Systematic Review and Meta-Analysis: Tuberculosis, TNFα Inhibitors, and Crohn's Disease

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SYSTEMATIC REVIEW AND META-ANALYSIS:
TUBERCULOSIS, TNFA INHIBITORS, AND CROHN’S DISEASE

by

BRENT L. CAO

A thesis submitted in partial fulfillment of the requirements
for the Honors in the Major Program in Biomedical Sciences
in the College of Medicine
and in The Burnett Honors College
at the University of Central Florida
Orlando, Florida

Summer Term, 2018

Thesis Chair: Saleh A. Naser, PhD
Some of the contents of this thesis can also be found at the following source:
Abstract

Inflammation is often a protective reaction against harmful foreign agents. However, in many disease conditions, the mechanisms behind the inflammatory response are poorly understood. Often times, the inflammation causes adverse effects, such as joint pain, abdominal pain, fever, fatigue, and loss of appetite. Thus, many treatments aim to inhibit the inflammatory response in order to control adverse symptoms. Such treatments include TNFα inhibitors. However, a major risk associated with drugs inhibiting tumor necrosis factor alpha (TNFα) is serious infection, including tuberculosis (TB).

Anti-TNFα therapy is used to treat patients with Crohn’s disease, for which the risk of tuberculosis may be even more concerning. Recent literature suggests Crohn’s might involve *Mycobacterium avium subspecies paratuberculosis* (MAP), an intracellular TB-like bacterium. This study seeks to investigate the risk of developing TB in patients with Crohn’s disease treated with TNFα inhibitors. A meta-analysis synthesized existing evidence. Evidence came from published randomized, double-masked, placebo-controlled trials of TNFα inhibitors for treatment of adult Crohn’s disease.

Twenty-three trials were identified, including 5,669 patients. The risk of tuberculosis was significantly increased in anti-TNFα treated patients, with a risk difference of 0.028 (95% confidence interval [CI], 0.0011-0.055). The odds ratio was 4.85 (95% CI, 1.02-22.99) when all studies were included and 5.85 (95% CI, 1.13-30.38) when studies reporting zero tuberculosis cases were excluded.

The risk of tuberculosis is increased in patients with Crohn’s disease treated with TNFα inhibitors. The medical community should be alerted about this risk and the potential for TNFα inhibitor usage favoring granulomatous infections and worsening the patient condition.
I would like to express my most sincere and profound gratitude to my family and friends, who have always supported me through thick and thin. I would also like to extend my thanks to my thesis committee members, Dr. Samsam and Dr. Singla, who have taken the time out of their day to provide guidance and advice on my endeavors.

Most of all, I would like to recognize and thank my thesis chair, Dr. Naser, whose mentorship and guidance proved invaluable.
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Introduction

Inflammation is a vital mechanism to protect against foreign agents. However, in many disease conditions, the immune system reacts for reasons that are not entirely understood. Often times, the response causes adverse symptoms, as is the case in rheumatoid arthritis, Crohn’s disease, ankylosing spondylitis, and psoriasis.\(^1\) Thus, many treatments aim to inhibit the inflammatory response in order to control adverse symptoms.\(^2\) Such treatments include TNF\(\alpha\) inhibitors. However, a major risk associated with drugs inhibiting tumor necrosis factor alpha (TNF\(\alpha\)) is serious infection, including tuberculosis (TB).\(^3\)

Anti-TNF\(\alpha\) therapy is used to treat patients with Crohn’s disease, for which the risk of developing TB infection may be even more relevant. Recent literature suggests Crohn’s disease involves *Mycobacterium avium subspecies paratuberculosis* (MAP), an intracellular TB-like bacterium.\(^4\)\(^-\)\(^6\) Thus, this study seeks to investigate the risk of developing TB in patients with Crohn’s disease treated with TNF\(\alpha\) inhibitors.

Crohn’s disease has become a global disease with a prevalence reaching 0.32% in Western Europe and North America and an increasing incidence in many other parts of the world.\(^7\) TNF\(\alpha\) inhibitors are the recommended therapy to treat moderate to severe Crohn’s disease, which has led to a rise in the development of biologic therapeutic drugs.\(^8\) As of 2018, three TNF\(\alpha\) inhibitors (not including biosimilars) have been approved by the European Medicines Agency (EMA) and the U.S. Food and Drug Administration (FDA) for treating patients with Crohn’s disease. These three drugs are adalimumab, certolizumab pegol, and
infliximab. The aforementioned anti-TNFα therapy have proven efficacy in relieving symptoms and are play an integral role in treatment options for Crohn’s disease.\textsuperscript{9,10}

However, suppressing the immune system carries many risks. Rare risks, especially, may not be characterized and studied well in the trials and investigations leading up to drug approval. Individual double-masked, randomized, placebo-controlled trials (RCTs) of TNFα inhibition therapy lack the power to analyze and determine whether there are increases in risk for developing uncommon adverse reactions, such as cancer or serious infection. However, these RCTs provide quality data for meta-analysis. Indeed, meta-analysis has previously demonstrated that risk of serious infection was increased for patients suffering from rheumatoid arthritis, psoriatic arthritis, and ankylosing spondylitis who elected to use TNFα inhibitors to manage their conditions.\textsuperscript{3} Furthermore, meta-analysis published in 2016 reported a significantly increased of opportunistic infections associated with the use of TNFα inhibitors for treatment of Crohn’s disease. However, the association between tuberculosis or serious infection and anti-TNFα therapy still remained unclear and was not reported.\textsuperscript{11} A major limitation to current analytical methods is the inability to deal with clinical trials reporting zero-event data in both arms of the studies (“double-zero studies”). Most often, meta-analytical studies excluded these double-zero studies.\textsuperscript{12,13} As will be discussed later, the number of aforementioned “double-zero studies” is high due to the low incidence of tuberculosis. In other scenarios, data from trials with zero-event data from just one group were often subject to modification (“continuity correction”) that, while having mathematical basis, lacks biological reasoning. Thus, previous analytical approaches cast uncertainty about whether or not sufficient evidence indicating an increased risk of developing TB infection from using TNFα inhibitors is provided.
Tuberculosis infection should be provided special attention, as aforementioned, Crohn’s disease might be caused by *Mycobacterium avium subspecies paratuberculosis* (MAP).\(^6\) TNF\(\alpha\) is of special importance due to its ability to contain granulomatous infections as an inflammatory cytokine.\(^6\) Therefore, TNF\(\alpha\) inhibitors can not only disrupt the human body’s ability to contain and deal with tuberculosis but also to MAP, further increasing the patient’s susceptibility to the MAP bacterium or even worsen their disease condition.\(^{14}\) Thus, a meta-analysis of RCTs to specifically quantify the risk of tuberculosis development in patients who suffer from Crohn’s disease and were treated with TNF\(\alpha\) inhibitors was conducted.
Background

