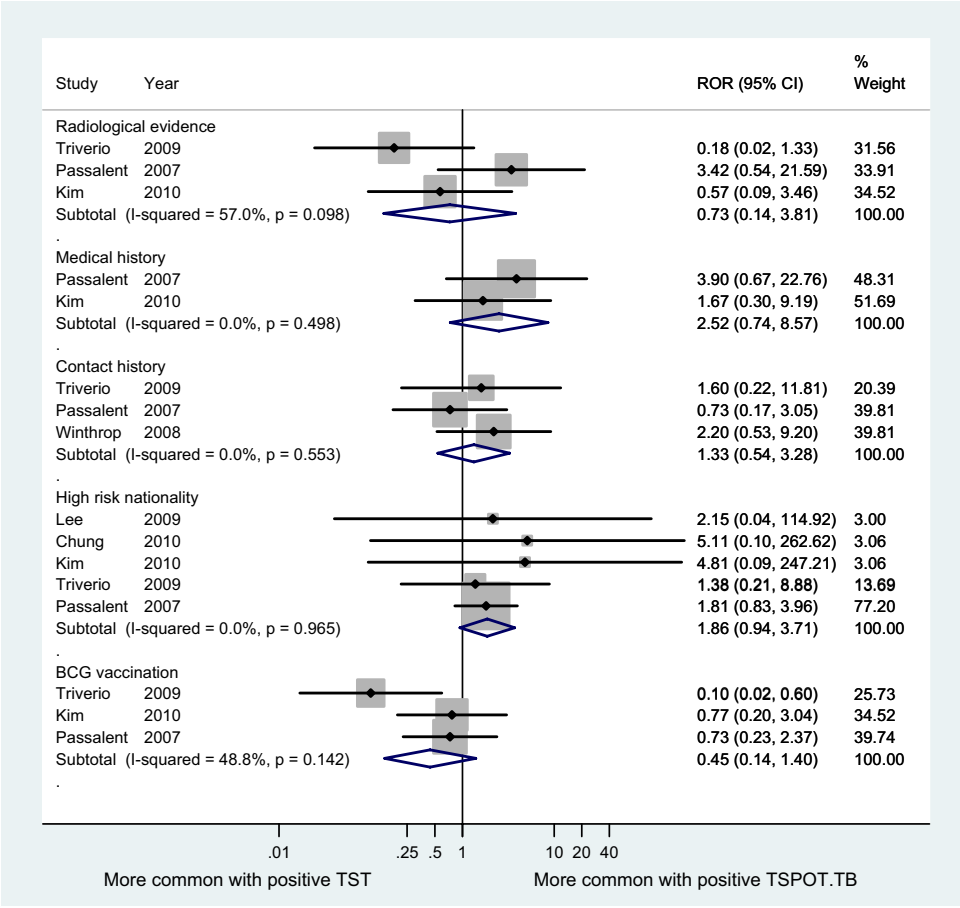

Figure 4. Tuberculin skin test (TST) versus enzyme-linked immunosorbent spot interferon γ release assay (T-SPOT.*TB*): relative association of a positive test result with risk factors for latent tuberculosis. Abbreviations: CI, confidence interval; ROR, relative odds ratio.

cytometry (using either ESAT-6 or Tuberkulin GT 100) and TST (RORs of 4.58 [95% CI, 0.09-244.796; $P = 0.5$] and 1.80 [95% CI, 0.033-98.112; $P = 0.8$], respectively). Two studies evaluated antibody detection kits. In one study, both MycoDot and Determiner TBGL Antibody test positivity were associated significantly with radiologic evidence of past TB (ORs of 3.47 [95% CI, 1.33-9.07; $P = 0.01$] and 2.18 [95% CI, 1.04-4.59; $P = 0.04$], respectively). The other study, which evaluated MycoDot only, showed no significant association of test positivity with BCG vaccination (OR, 0.44; 95% CI, 0.01-22.61; $P = 0.7$).

