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Review

Diagnostic Performance of Western Blot for Congenital Toxoplasmosis: A Systematic Review and Meta-Analysis

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Abstract

Background: Congenital toxoplasmosis results from infection with the parasite Toxoplasma gondii, which is transmitted from mother to child during pregnancy. Although Western blot is considered the most sensitive diagnostic tool for congenital toxoplasmosis, its diagnostic performance has not been subjected to meta-analysis. **Methods:** We conducted a systematic review and meta-analysis by performing literature searches across PubMed, Scopus, and Web of Science. The search strategy included the terms "western blot OR immunoblot" AND "congenital toxoplasmosis." The selected studies were required to meet specific inclusion criteria, which involved comparing the performance of the western blot test against the gold standard criteria for permanence of IgG after 10 months of age. These studies had to be case and control studies. The data obtained from the studies were then organized into an evidence synthesis table and the sensitivity, specificity, and Diagnostic Odds Ratio (DOR) index were calculated. This meta-analysis was performed in compliance with the recommendations of PRISMA guidelines. **Results:** After evaluating the selection criteria, we identified 44 articles; however, only 10 were selected for the meta-analysis. Western blot assay demonstrated a pooled sensitivity of 93.8% (95% CI: 79.2-98.4) and a pooled specificity of 96.6% (95% CI: 89.8-98.9) for the diagnosis of congenital toxoplasmosis. Six of the 10 studies had a DOR higher than 300, whereas the in-house method yielded a lower DOR of 1.2. **Conclusions:** This meta-analysis confirmed the utility of well-standardized western blot tests as a dependable diagnostic approach for congenital toxoplasmosis in terms of both sensitivity and specificity.

Introduction

Toxoplasmosis is a disease caused by the parasite Toxoplasma gondii, which has a distinct reproductive cycle. The extraintestinal asexual cycle occurs in intermediate hosts, such as humans, whereas the intraintestinal sexual cycle occurs in definitive hosts, such as felines. The parasite has different stages, including tachyzoites that replicate during acute infection, bradyzoites that multiply slowly during latent tissue infections, and oocysts that are excreted in cat feces.[1] Infection can occur through the consumption of contaminated food, water, and other materials that contain the resistant form of the parasite, oocysts.[1] There is also a possibility of transmission through blood transfusions or organ transplants.[1] The human host is where the parasite forms cysts in tissues, particularly in the muscles, heart, brain, and eyes. The transmission of the parasite to the fetus can occur, causing severe damage if the mother is infected for the first time during pregnancy.[1]

The symptoms of toxoplasmosis in immunocompetent adults vary and can be mild or flu-like in the presence of cervical lymphadenopathy.[1] Complications of the central nervous system, such as hemiplegia, may occur in immunocompromised individuals.[1] Infected newborns can develop permanent sequelae such as hydrocephalus, intracranial calcifications, seizures and chorioretinitis.[1] Its diagnosis is particularly difficult because as many as 40% of children infected with T. gondii do not exhibit the traditional diagnostic markers, such as the presence of IgM or IgA anti-Toxoplasma antibodies. Additionally, a significant number of cases are asymptomatic at birth, but may develop retinal lesions later in life.[2] The western blot technique has become a key support for making an early diagnosis, allowing, according to some authors, the identification of up to 90% of cases before the first month of life.[2] In newborns, the diagnosis can be challenging, especially if the mother receives prenatal treatment and the newborn is asymptomatic.[1] It is recommended to combine tests for diagnosis in newborns, such as IgA anti-Toxoplasma and PCR in cerebrospinal fluid.[3] It is important to note that a definitive diagnosis of congenital toxoplasmosis is achieved if IgG anti-Toxoplasma antibodies are still present in the newborn at ten months of age, indicating that these are not IgG transmitted by the mother and confirm newborn production. This is particularly important in asymptomatic newborns, where treatment is difficult to justify if no congenital infection is demonstrated, which can

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delay the early diagnosis and initiation of treatment.[2] It should be noted that early diagnosis will help children receive timely treatment and thus reduce the possible consequences of congenital toxoplasmosis in the future.[3]

Currently, no meta-analysis of congenital toxoplasmosis related to Western Blot diagnostic test performance has been found in the databases consulted, compared to other diagnostic methods such as PCR and serology, of which meta-analyses were found. This highlights the need to evaluate diagnostic performance through a critical and systematic analysis of the studies that evaluated this diagnostic test. Therefore, the present meta-analysis aimed to evaluate the western blot test's performance in diagnosing congenital toxoplasmosis and assess its reliability as a tool for the early detection of the disease.