Concentrations of TNFα have been found to be elevated in the stool, mucosa, and blood of patients suffering from inflammatory bowel disease. Thus, monoclonal antibodies to TNFα were synthesized to treat and manage the symptoms of patients.\(^8\)

Although anti-TNFα therapies have brought great change to the treatment of IBD, there are theoretical and realized safety concerns. Case reports often initially raise awareness and need for research regarding novel therapies and their unexpected adverse side effects. Often, it is these reports that lead to larger powered studies and to hypothesis generation.\(^{15}\)

Large scale studies done on TNFα inhibitors as a whole has discovered that the immunosuppressant therapy does increase patient risk of developing infections and even cancer.\(^{16}\) Indeed, pharmaceutical companies have funneled large sums of money not only to conduct clinical trials but to also explore all the possible consequences of biologic medicines.\(^2\)

Previous studies have found increased risk of tuberculosis, opportunistic infections, malignancies, and serious infections in various disease groups and over a drug class as a whole. In fact, adverse events and side effects from the use of biologics are well documented and well known.\(^{14}\) However, many of these studies are outdated, not comprehensive, or cannot deal with rare events. Specifically, previous meta-analyses have not investigated whether TNFα inhibitors increase TB infection risk in patients suffering from Crohn’s disease.\(^{12}\) Up until 2018, there was not enough literature or lack of tools to investigate such a topic. Previous analyses have been able to draw conclusions about rheumatoid arthritis and psoriatic disease due to the abundance of literature. Furthermore, some studies draw counterintuitive results.\(^{17}\) A meta-analysis found that
there was no increased risk of overall infections in patients suffering from CD where were treated with TNFα inhibitors versus with placebos.13

With TNFα inhibitors that are approved relatively recently, there are even less studies and data to draw conclusions from. Conversely, recently approved FDA or EMA drugs necessitate the greatest amount of scrutiny. These studies often lack the power to draw definitive assessments, which makes it difficult to come discover and warn the general public of the potential side effects that the therapies may bring.12

With numerous RCTs of TNFα inhibitors published now, it is possible to draw a more accurate conclusion and pooled estimate on the risk of TB infection. In addition, a quantified measurement of risk may be determined.
Methods

In an effort to make the data and the work more transparent, this meta-analysis was registered online in the prospective register of systematic reviews (PROSPERO) international database on February 8th, 2018 (PROSPERO ID: CRD42018087548). The following methodology adhered closely to the preferred reporting items for systematic reviews and meta-analyses (PRISMA) checklist (Appendix A).

Data Source and Search Strategy

A database search of PubMed was conducted up until January 21, 2018. The search terms and methodology used are as follows: biologic(s), tuberculosis, adalimumab, certolizumab, infliximab, TNFα inhibitors, anti-TNFα, or TNFα in conjunction with Crohn’s disease. The results were restricted to only double-masked, randomized, placebo-controlled trials. ClinicalTrials.gov supplemented the search methodology in the event of completed clinical trials with unpublished data. Studies that were irrelevant were screened out after reviewing the title and abstracts. Studies that made it past the initial screening had their full text and abstracts evaluated more closely.

Selection

Only sources written in English were considered. Studies qualified for inclusion if they were randomized, placebo-controlled, double-masked trials with adequate exposure in adult populations. Exposure was defined as receiving treatment of TNFα inhibitors (certolizumab pegol, adalimumab, and infliximab) that were approved for the treatment of adult (18 years or
older) Crohn’s disease by the European Medicines Agency and the U.S. Food and Drug Administration. As mentioned in the introduction, non-approved drugs, including those not in phase 3 clinical trials, and biosimilars of anti-TNFα therapy were excluded from the meta-analysis. All drug doses were included in the study. Duplicates and observational studies were screened out. Single studies with both an induction and maintenance phase but reported distinct patient groups were analyzed as two unique trials.

**Data Extraction**

Data was extracted onto a Microsoft Excel spreadsheet. First author, year of publication, study duration, number of participants in treatment and control groups, patient characteristics, treatment parameters (i.e. TNFα inhibitor and placebo), events in treatment and control groups, and screening method were recorded from each study. Studies found via ClinicalTrials.gov were also analyzed, with the aforementioned characteristics also recorded. Cases of TB infection was the primary outcome assessed in this meta-analysis. TB infection was defined as diagnosis of active tuberculosis by the clinician or other medical professional participating in the respective clinical trial.

**Risk difference**

Arcsine differences (ASD) were used as the measurement of risk differences. For a trial with $N_T$ subjects in the anti-TNFα treatment group, $N_C$ subjects in the control group, and $a$ and $b$ (following a standard 2x2 table; table 1) being the number of reported tuberculosis cases, respectively, the ASD for each individual trial can be calculated with Equation 1:
Table 1: Common 2x2 table

<table>
<thead>
<tr>
<th>Group</th>
<th>Yes</th>
<th>No</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>a</td>
<td>b</td>
<td>n_T</td>
</tr>
<tr>
<td>Control</td>
<td>c</td>
<td>d</td>
<td>n_C</td>
</tr>
<tr>
<td>Total</td>
<td>a + c</td>
<td>b + d</td>
<td>n</td>
</tr>
</tbody>
</table>

\[
\overline{ASD} = \arcsin\left(\frac{a}{n_T}\right) - \arcsin\left(\frac{b}{n_C}\right)
\]  

(Equation 1)

The use of arcsine difference for analysis can be dated as far back as the 1940s.\textsuperscript{20,21} The notable advantages to using ASD are that the variance of the point estimate (i.e. \(\overline{ASD}\)) is determined only by the sample size and that it deals with occurrences of 0 counts naturally, allowing for trials with 0 events in both arms of the study to be incorporated into meta-analyses.\textsuperscript{22} A nice graphical representation of arcsine transformation is shown below (figure 1).
Relative odds

The Yusuf-Peto method calculated odds ratios (ORs).\textsuperscript{23} Although commonly used, the Mantel-Haenszel method was not utilized because it cannot include deal with double-zero studies without substituting zero with a non-zero number. The Yusuf-Peto method provides a distinct advantage in that it includes single-zero studies; therefore, the Yusuf-Peto odds ratio ($OR_{Peto}$) was recognized as a (relatively) more efficient estimator, especially since treatment effects from trials are not large in number or the size of samples are similar between two groups.\textsuperscript{24} The $OR_{Peto}$ for an individual trial was calculated according to Equation 2:

\[
\text{Log}\, OR_{Peto} = (a - \frac{a+b}{n_T+n_C} * n_T)^* \frac{(n_T+n_C)^2/(n_T+n_C-1)}{(a+b)(n_T-a+n_C-b)n_T n_C} \quad \text{(Equation 2)}
\]

where $n_T, n_C, a$ and $b$ denote the same as in Equation 1.
However, the Yusuf-Peto method, once again, cannot deal with double-zero studies. As mentioned previously, known approaches used for meta-analysis on TNFα inhibitors are to exclude double-zero studies altogether and, in the event of single-zero studies, to change recorded zero counts by adding either 0.5 events or a number, usually around 0.5 in magnitude, that calculated based on inverse proportions to the relative size of the opposite drug (treatment or placebo) group.\textsuperscript{3,11,25} These analytical treatments lack any basis in biology or epidemiology.