DISCUSSION

Screening for latent TB in the end-stage kidney disease population allows treatment to be targeted at persons with the highest risk of active TB and who will benefit most from prophylaxis. The key finding of this review is that compared to the TST, ELISA IGRA positivity was associated more strongly with clinical risk factors for latent TB, while associated less strongly with prior BCG vaccination. This suggests that ELISA IGRA is both more sensitive and specific than the TST in the context of end-stage kidney disease. Global guidelines for latent TB screening in immunocompromised populations currently recommend using an IGRA as a supplementary or replacement test to the TST.[11] The results of this review support replacement of the TST with the ELISA IGRA. Not enough data were available for the relative performance of ELISPOT IGRA with the TST or ELISPOT IGRA with ELISA IGRA to make conclusions about the ELISPOT IGRA.

Data analyzed in the present study were restricted largely to the dialysis population (3,700/4,546 [81.4%]). Although this may be considered a limitation, performance of tests for latent TB in the dialysis population is most clinically relevant because assessment for latent TB generally occurs prior to starting dialysis therapy or during clinical evaluation leading up to kidney transplantation.[61]

Compared with both the TST and ELISPOT IGRA, the ELISA IGRA showed the strongest overall association with clinical risk for latent TB, including radiologic evidence of past TB (OR, 2.97; Fig S2), medical evidence of past TB (OR, 6.01; Fig S3), and contact with a person with active TB (OR, 3.52; Fig S4). Data were less conclusive for the utility of the ELISPOT IGRA, although positive results from this assay were associated with medical evidence of past TB (OR,

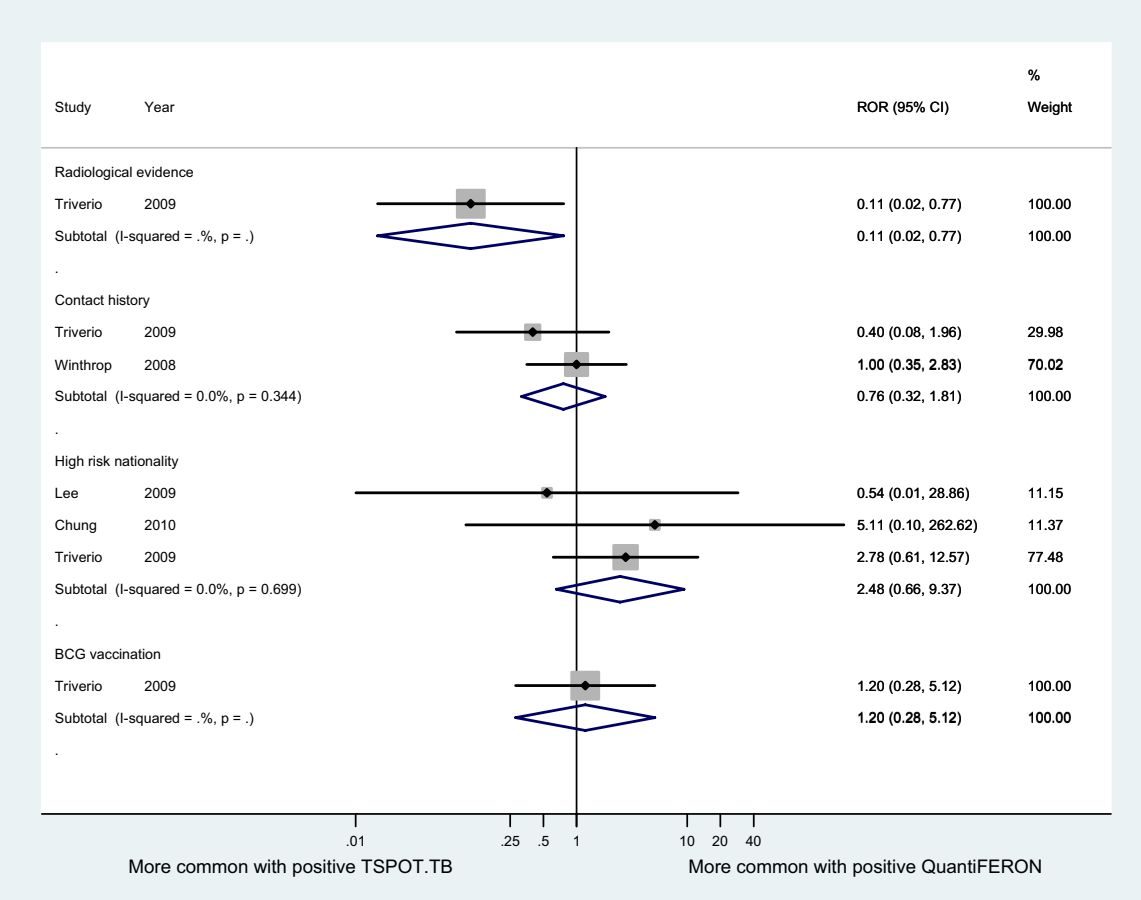

Figure 5. Enzyme-linked immunosorbent spot interferon γ release assay (IGRA; T-SPOT.*TB*) versus enzyme-linked immunosorbent assay IGRA (QuantiFERON): relative association of a positive test with risk factors for latent tuberculosis. Abbreviations: CI, confidence interval; ROR, relative odds ratio.

5.02; Fig S3). We found no association between nationality and test positivity for any of the tests; however, in these analyses, data were limited to studies conducted in countries with low to moderate TB burden. The clinical value of IGRAs for detecting latent TB in different patient populations in high-prevalence regions warrants study.

Studies that met the inclusion criteria but did not provide sufficient data to contribute to meta-analysis represent a potential source of bias in this review. These studies accounted for 36.2% (17/47) of included studies and 33.4% (2,282/6,828) of all participants. Due to missing data for study design and patient characteristics, it was not possible to formally compare differences between studies that contributed to meta-analysis and those that did not (Table 1; Table S4). The available data suggest that patient characteristics within the dialysis and transplantation populations are similar between studies that contributed to meta-analysis and those that did not, but there appear to be proportionately more transplant recipients in studies that did not contribute to meta-analysis.

Another key finding of the present study is that based on the best available evidence, the preferred IGRA for diagnosing latent TB is the ELISA IGRA, although comparison of either the ELISA or ELISPOT IGRAs with the TST and each other was limited by the small number of evaluable studies (n ≤10 for each comparison; Figs 3-5). Data from 6 studies indicate that compared to the TST, ELISA IGRA positivity was associated more strongly with clinical risk factors for latent TB, whereas comparing ELISPOT IGRA and TST, no statistical differences were observed in ROR for any clinical risk factors.[32,34-36,56,57] Only a single study of 4 studies comparing the performance of ELISPOT and ELISA IGRAs in end-stage kidney disease showed that the former was associated more strongly with radiologic evidence of past TB.[35]

Although the utility of assays other than the ELISA IGRA, TST, and ELISPOT IGRA have been studied in the context of diagnosing latent TB, neither serologic detection of antibody to *Mycobacterium tuberculosis* or flow cytometry studies have been shown to be clinically useful and sensitivity analyses have been

limited by the small number of reports.[10,38,39,54,55] Based on the present available data, these assays cannot be recommended for assisting in the diagnosis of latent TB.

Systematic reviews are the preferred format for summarizing evidence because they use explicit and reproducible methods to limit bias. We acknowledge that the validation of test results against clinical risk factors has several limitations. Although this approach allows us to make inferences about the accuracy of tests in relative terms, it does not allow us to calculate absolute measures of test accuracy. To derive test accuracy characteristics, previous reviews in the general population have overcome the lack of a reference standard by using active TB and low-risk individuals as surrogates for positive and negative latent TB status.[19-21] This method is dubious because active and latent TB are distinct disease states that elicit different responses from the host immune system and therefore it may be inappropriate to use active TB as an immunologic model for latent TB.[1,62] Several studies have shown that responses to the TST and IGRAs diminish during untreated active TB infection, but rapidly increase after treatment, suggesting that active TB may suppress the host immune response to these tests.[63-66]

Another limitation of this review is that the implications of our results rely on the assumption that a high proportion of people with clinical risk factors for latent TB actually have latent TB. Although this assumption is difficult to prove given the lack of a reference standard for latent TB, there is evidence that active TB is more likely to develop in dialysis patients with old TB on chest radiograph or immunosuppressive diseases, including diabetes mellitus and HIV infection.[67] Risk factors for the transmission of latent TB also may be inaccurate and difficult to quantify and therefore a potential source of heterogeneity between studies.[22] For example, studies included in this review assessed contact with active TB as a dichotomous risk factor only, whereas from epidemiologic studies, we know that the likelihood of transmission is determined by both time and proximity to a person with active TB.[22] Assessing nationality as a dichotomous risk factor is similarly problematic because it assumes that all individuals from a country with high TB burden have the same risk of transmission, whereas in reality, transmission requires an interplay of several factors, including socioeconomic status, time spent in the country, and where in the country that time was spent.