**Epidemiologically-Based Background Correction**

Through the exclusion of double-zero studies, the obtained results will be biased away from the null hypothesis. Likewise, a continuity correction may bias the results towards the null. Thus, an epidemiologically-based background correction (EBC) was proposed and utilized. This approach estimated an expected number of cases (e.g., if there was an expected 0.01 tuberculosis case from an experimental arm, such a case would very likely not be observed. However, using 0.01 events to replace a 0 event would more adequately reflect the underlying epidemiology as opposed to continuity corrections of around 0.5). The epidemiologically-based background correction assumed an incidence of tuberculosis of 20 cases/100,000 person-years, which was the incidence reported for patients suffering from inflammatory bowel disease (IBD) in the United Kingdom.\textsuperscript{26} EBC was subsequently calculated according to Equation 3 below.

\[
\text{EBC} = n \times \frac{\text{Follow up duration (years)}}{100,000 \text{ person-years}} \times \frac{20 \text{ cases}}{100,000 \text{ person-years}} ~ \text{(Equation 3)}
\]

where \(n\) equals the number of subjects enrolled in either the treatment or the placebo groups.
The mathematics and reasoning behind the correction will be described in the following statements. The number of people in the control or treatment groups was multiplied by the length of time of TNFα inhibitor (or placebo) exposure (weeks) to produce exposure length in person-weeks. This was converted to person-years by dividing by 54 weeks. The converted exposure length (person years) was then multiplied by the incidence of TB in the United Kingdom among populations with inflammatory bowel disease, which was 20 cases/100,000 person years (Aberra et al., 2007). The end result were cases that were less than 1 but greater than zero. This allowed for sparse events, such as TB incidence, to be dealt with without using the continuity correction of 0.5. The EBC was then calculated and added into event counts recording both tuberculosis and non-tuberculosis for clinical trials with zero event occurrences.

Statistical analysis

Statistical analysis used the intent-to-treat principle. R version 3.4.3\(^2\), along with the “meta” package, was utilized to graph plots and calculate the Yusuf-Peto OR and the ASD along with corresponding confidence intervals (manual calculation on a Microsoft Excel spreadsheet verified calculation accuracy). Contribution weight from individual studies to the overall, pooled estimate was calculated based of the inverse variance of the point estimate from individual studies. The DerSimonian-Laird method calculated inter-study variance\(^2\). Two-sided \(P\) values of less than 0.05 with the 95% confidence interval (95% CI) excluding the null was statistically significant. Plots were recorded and taken using written code (Appendix B).
Meta-analysis

The beauty of meta-analysis is its ability to pool multiple low-powered studies to increase the overall power and synthesize data to generate a more conclusive conclusion than single studies can alone. Such ability to pool data lies heavily in statistics and mathematics. With meta-analysis, two models are primarily followed: fixed-effects and random effects.\(^{28}\) Under the fixed-effects model, population groups across studies are assumed to be relatively similar, with little variation. Under the random-effects model, populations are assumed to be heterogeneous and differ from one study to the next.\(^{29}\) In this investigation, the data synthesis will follow the random-effects model, as study participants were not only in different stands but also in different countries.

Thus, the overall point estimate, variance, and confidence intervals were calculated. The overall concept remained similar regardless of which whether the ASD or Yusuf-Peto method was utilized. Point estimates and variances were generated from each individual study. Weights were assigned to studies based on an inverse relationship with the respective variance. Thus, studies that had low numbers of study participants tended to have high variances, which subsequently lowered their weights. Studies that had many participants had lower variances and, subsequently, more weight.\(^{30}\)

The calculations used for the pooled ASD point estimate are as follows.

For an individual study, \(k\), the 2-arm variance can be estimated as \(v\), where \(v\) is calculated as

\[
v = \frac{1}{4n_T} + \frac{1}{4n_C},
\]

(Equation 4)
where \( n_T \) and \( n_C \) are defined as denoted in table 1.

Assume that there are \( K \) studies. For \( k = 1, \ldots, K \), the estimated treatment effect for the \( k^{th} \) study is \( \hat{\theta}_k \), and the standard error of this particular estimate is \( s_k \). The individual \( s_k \) is calculated by the following conversion

\[
s_k = \sqrt{v} \quad \text{(Equation 5)}
\]

The weight assigned to the estimates from the \( k^{th} \) study is denoted by \( w_k = \frac{1}{s_k^2} \).

Thus, the overall treatment effect point estimate is provided by

\[
\hat{\theta} = \frac{\sum_{k=1}^{K} \omega_k \hat{\theta}_k}{\sum_{k=1}^{K} \omega_k}, \quad \text{(Equation 6)}
\]

and the standard error, SE, is provided by

\[
SE = \sqrt{\frac{1}{\sum_{k=1}^{K} \omega_k}}. \quad \text{(Equation 7)}
\]

The calculations used for the pooled Yusuf-Peto odds ratio point estimate are as follows.
For an individual study, $k$, the variance can be estimated by $v$, where $v$ is calculated as

$$v = \frac{(a+c)(b+d)n_Tn_C}{n^2(n-1)}.$$  \hspace{1cm} \text{(Equation 8)}

and $E$ calculated as

$$E = \frac{(a+c)(b+d)}{n}$$ \hspace{1cm} \text{(Equation 9)}

where $a, c, b, d, n, n_T$, and $n_C$ are all defined once again by table 1.