Future research in this area should pursue 3 directions. First, this review demonstrated a gap in evidence for the relative test performance of the ELISA and ELISPOT IGRAs. More studies are required to assess the relative performance of these tests in the end-stage kidney disease population. Second, a study assessing the active TB rate in patients after test-directed treatment would be helpful to derive the relative clinical value of the TST and ELISA and ELISPOT IGRAs. Third, a cost-effectiveness evaluation is needed to determine whether the reduction in false-positive and negative results that occurs when ELISA IGRA replaces the TST is worth the trade-off in any cost increase that also may occur.

In conclusion, we determined that compared to the TST, ELISA IGRA positivity was associated more strongly with clinical risk factors for latent TB in end-stage kidney disease and therefore is likely to be a more accurate diagnostic tool for latent TB in end-stage kidney disease. This finding is consistent with previous systematic reviews conducted in the general population that showed that IGRA results correlate better with the intensity of TB exposure compared to the TST while remaining independent of BCG vaccination status[22] and that IGRAs are more sensitive and specific and thus the preferred tests.[19-21] On the basis of best available evidence, we propose that the ELISA IGRA should be the test of choice for screening for latent TB, and a review of clinical practice guidelines for managing latent TB in the end-stage kidney disease population is warranted.

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SUPPLEMENTARY MATERIAL

Table S1: Electronic search strategy for MEDLINE and EMBASE.

Table S2: Standard definitions of risk factors.

Table S3: QUADAS working definitions.

Table S4: Characteristics of included studies that did not provide sufficient data to contribute to meta-analysis.

Figure S1: Methodological quality of studies contributing to the meta-analysis.

Figure S2: Association of test positivity with radiological evidence of past TB.

Figure S3: Association of test positivity with medical evidence of past TB.

Figure S4: Association of test positivity with active TB contact.

Figure S5: Association of test positivity with BCG vaccination.

Figure S6: Association of test positivity with immunosuppression.

Figure S7: Association of test positivity with high-risk nationality.

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REFERENCES

1. Mack U, Migliori GB, Sester M, et al. LTBI: latent tuberculosis infection or lasting immune responses to *M. tuberculosis*? A TBNET consensus statement. *Eur Respir J*. 2009;33(5):956-973.

2. Cengiz K. Increased incidence of tuberculosis in patients undergoing hemodialysis. *Nephron*. 1996;73(3):421-424.

3. Hussein MM, Mooij JM, Roujouleh H. Tuberculosis and chronic renal disease. *Semin Dial*. 2003;16(1):38-44.

4. Dobler CC, McDonald SP, Marks GB. Risk of tuberculosis in dialysis patients: a nationwide cohort study. *PLoS ONE*. 2011;6(12):e29563.

5. Jasmer RM, Nahid P, Hopewell PC. Clinical practice. Latent tuberculosis infection. *N Engl J Med*. 2002;347(23):1860-1866.

6. Blumberg HM, Burman WJ, Chaisson RE, et al. American Thoracic Society/Centers for Disease Control and Prevention/Infectious Diseases Society of America: treatment of tuberculosis. *Am J Respir Crit Care Med*. 2003;167(4):603-662.

7. Sousa M, Pozniak A, Boffito M. Pharmacokinetics and pharmacodynamics of drug interactions involving rifampicin, rifabutin and antimalarial drugs. *J Antimicrob Chemother*. 2008;62(5):872-878.

8. Niemi M, Backman JT, Fromm MF, Neuvonen PJ, Kivisto KT. Pharmacokinetic interactions with rifampicin: clinical relevance. *Clin Pharmacokinet*. 2003;42(9):819-850.

9. Thompson NP, Caplin ME, Hamilton MI, et al. Antituberculosis medication and the liver: dangers and recommendations in management. *Eur Respir J*. 1995;8(8):1384-1388.

10. Sester M, Sester U, Clauer P, et al. Tuberculin skin testing underestimates a high prevalence of latent tuberculosis infection in hemodialysis patients. *Kidney Int*. 2004;65(5):1826-1834.

11. Segall L, Covic A. Diagnosis of tuberculosis in dialysis patients: current strategy. *Clin J Am Soc Nephrol*. 2010;5(6):1114-1122.

12. Denkinger CM, Dheda K, Pai M. Guidelines on interferon-gamma release assays for tuberculosis infection: concordance, discordance or confusion? *Clin Microbiol Infect*. 2011;17(6):806-814.

13. Mazurek GH, Jereb J, Vernon A, et al. Updated guidelines for using interferon gamma release assays to detect *Mycobacterium tuberculosis* infection—United States, 2010. *MMWR Recomm Rep*. 2010;59(RR-5):1-25.

14. Canadian Tuberculosis Committee. Updated recommendations on interferon gamma release assays for latent tuberculosis infection. An Advisory Committee Statement (ACS). *Can Commun Dis Rep*. 2008;34(ACS-6):1-13.

15. National Institute for Health and Clinical Excellence. Clinical diagnosis and management of tuberculosis, and measures for its prevention and control. CG117. London: National Institute for Health and Clinical Excellence; 2011.

16. British Thoracic Society Standards of Care Committee. Guidelines for the prevention and management of *Mycobacterium tuberculosis* infection and disease in adult patients with chronic kidney disease. *Thorax*. 2010;65(6):557-570.

17. Health Protection Agency. *Health Protection Agency Position Statement on the Use of Interferon Gamma Release Assay (IGRA) Tests for Tuberculosis (TB)*. London: Health Protection Agency; 2007.

18. Swiss Lung Association. *Tuberculosis in Switzerland*. Berne: Swiss Lung Association; 2011.

19. Pai M, Riley LW, Colford JM Jr. Interferon-gamma assays in the immunodiagnosis of tuberculosis: a systematic review. *Lancet Infect Dis*. 2004;4(12):761-776.

20. Pai M, Zwerling A, Menzies D. Systematic review: T-cell-based assays for the diagnosis of latent tuberculosis infection: an update. *Ann Intern Med*. 2008;149(3):177-184.

21. Chang KC, Leung CC. Systematic review of interferon-gamma release assays in tuberculosis: focus on likelihood ratios. *Thorax*. 2010;65(3):271-276.

22. Dinnes J, Deeks J, Kunst H, et al. A systematic review of rapid diagnostic tests for the detection of tuberculosis infection. *Health Technol Assess*. 2007;11(3):1-196.

23. Whiting P, Rutjes AWS, Reitsma JB, Bossuyt PMM, Kleijnen J. The development of QUADAS: a tool for the quality assessment of studies of diagnostic accuracy included in systematic reviews. *BMC Med Res Methodol*. 2003;3:25.

24. Agresti A. *An Introduction to Categorical Data Analysis*. New York, NY: Wiley; 1996.

25. World Health Organisation. *Global Tuberculosis Control*. Geneva, Switzerland: WHO; 2010.

26. Linquist JA, Rosaia CM, Riemer B, Heckman K, Alvarez F. Tuberculosis exposure of patients and staff in an outpatient hemodialysis unit. *Am J Infect Control*. 2002;30(5):307-310.

27. Hickstein L, McPherson C, Kwalick D, et al. Tuberculosis transmission in a renal dialysis center—Nevada, 2003. *MMWR Morb Mortal Wkly Rep*. 2004;53(37):873-875.

28. Inoue T, Nakamura T, Katsuma A, et al. The value of QuantiFERON TB-Gold in the diagnosis of tuberculosis among dialysis patients. *Nephrol Dial Transplant*. 2009;24(7):2252-2257.

29. Lee SSJ, Chou KJ, Dou HY, et al. High prevalence of latent tuberculosis infection in dialysis patients using the interferon-release assay and tuberculin skin test. *Clin J Am Soc Nephrol*. 2010;5(8):1451-1457.

30. Lee S, Chou KJ, Su IJ, et al. High prevalence of latent tuberculosis infection in end-stage renal disease patients on hemodialysis, using QuantiFERON-GOLD In-Tube Test and tuberculin skin test. Paper presented at: 49th Interscience Conference on Antimicrobial Chemotherapy. September 12-15, 2009; San Francisco, CA.