The overall pooled point estimate is subsequently calculated as

$$OR_{pco} = \frac{\sum_{k=1}^{K}(a_k-E_k)}{\sum_{k=1}^{K}v_k},$$ \hspace{1cm} \text{(Equation 10)}

and the standard error, $SE$, calculated as

$$SE = \sqrt{\frac{\sum_{k=1}^{K}v_i}{\sum_{k=1}^{K}v_i}}.$$ \hspace{1cm} \text{(Equation 11)}
Results

Search Results

From PubMed, a total of 748 articles were located. Titles and abstracts were reviewed to determine eligibility. Studies that were clearly not relevant, 706 studies in total, to the meta-analysis were excluded. The remaining 42 articles were more closely examined to determine inclusion in the analysis. Six studies were excluded because they were not head-to-head or placebo-controlled, 5 did not study FDA or EMA approved drugs, 4 were not placebo-controlled, and 3 studies were duplicates in that they measured the same sample. All-in-all, 19 of the 42 studies were not included in the analysis. Two additional trials were located through clinicaltrials.gov; of which, one (NCT00291668) did not post the results and was excluded. Thus, a total of 23 studies were included in the meta-analysis. The selection process was summarized in Figure 2 below.
The 23 studies evaluated adalimumab (number of studies (n) = 7; 1726 patients), certolizumab pegol (n = 6; 2008), and infliximab (n = 10; 1935). For this analysis, both induction and maintenance studies were analyzed. A total of 5669 patients were enrolled in the clinical trials. Of those enrolled, a total of 3275 patients were in the treatment group and a total of 2394...
patients were in the control group. The treatment arm sizes were mostly similar to control arm sizes, with a median ratio being 1.03:1. Nonetheless, some studies had unbalanced arm sizes (maximum ratio = 3.32:1, average ratio = 1.64:1). Follow-up duration ranged anywhere from 4 to 104 weeks (mean follow-up duration = 32 weeks). A grand total of approximately 79,000 person-years was exposed to either anti-TNFα therapy or placebo throughout the duration of all the clinical trials (treatment = 46,000 person-years, control = 33,000 in person-years). Dates of publication ranged from 1997 to 2016. Six cases of tuberculosis were recorded, and all of these cases were reported in treatment groups. Two cases of TB were recorded in trials using adalimumab, 1 using certolizumab pegol, and 3 using infliximab. A summary of the trials’ specifics – follow-up duration, treatment, cases, and publication year – is presented in table 2.

Table 2. Summary of randomized, placebo-controlled, double-masked trials included. N indicated number of total subjects; n indicated number of tuberculosis cases.

<table>
<thead>
<tr>
<th>Study</th>
<th>Follow-up Duration (weeks)</th>
<th>Anti-TNFα Treatment</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>N</td>
<td>n</td>
</tr>
<tr>
<td><strong>Adalimumab</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hanauer et al., 200647</td>
<td>4</td>
<td>0</td>
<td>225</td>
</tr>
<tr>
<td>Colombel et al., 200750</td>
<td>52</td>
<td>2</td>
<td>517</td>
</tr>
<tr>
<td>Sandborn et al., 2007a38</td>
<td>52</td>
<td>0</td>
<td>37</td>
</tr>
<tr>
<td>Sandborn et al., 2007b37</td>
<td>4</td>
<td>0</td>
<td>159</td>
</tr>
</tbody>
</table>

17
<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>N1</th>
<th>N2</th>
<th>N3</th>
<th>N4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rutgeerts et al., 2012⁴⁰</td>
<td>48</td>
<td>0</td>
<td>64</td>
<td>0</td>
<td>65</td>
</tr>
<tr>
<td>Watanabe et al., 2012³²</td>
<td>52</td>
<td>0</td>
<td>25</td>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td>Watanabe et al., 2012³²</td>
<td>4</td>
<td>0</td>
<td>67</td>
<td>0</td>
<td>23</td>
</tr>
<tr>
<td>Total:</td>
<td>1094</td>
<td>632</td>
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**Certolizumab Pegol**

<table>
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<th>N2</th>
<th>N3</th>
<th>N4</th>
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</thead>
<tbody>
<tr>
<td>Winter et al., 2004³¹</td>
<td>12</td>
<td>0</td>
<td>66</td>
<td>0</td>
<td>24</td>
</tr>
<tr>
<td>Schreiber et al., 2005³³</td>
<td>20</td>
<td>0</td>
<td>145</td>
<td>0</td>
<td>73</td>
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<tr>
<td>Sandborn et al., 2007c³⁹</td>
<td>26</td>
<td>0</td>
<td>331</td>
<td>0</td>
<td>329</td>
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<tr>
<td>Schreiber et al., 2007³⁴</td>
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<td>1</td>
<td>216</td>
<td>0</td>
<td>212</td>
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<td>Sandborn et al., 2011³⁶</td>
<td>6</td>
<td>0</td>
<td>223</td>
<td>0</td>
<td>215</td>
</tr>
<tr>
<td>NCT00349752, 2014⁴⁵</td>
<td>36</td>
<td>0</td>
<td>87</td>
<td>0</td>
<td>87</td>
</tr>
<tr>
<td>Total:</td>
<td>1068</td>
<td>940</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

**Infliximab**

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
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<th>N2</th>
<th>N3</th>
<th>N4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Targan et al., 1997¹⁶</td>
<td>12</td>
<td>0</td>
<td>83</td>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td>D’Haens et al., 1999⁴⁹</td>
<td>4</td>
<td>0</td>
<td>22</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>Present et al., 1999⁴⁴</td>
<td>18</td>
<td>0</td>
<td>63</td>
<td>0</td>
<td>31</td>
</tr>
<tr>
<td>Rutgeerts et al., 1999⁴¹</td>
<td>36</td>
<td>0</td>
<td>37</td>
<td>0</td>
<td>36</td>
</tr>
<tr>
<td>Hanauer et al., 2002⁴⁸</td>
<td>44</td>
<td>1</td>
<td>385</td>
<td>0</td>
<td>188</td>
</tr>
</tbody>
</table>
The risk difference between anti-TNFα therapy and placebo was found to be 0.028 (95% CI, 0.0011-0.05; \( P < 0.05 \)) (Table 3). The random-effect model results are presented for the ASD. The fixed effects results for the Peto OR are also presented, although the inter-study variance did not additionally contribute to the total variance of the pooled OR (the DerSimonian-Laird estimate of between study variance was zero).

Table 3. Risk of TB associated with the use of TNFα inhibitors in patients with Crohn’s disease.

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Risk estimate</th>
<th>95% Confidence interval</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Risk difference</strong></td>
<td>23</td>
<td>0.028</td>
<td>(0.0011, 0.055)</td>
<td>0.042</td>
</tr>
<tr>
<td><strong>Odds ratio</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Including double-zero studies</td>
<td>23</td>
<td>4.85</td>
<td>(1.02, 22.99)</td>
<td>0.047</td>
</tr>
<tr>
<td>Excluding double-zero studies</td>
<td>5</td>
<td>5.85</td>
<td>(1.13, 30.38)</td>
<td>0.036</td>
</tr>
</tbody>
</table>
The respective weights for each drug, adalimumab, certolizumab pegol, and infliximab, were 28.9%, 36.9%, and 34.2%. The risk differences were 0.028, 0.015, and 0.042 respectively (Figure 3).
Figure 3. Risk difference of tuberculosis infection between patients suffering from Crohn’s disease treated with TNFα inhibitors and those treated with placebo.