31. Seyhan EC, Sokucu S, Altin S, et al. Comparison of the QuantiFERON-TB Gold In-Tube test with the tuberculin skin test for detecting latent tuberculosis infection in hemodialysis patients. *Transplant Infect Dis*. 2010;12(2):98-105.

32. Kim SH, Lee SO, Park IA, et al. Diagnostic usefulness of a T cell-based assay for latent tuberculosis infection in kidney transplant candidates before transplantation. *Transplant Infect Dis*. 2010;12(2):113-119.

33. Kim S, Lee S, Park I, et al. A prospective longitudinal study of usefulness of a T cell-based assay for latent tuberculosis infection (LTBI) in renal transplant recipients. Paper presented at: 48th Meeting of the Infectious Diseases Society of America. October 21-24, 2010; Vacouver, BC.

34. Chung WK, Zheng ZL, Kim H-S, et al. Serial testing of interferon-gamma-release assays for the diagnosis of latent tuberculosis in hemodialysis patients. *J Infect*. 2010;61(2):144-149.

35. Triverio P-A, Bridevaux P-O, Roux-Lombard P, et al. Interferon-gamma release assays versus tuberculin skin testing for detection of latent tuberculosis in chronic haemodialysis patients. *Nephrol Dial Transplant*. 2009;24(6):1952-1956.

36. Lee SSJ, Chou KJ, Su IJ, et al. High prevalence of latent tuberculosis infection in patients in end-stage renal disease on hemodialysis: comparison of QuantiFERON-TB GOLD, ELISPOT, and tuberculin skin test. *Infection*. 2009;37(2):96-102.

37. Lee S, Ni Y, Huang T, et al. QuantiFERON-TB GOLD for diagnosis of latent tuberculosis infection in patients with end-stage renal disease in Taiwan. Paper presented at: 46th Interscience Conference on Antimicrobial Chemotherapy. September 27-30, 2006; San Francisco, CA.

38. Eleftheriadis T, Tsiaga P, Antoniadi G, et al. The value of serum antilipoarabinomannan antibody detection in the diagnosis of latent tuberculosis in hemodialysis patients. *Am J Kidney Dis*. 2005;46(4):706-712.

39. Yanai M, Uehara Y, Takeuchi M, et al. Evaluation of serological diagnosis tests for tuberculosis in hemodialysis patients. *Ther Apher Dial*. 2006;10(3):278-281.

40. Wauters A, Peetermans WE, Van den Brande P, et al. The value of tuberculin skin testing in haemodialysis patients. *Nephrol Dial Transplant*. 2004;19(2):433-438.

41. Shankar MSR, Aravindan AN, Sohal PM, et al. The prevalence of tuberculin sensitivity and anergy in chronic renal failure in an endemic area: tuberculin test and the risk of post-transplant tuberculosis. *Nephrol Dial Transplant*. 2005;20(12):2720-2724.

42. Fang HC, Chou KJ, Chen CL, et al. Tuberculin skin test and anergy in dialysis patients of a tuberculosis-endemic area. *Nephron*. 2002;91(4):682-687.

43. Yildiz A, Akkaya V, Turk S, Sever MS, Ark E, Yildiz P. Tuberculin responsiveness in hemodialysis patients. *Chest*. 1998; 114(3):947-948.

44. Yildiz A, Sever MS, Yildiz P, et al. Tuberculin (PPD) reactivity in hemodiaylsis patients (HDp). *Nephrol DialTransplant*. 1997;12(9):A148.

45. Ates G, Yildiz T, Danis R, et al. Incidence of tuberculosis disease and latent tuberculosis infection in patients with end stage renal disease in an endemic region. *Ren Fail*. 2010;32(1):91-95.

46. Ates G, Ozekinci T, Yildiz A, Danis R. Comparison of interferon-gamma release assay versus tuberculin skin test for latent tuberculosis screening in hemodialysis patients. *Biotechnol Biotechnol Equip*. 2009;23(2):1242-1246.

47. Habesoglu MA, Torun D, Demiroglu YZ, et al. Value of the tuberculin skin test in screening for tuberculosis in dialysis patients. *Transplant Proc*. 2007;39(4):883-886.