The risk difference was calculated via arcsine transformation of TB infection incidence (Arcus Sinus Difference, ASD) and was indicated by the numbers on x-axis. Weight indicated the percentage contribution of the individual study to the pooled estimation. This weight corresponded to the size of the black box. The x-axis indicated risk difference. Vertical dashed line indicated the pooled point estimate. Solid horizontal lines described the 95% confidence interval (CI). The solid diamond shows the overall, pooled confidence interval for each individual TNFα inhibitor and for the entire study.

A funnel plot of the ASDs (Figure 4) was also graphed, indicating that trials that had smaller risk differences were more often published. However, this does not suggest publication bias because risk of tuberculosis was neither a reason for publishing the clinical trials nor the
primary focus of the studies. To the contrary, the funnel plot showed that larger studies had a higher likelihood of detecting rare risks (TB infection) than smaller ones.

Figure 4. Relationship between the estimated tuberculosis risk difference and the corresponding standard error of the estimate.

The center, dashed vertical line indicated the pooled calculated. The diagonal lines indicated the respective 95% confidence intervals associated with the expected mean ASD for clinical trials enrolling a variable number of study subjects.

Relative odds

The treatment arm sizes were comparable to the control arm sizes. A median ratio of treatment arm size to control arm size was calculated at 1.03. However, specific studies had particularly unbalanced arm sizes, with a maximum ratio of 3.32:1 and an average ratio = 1.64:1.

The odds ratio was calculated at 4.85 (95% CI, 1.02-22.99; \( P<0.05 \)) with the EBC and 5.85 (95% CI, 1.13-30.38; \( P<0.05 \)) without the EBC (Table 3). The random effects model could not be used because the Yusuf-Peto odds ratio calculations follows the assumption of a fixed effects model.\(^{23} \) Weights for adalimumab, certolizumab pegol, and infliximab were 31.1%,
18.2%, and 50.7% with EBC, respectively (Figure 5). Without the EBC, only 5 studies could be included (1 adalimumab trial, 1 certolizumab pegol trial, and 3 infliximab trials).
Figure 5. The odds of patients developing active TB infection when treated with TNFα inhibitors for their Crohn’s disease relative to those treated with placebo.

<table>
<thead>
<tr>
<th>Study</th>
<th>Anti-TNF</th>
<th>Control</th>
<th>Odds Ratio</th>
<th>OR</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TB cases</td>
<td>Total</td>
<td>TB cases</td>
<td>Total</td>
<td></td>
</tr>
<tr>
<td>Adalimumab</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hanauer et al., 2006</td>
<td>0.0035</td>
<td>225</td>
<td>0.0011</td>
<td>74</td>
<td>1.05</td>
</tr>
<tr>
<td>Colombe et al., 2007</td>
<td>2.1031</td>
<td>517</td>
<td>0.0521</td>
<td>281</td>
<td>4.05</td>
</tr>
<tr>
<td>Sandborn et al., 2007a</td>
<td>0.0074</td>
<td>37</td>
<td>0.0036</td>
<td>18</td>
<td>1.00</td>
</tr>
<tr>
<td>Sandborn et al., 2007b</td>
<td>0.0024</td>
<td>159</td>
<td>0.0025</td>
<td>166</td>
<td>1.00</td>
</tr>
<tr>
<td>Rutgeerts et al., 2012</td>
<td>0.0118</td>
<td>64</td>
<td>0.0120</td>
<td>65</td>
<td>1.00</td>
</tr>
<tr>
<td>Watanabe et al., 2012</td>
<td>0.0050</td>
<td>25</td>
<td>0.0050</td>
<td>25</td>
<td>1.00</td>
</tr>
<tr>
<td>Watanabe et al., 2012</td>
<td>0.0010</td>
<td>67</td>
<td>0.0004</td>
<td>23</td>
<td>0.85</td>
</tr>
<tr>
<td>Fixed effect model</td>
<td>1094</td>
<td>632</td>
<td></td>
<td></td>
<td>3.90</td>
</tr>
<tr>
<td>Certolizumab pegol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Winter et al., 2004</td>
<td>0.0030</td>
<td>66</td>
<td>0.0011</td>
<td>24</td>
<td>0.99</td>
</tr>
<tr>
<td>Schreiber et al., 2005</td>
<td>0.0111</td>
<td>145</td>
<td>0.0056</td>
<td>73</td>
<td>1.00</td>
</tr>
<tr>
<td>Sandborn et al., 2007c</td>
<td>0.0330</td>
<td>331</td>
<td>0.0328</td>
<td>329</td>
<td>1.00</td>
</tr>
<tr>
<td>Schreiber et al., 2007</td>
<td>1.0166</td>
<td>216</td>
<td>0.0163</td>
<td>212</td>
<td>6.81</td>
</tr>
<tr>
<td>Sandborn et al., 2011</td>
<td>0.0051</td>
<td>223</td>
<td>0.0049</td>
<td>215</td>
<td>1.00</td>
</tr>
<tr>
<td>NCT00349752, 2014</td>
<td>0.0120</td>
<td>87</td>
<td>0.0120</td>
<td>87</td>
<td>1.00</td>
</tr>
<tr>
<td>Fixed effect model</td>
<td>1068</td>
<td>940</td>
<td></td>
<td></td>
<td>5.59</td>
</tr>
<tr>
<td>Infliximab</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Targan et al., 1997</td>
<td>0.0038</td>
<td>83</td>
<td>0.0012</td>
<td>25</td>
<td>0.95</td>
</tr>
<tr>
<td>D'haens et al., 1999</td>
<td>0.0003</td>
<td>22</td>
<td>0.0001</td>
<td>8</td>
<td>1.09</td>
</tr>
<tr>
<td>Present et al., 1999</td>
<td>0.0043</td>
<td>63</td>
<td>0.0021</td>
<td>31</td>
<td>1.01</td>
</tr>
<tr>
<td>Rutgeerts et al., 1999</td>
<td>0.0551</td>
<td>37</td>
<td>0.0050</td>
<td>36</td>
<td>0.99</td>
</tr>
<tr>
<td>Hanauer et al., 2002</td>
<td>1.0850</td>
<td>385</td>
<td>0.0317</td>
<td>188</td>
<td>3.89</td>
</tr>
<tr>
<td>Sands et al., 2004</td>
<td>0.0213</td>
<td>139</td>
<td>0.0219</td>
<td>143</td>
<td>1.00</td>
</tr>
<tr>
<td>Léman et al., 2006</td>
<td>0.0114</td>
<td>57</td>
<td>0.0116</td>
<td>58</td>
<td>1.00</td>
</tr>
<tr>
<td>Colombe et al., 2010</td>
<td>1.0194</td>
<td>169</td>
<td>0.0196</td>
<td>170</td>
<td>6.89</td>
</tr>
<tr>
<td>Regueiro et al., 2011</td>
<td>0.0022</td>
<td>11</td>
<td>0.0026</td>
<td>13</td>
<td>1.00</td>
</tr>
<tr>
<td>Regueiro et al., 2016</td>
<td>1.0586</td>
<td>147</td>
<td>0.0598</td>
<td>150</td>
<td>6.09</td>
</tr>
<tr>
<td>Fixed effect model</td>
<td>1113</td>
<td>822</td>
<td></td>
<td></td>
<td>5.26</td>
</tr>
<tr>
<td>Fixed effect model</td>
<td>3275</td>
<td>2394</td>
<td></td>
<td></td>
<td>4.85</td>
</tr>
</tbody>
</table>