48. Taskapan H, Oymak O, Utas C. Tuberculin and anergy testing in CAPD patients. *Perit Dial Int*. 2000;20(6):807-809.

49. Dogan E, Erkoc R, Sayarlioglu H, Uzun K. Tuberculin skin test results and the booster phenomenon in two-step tuberculin skin testing in hemodialysis patients. *Ren Fail*. 2005;27(4):425-428.

50. Cengiz K, Seker A. Boosted tuberculin skin testing in hemodialysis patients. *Am J Infect Control*. 2006;34(6):383-387.

51. Woeltje KF, Mathew A, Rothstein M, Seiler S, Fraser VJ. Tuberculosis infection and anergy in hemodialysis patients. *Am J Kidney Dis*. 1998;31(5):848-852.

52. Smirnoff M, Patt C, Seckler B, Adler JJ. Tuberculin and anergy skin testing of patients receiving long-term hemodialysis. *Chest*. 1998;113(1):25-27.

53. Poduval RD, Hammes MS. Tuberculosis screening in dialysis patients—is the tuberculin test effective? *Clin Nephrol*. 2003; 59(6):436-440.

54. Sester U, Junker H, Hodapp T, et al. Improved efficiency in detecting cellular immunity towards *M. tuberculosis* in patients receiving immunosuppressive drug therapy. *Nephrol Dial Transplant*. 2006;21(11):3258-3268.

55. Sester U, Wilkens H, Van Bentum K, et al. Impaired detection of *Mycobacterium tuberculosis* immunity in patients using high levels of immunosuppressive drugs. *Eur Respir J*. 2009;34(3):702-710.

56. Passalent L, Khan K, Richardson R, Wang J, Dedier H, Gardam M. Detecting latent tuberculosis infection in hemodialysis patients: a head-to-head comparison of the T-SPOT.TB test, tuberculin skin test, and an expert physician panel. *Clin J Am Soc Nephrol*. 2007;2(1):68-73.

57. Winthrop KL, Nyendak M, Calvet H, et al. Interferon-gamma release assays for diagnosing *Mycobacterium tuberculosis* infection in renal dialysis patients. *Clin J Am Soc Nephrol*. 2008; 3(5):1357-1363.

58. Kantarci G, Altinoz H, Sahin S, Manga G, Tasan G. Tuberculin positivity: a serious problem before transplantation in Turkey. *Transplant Proc*. 2006;38(2):646-648.

59. Aydogan O, Gurgun A, Basoglu OK, et al. [Tuberculin skin test reactivity in patients with chronic renal failure] [in Turkish]. *Tuberk Toraks*. 2009;57(3):268-276.

60. Basoglu OK, Atasever A, Gunduz Telli C, et al. [T-lymphocyte subgroups and tuberculin skin test reactivity in patients with chronic renal failure]. *Tuberk Toraks*. 2006;54(1):5-10.

61. Mycobacterium tuberculosis. *Am J Transplant*. 2004; 4(suppl 10):37-41.

62. Rothel JS, Radford AJ. Comparison of tuberculosis tests: finding truth or confirming prejudice? *Clin Infect Dis*. 2003;36(9): 1206-1207; author reply 1209-1210.

63. Pathan AA, Wilkinson KA, Klenerman P, et al. Direct ex vivo analysis of antigen-specific IFN-gamma-secreting CD4 T cells in *Mycobacterium tuberculosis*-infected individuals: associations with clinical disease state and effect of treatment. *J Immunol*. 2001;167(9):5217-5225.

64. Shams H, Wizel B, Weis SE, Samten B, Barnes PF. Contribution of CD8(+) T cells to gamma interferon production in human tuberculosis. *Infect Immun*. 2001;69(5):3497-3501.

65. Rooney JJ Jr, Crocco JA, Kramer S, Lyons HA. Further observations on tuberculin reactions in active tuberculosis. *Am J Med*. 1976;60(4):517-522.

66. Hirsch CS, Toossi Z, Othieno C, et al. Depressed T-cell interferon-gamma responses in pulmonary tuberculosis: analysis of underlying mechanisms and modulation with therapy. *J Infect Dis*. 1999;180(6):2069-2073.

67. Christopoulos AI, Diamantopoulos AA, Dimopoulos PA, Goumenos DS, Barbalias GA. Risk factors for tuberculosis in dialysis patients: a prospective multi-center clinical trial. *BMC Nephrol*. 2009;10:36.