Odds ratio (OR) was calculated using the Yusuf-Peto method and indicated by the numbers on the x-axis. Number of tuberculosis infection cases was corrected with a background TB incidence, the EBC. Once again, the weight was the percentage contribution of an individual study to the pooled estimation. The size of the black box is proportional to the weight of the study. The vertical dashed line indicated the pooled odds ratio. The solid horizontal lines show the respective 95% confidence interval (CI). The black diamond shows the overall confidence interval for each biologic and for the entire analysis.

**Number Needed to Harm**

With a background TB infection incidence in patients equivalent to 20 cases/100,000 person-years, one active tuberculosis infection case would be expected from a total of 5,000 Crohn’s disease patients who do not use TNFα inhibitors within one year. An ASD of 0.028 would mean a tuberculosis incidence of about 177 cases/100,000 person-years; thus, 1 active TB infection
case would be expected when treating 565 patients with anti-TNFα therapy for one year (Table 4). If the harmful effects of TNFα inhibitors are described on a multiplicative scale, as is the case with the pooled Yusuf-Peto odds ratio, the numbers of patients treated to expect 1 tuberculosis case can be anywhere from 855 to 1031 (Table 4).

Table 4. Estimated incidence of active TB infection in patients with Crohn’s disease treated with anti-TNFα therapy.

<table>
<thead>
<tr>
<th>Incidence of tuberculosis with TNFα treatment (cases/person-years)</th>
<th>Number of patients treated to see one tuberculosis case in one year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Based on risk difference</td>
<td>177/100,000</td>
</tr>
<tr>
<td>Based on relative odds estimated</td>
<td>97/100,000</td>
</tr>
<tr>
<td>with background correction</td>
<td></td>
</tr>
<tr>
<td>Based on relative odds estimated</td>
<td>117/100,000</td>
</tr>
<tr>
<td>without background correction</td>
<td></td>
</tr>
</tbody>
</table>

The number of patients with Crohn’s disease treated with TNFα inhibitors to expect one active TB infection case is also shown. The background incidence was assumed to be 20 cases/100,000 person-years.20
Discussion

Summary

This investigation advances the current state of knowledge on the association between anti-TNF\(\alpha\) therapy and active tuberculosis infection in patients with Crohn’s disease. First, a non-biased estimation of active tuberculosis infection risk associated with the use of TNF\(\alpha\) inhibitors for the treatment of Crohn’s disease was performed by using arcsine transformations of tuberculosis infection incidence, which enabled all qualified studies, including double-zero studies, to be included in the meta-analysis. Secondly, a novel, epidemiologically-based background correction was developed and used to adjust for zero count events, enabling the inclusion of double-armed zero event studies into the estimation of the relative effect (odds ratio). Finally, with the use of the aforementioned analytical approaches, a significant increase of tuberculosis infection risk with the usage of TNF\(\alpha\) inhibitors for the treatment of adult Crohn’s disease was demonstrated from existing evidence, challenging the findings of previous studies.

Meta-analysis

In this investigation, all qualified clinical trials with public data were included, amounting to 23 studies in total. Among these 23 studies, 18 (78\%) had no reported cases of tuberculosis infection from either the TNF\(\alpha\) inhibitor treatment or the placebo treatment. These 18 studies would have been excluded if the arcsine difference was not used and had the analysis followed methods that previous meta-analyses regarding this particular area of interest took. The abundance of double-zero observations was expected. Tuberculosis infection, while a relatively more common bacterial infection, still qualifies as a rare event in the Americas, Europe, Austria,
South Africa, and Japan, which was where these RCTs were conducted. In meta-analysis, a rare event has less than a 1/1,000 chance of occurrence. The median sample size of the control groups across the 23 studies was 73 people, and the median follow-up duration was 30 weeks. About 0.0084 tuberculosis cases would be expected, mathematically, in control group if the background tuberculosis followed the incidence of 20 cases/100,000 person-years as reported by Aberra et al. For reference, if TNFα inhibitors had increased the risk of tuberculosis infection by 5 times, there would be about a 4% chance to observe 1 event in the anti-TNFα treatment arm. Meta-analysis provides the ability and opportunity to pool multiple studies together to increase the probability of observing a single tuberculosis infection case. Furthermore, discarding double-zero studies (78% of the studies in our analysis) might decrease the value of meta-analysis.

The risk difference calculated using the arcsine transformation of incidence was considered as the primary results. The ASD method does not need any correction for zero-event counts. Additionally, the ASD estimate provides another advantage in that it is not contingent on the effect size or the balance of sample sizes between the treatment and control groups. These analytical features greater utility over either the Yusuf-Peto method or the Mantel-Haenszel method. However, a more intuitive understanding of risk of TB infection may be better described on a multiplicative scale as a ratio. Thus, further analysis was performed, for which the Yusuf-Peto method was the approach of choice because, compared to the Mantel-Haenszel method, it can handle zero events in single arms. Even with non-zero events, the Mantel-Haenszel method lacks sufficient power and can be substantially worse than other statistical methods when
Funnels are less than 1 in 1,000. Unfortunately, the Yusuf-Peto method cannot handle double-zero studies.

**Funnel Plot**

A funnel plot graphing ASDs of the studies (figure 3) versus their standard errors was relatively symmetric and followed an inverted funnel shape, showing a low risk of publication bias, selective outcome reporting, or selective analysis reporting (Egger et al., 1997). However, it is still unclear if funnel plots really diagnose publication bias (Lau et al., 2006). Since risk of TB was not the primary reason the clinical trials were conducted, it is more likely that publication bias is less of a concern.

**Epidemiological Background Correction**

An epidemiologically based background correction (i.e., EBC) was proposed in order to mathematically replace zero events. The formula to calculate the corrections was based off the exposure length (person-years) and the UK TB incidence rate in IBD populations to produce a non-zero number of TB cases. In the event that metrics other than ASD (i.e. odds ratio, hazards ratio or rate ratio) are estimated and that the event of interest is so rare that even one occurrence would not be expected, the EBC is recommended for continuity corrections instead of adding 0.5, similar corrections based on the ratio of the sample size between treatment and control groups, or statistical-model based estimates. The latter approaches are not rooted in epidemiology and lack biological considerations. In the case of adding 0.5 or a similar modifier, the correction artificially makes a much larger background incidence than there actually is.
example, in this study, it would have boosted the background incidence by about a factor of 60. Thus, the common artificial continuity correction was not used was due to its inaccuracy and invalid conclusion regarding small sample size, small number of studies, or low event rates.52

Limitations

All data from all clinical trials were assumed to be available and that no bias due to unpromising results not being reported or due to patient withdrawals were present. Additionally, if a study did not report TB outcomes, then it was assumed that that there were 0 cases of TB in that study. The FDA mandates that serious infections are reported in clinical trials, which TB is defined as54. However, it is possible that cases of TB were reported under serious infections or opportunistic infections without being reported in the study.

There are some notable limitations with the use of the EBC. The EBC was based on the tuberculosis incidence rate in the UK IBD populations up until 1997, before the first TNFα inhibitors were available to the public. In the decades since then, it is possible that TB incidences in these UK populations have changed. The reported incidence also takes into account that the Crohn’s patients likely used certain medications that increased their risk of infection overall, such as corticosteroids. Additionally, although most of the studies were conducted in Western countries, the TB infection incidence of Crohn’s patients in the United Kingdom may also not truly represent the average TB infection incidence of the countries in which the clinical trials were conducted. Furthermore, the TB infection incidence rate was that found in populations with IBD overall, which may not be representative of TB infection incidence in populations with
Crohn’s disease. Since the correction assumed the incidence of TB is identical with or without TNFα inhibitor usage in the clinical trials, the results are also biased towards the null.

In the TB incidence is closer to that found in the general population in the UK, which is 9 cases/100,000 person-years, then the double-zero studies included in the meta-analysis would have a lower calculated weight. That would result in the five studies with reported cases of TB infection having a higher contribution to the pooled estimate, leading to an overall ratio that is higher (closer to the 8.85 times increased risk) with a larger confidence interval than the Peto OR calculated with the determined correction but smaller than the Peto OR calculated without the double-zero studies.

Aside from the approaches to avoid Simpson’s paradox, the ability to pool distinct results from individual clinical trials in this meta-analysis resided in the fact that each study had a placebo-treatment arm. Therefore, the impact from having different study populations was largely minimized, as either the end point (risk difference or odds ratio) mostly reflected the effect of TNFα inhibitors alone (the impact of confounders was either subtracted out [for ASD] or divided out [for ORs]). Thus, between-study heterogeneity such as geographic location, population characteristics, exposure, maintenance vs. induction trials, and screening methods is assumed to be minimized. Within study heterogeneity such as age, geographic location, exposure, and race is assumed to be reduced by the random assignment of participants to treatment and control groups.

All the studies were in English, so the generalizability of the results may be limited, especially considering that the demographics and trends of both Crohn’s disease and tuberculosis infection differ between western and eastern countries. The included studies were also mainly
EMA- and FDA-regulated clinical trials conducted in western countries. In fact, only one study that enrolled largely Asian populations was included in this meta-analysis. In this regard, EBC may also be compromised, since the prevalence and incidence of tuberculosis is higher in Asian countries. Thus, much caution should be taken when extrapolating and applying results from this analysis to predict TB infection risk of using anti-TNFα therapy to treat Crohn’s disease in non-western countries.

**Screening**

Special attention should be paid to screening. Patients could have had either latent TB infection that was reactivated or acquired TB infection through exposure. The screening methods varied by trial and often went unreported. Additionally, screening out patients based on a positive tuberculin skin test may bias certain patient population groups due to the prevalence of BCG vaccinations in non-western countries. Furthermore, screening out patients based on a positive tuberculin skin test may have different impacts on the tuberculosis occurrence due to the different practices of Bacillus Calmette-Guérin vaccinations. Thus, certain study groups may have less immigrant participants enrolled. That would therefore lower the expected number of TB infection cases. Finally, two studies did not report screening methods. Closer examination of the details of trial screenings may provide additional insight on the nature of TB infection – whether it is acquired or reactivated.
Future Research

Much still remains uncertain about the etiology Crohn’s disease. Current literature suggests that Crohn’s disease might be caused by an immune response to enteric bacteria. Recent research also points to Crohn’s disease being intimately linked with MAP, a TB-like bacterium. The use of anti-TNFα therapy in these patients could even favor MAP infection and worsen the patient condition, which is what was found in this study. Currently, it is difficult to come to conclusions about possible linkages, considering that the RCTs did screen or test for MAP infection – much less reported it. Additional research could be done on analyzing patient outcomes and determining which patients were infected with MAP and their susceptibility to overall, serious, or TB infection was.

Much more research could be done on the EBC. Specifically, mathematically modeling to determining the validity of such a correction as compared to previous existing methodologies would elucidate the strengths and weaknesses of such an approach. This is not the first time a novel correction was introduced. In a 2016 study, Bai et al. used a similar correction that could adjust for zero outcomes when events are rare. Thus, more needs to be elucidated on the current meta-analytical approaches, tools, and corrections so that data from clinical trials can be more adequately assessed.

Additional models can be explored as an avenue to analyze rare event data. The beta-binomial model holds much promise; however, its programming and transformations can be difficult to interpret. Nonetheless, future research and analyses could explore and investigate the pros and cons to using the beta-binomial method.
Finally, future meta-analysis could analyze other adverse events reported by clinical trials. Such topics of interest include risk of serious infection and overall infection. The topic of interest could be extended or narrowed. For example, further analysis could be done on anti-TNFα therapy as a whole or study pediatric subpopulations.
Conclusion

All in all, this study concluded that there is sufficient evidence to support the association between TNFα inhibitors use and an increased the risk of developing tuberculosis in patients with Crohn’s disease. Twenty-three studies were analyzed with multiple statistical methods consistently providing significant risk. To current knowledge, all these 23 studies represent the most comprehensive and appropriate literature available for the topic at hand, with an extensive search and careful review conducted. No studies written in English were excluded, provided that a placebo control was present and that they were randomized and masked. The randomization of patients and clinicians minimized potential confounding agents such as age, duration of IBD, and disease activity.

The results challenge findings from previous studies, which all reported no significantly increased risk of tuberculosis infection associated with TNFα inhibitor usage among patients with Crohn’s disease.\textsuperscript{11,13} Based on the pooled risk difference (ASD) calculated in this study, on average 565 patients treated with TNFα inhibitors would be expected to result in 1 patient getting infected with TB, versus 5000 patients electing therapy other than TNFα inhibitors producing 1 case of TB, assuming the background incidence of tuberculosis in moderately severe Crohn’s disease is similar to the reported rates in the UK IBD population.
Appendix A: PRISMA Checklist for Meta-Analysis
### PRISMA 2009 Checklist

<table>
<thead>
<tr>
<th>Section/Topic</th>
<th># Checklist Item</th>
<th>Reported on page</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TITLE</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Title</td>
<td>Identify the report as a systematic review, meta-analysis, or both.</td>
<td></td>
</tr>
<tr>
<td><strong>ABSTRACT</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abstract</td>
<td>Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations, conclusions and implications of key findings; systematic review registration number.</td>
<td></td>
</tr>
<tr>
<td><strong>INTRODUCTION</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rationale</td>
<td>Describe the rationale for the review in the context of what is already known.</td>
<td></td>
</tr>
<tr>
<td>Objectives</td>
<td>Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICO5).</td>
<td></td>
</tr>
<tr>
<td><strong>METHODS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protocol and registration</td>
<td>Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.</td>
<td></td>
</tr>
<tr>
<td>Eligibility criteria</td>
<td>Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.</td>
<td></td>
</tr>
<tr>
<td>Information sources</td>
<td>Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies in the search and data last searched).</td>
<td></td>
</tr>
<tr>
<td>Search</td>
<td>Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.</td>
<td></td>
</tr>
<tr>
<td>Study selection</td>
<td>State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).</td>
<td></td>
</tr>
<tr>
<td>Data collection process</td>
<td>Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.</td>
<td></td>
</tr>
<tr>
<td>Data items</td>
<td>List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.</td>
<td></td>
</tr>
<tr>
<td>Risk of bias in individual studies</td>
<td>Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.</td>
<td></td>
</tr>
<tr>
<td>Summary measures</td>
<td>State the principal summary measures (e.g., risk ratio, difference in means).</td>
<td></td>
</tr>
<tr>
<td>Synthesis of results</td>
<td>Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I2) for each meta-analysis.</td>
<td></td>
</tr>
<tr>
<td><strong>RESULTS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Risk of bias across studies</td>
<td>Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).</td>
<td></td>
</tr>
<tr>
<td>Additional analyses</td>
<td>Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.</td>
<td></td>
</tr>
<tr>
<td><strong>DISCUSSION</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Summary of evidence</td>
<td>Summarize the main findings including the strength of evidence for each main outcome, consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).</td>
<td></td>
</tr>
<tr>
<td>Limitations</td>
<td>Discuss limitations at study and outcome level (e.g., risk of bias) and at review level (e.g., incomplete retrieval of identified research, reporting bias).</td>
<td></td>
</tr>
<tr>
<td>Conclusions</td>
<td>Provide a general interpretation of the results in the context of other evidence, and implications for future research.</td>
<td></td>
</tr>
<tr>
<td><strong>FUNDING</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Funding</td>
<td>Describe sources of funding for the systematic review and other support (e.g., supply of data, role of funders for the systematic review).</td>
<td>36</td>
</tr>
</tbody>
</table>
Appendix B: R Code for Plot and Image Generation
Appendix B-1: Arcsine R Code (forest plot and funnel plot)

dat<-read.csv(file="<file location>", header=TRUE, sep=" ");

CD <- metabin(event.e=ai, n.e=n1, event.c=ci, n.c=n2, data = dat, sm = "ASD", byvar=Drug, studlab=paste(Authors), print.byvar=FALSE); CD;

pdf(file = "<file location/figure name.pdf>", width = 8.5, height = 11);
forest(CD, leftcols=c("studlab"), comb.fixed=FALSE, hetstat =FALSE, digits=4, col.by="black", col.diamond="black", col.square="black"); dev.off();

jpeg(file="<file location/figure name.jpeg>", width = 10, height = 10, units = 'in', res = 600);
forest(CD, leftcols=c("studlab"), comb.fixed=FALSE, hetstat =FALSE, digits=4, col.by="black", col.diamond="black", col.square="black"); dev.off();

tiff(file="<file location/figure name.tiff>", width = 10, height = 10, units = 'in', res = 600);
forest(CD, leftcols=c("studlab"), comb.fixed=FALSE, hetstat =FALSE, digits=4, col.by="black", col.diamond="black", col.square="black"); dev.off();

jpeg(file="<file location/figure name_funnel.jpeg>", width = 10, height = 8, units = 'in', res = 600);
par(ps = 13, cex = 1.5, cex.main = 1); funnel(CD, bg="black", axes=FALSE); axis(1, at=seq(-0.4, 0.5, 0.1)); axis(2); dev.off();
Appendix B-2: Yusuf-Peto R Code (forest plot)

dat<‐read.csv(file="<file location>","); dat;

CD <‐ metabin(event.e=ai, n.e=n1, event.c=ci, n.c=n2, data = dat, sm = "OR", method = "Peto",
byvar=Drug, studlab=paste(Authors), print.byvar=FALSE); CD;

pdf(file = "<file location/figure name.pdf>", width = 8.5, height = 11);
forest(CD, comb.random=FALSE, hetstat =FALSE, col.by="black", col.diamond="black",
col.square="black", rightcols=c("w.fixed"), xlim=c(0.01, 400), colgap="4mm", digits.weight=3);
dev.off();

jpeg(file=""<file location/figure name.jpeg> ",
width = 10, height = 10, units = 'in', res = 600);
forest(CD, comb.random=FALSE, hetstat =FALSE, col.by="black", col.diamond="black",
col.square="black", rightcols=c("w.fixed"), xlim=c(0.01, 400), colgap="4mm", digits.weight=3);
dev.off();

tiff(file="<file location/figure name.tiff>" , width = 10, height = 10, units = 'in', res = 600);
forest(CD, comb.random=FALSE, hetstat =FALSE, col.by="black", col.diamond="black",
col.square="black", rightcols=c("w.fixed"), xlim=c(0.01, 400), colgap="4mm", digits.weight=3);
dev.off().
References