Methods

A literature search was conducted in April 2023 on digital platforms, such as PubMed, Scopus, and Web of Science. This research was carried out on the usefulness of the western blot test in diagnosing congenital toxoplasmosis, with the search terms "western blot", "Immunoblot" and "Congenital toxoplasmosis". It is important to clarify that there was no contact with the authors to seek additional information. The articles selected were original case-control studies evaluating the diagnostic properties of western blot assays presenting primary data.

Selection: When the search was completed, articles that contained cases and controls and compared the western blot test with the Gold Standard (follow-up of children with anti-Toxoplasma IgG after 10 months of life) were independently selected by two researchers. Only studies with follow-up of children with negativization of specific IgG after 10 months of life for non-infected newborns (true negative) and persistence of specific IgG positivity after 10 months of life (true positive) were included. Only articles published in English were included in this study.

Initially, 172 articles were found in PubMed, Scopus, and the Web of Science. Of these 172, 55 were excluded because they were duplicates in the databases for a total of 117 unique articles. The titles and abstracts of the 117 articles were reviewed, seeking to evaluate the western blot test, and they were case-control studies. Consequently, 104 articles were excluded because only 13 met this criterion, which was later reviewed in the full text. Of the remaining 13 articles, 3 did not report data that would allow the evaluation of sensitivity, specificity, and DOR. Therefore, 10 articles published between 2001 and 2016 were included in the meta-analysis.

Organization: The data from the articles were organized into a matrix of evidence tables or comparative property tables, which were arranged in the following fields:

- Platform and date of consultation.
- Study population and definitions of the cases and controls.
- Criteria used to determine the negativity or positivity of cases and controls by WB.
- True positives (patients infected with congenital toxoplasmosis who were correctly identified as positive for persistent IgG beyond 12 months).
- False positives (uninfected newborns who were incorrectly identified as positive by the positive IgG test at birth but disappeared before 12 months).
- True negatives (uninfected newborns who are correctly identified as IgM-negative and IgG-positive at birth but disappear before 6–12 months).
- False negative (newborns infected with congenital toxoplasmosis who were incorrectly identified as negative).

This matrix of evidence allowed all researchers to analyze the data objectively at any time, without allowing for the detection of observational bias. In addition, we organized the search dates for the information, authors, and citations of the selected works. The main objective was established and analyzed in the evidence matrix using the information available in the reference bibliographic databases. In this way, the population of each study (infants exposed and not exposed to seroconversion during pregnancy), the diagnostic criteria stipulated in each study (different bands between mother and child, increased number of bands or persistent IgG in the baby, IgM in the baby's serum, positive PCR in amniotic fluid, and parasitological detection by inoculation in mice), and the use of the western blot test in each study were identified.

The statistical analyses were performed as follows:

A. The sensitivity was calculated using the following formula: $\frac{POSITIVE\ CASES\ WITH\ WB}{POSITIVE\ CASES\ (CHILDREN\ WITH\ CONFIRMED\ CONGENITAL\ TOXOPLASMOSIS)}$

B. Specificity was calculated using the following formula: $\frac{NEGATIVE\ CASES\ WITH\ WB}{NEGATIVE\ CASES\ DUE\ TO\ THE\ CRITERIA\ ESTABLISHED\ BY\ THE\ STUDY}$

C. The Diagnostic Odds Ratio was calculated using the following formula: $\frac{\#\ TRUE\ POSITIVES}{\#\ FALSE\ POSITIVES} / \frac{\#FALSE\ POSITIVES}{\#TRUE\ NEGATIVES}$ (#True positives/# false positives/# false positives/# true negatives). When the number was zero, it was replaced with one to avoid infinite values.

D. Confidence intervals of 95% (95% CI) were estimated for sensitivity, specificity, and DOR by applying the formula $p \pm z\sqrt{\frac{\hat{d}p(1-\hat{}p)/}{n}}$, where p= percentage, n=sample size, and z=1.96.

Data were examined for normality using the Kolmogorov-Smirnov test, correlation with Pearson's test, and means comparison by ANOVA using SPSS (IBM Corp. Released 2021. IBM SPSS Statistics for Windows, Version 28.0. Armonk, NY: IBM Corp). Calculation of pooled sensitivity and poled specificity were made on the software Open Meta [Analyst] downloaded at: http://www.cebm.brown.edu/openmeta/index.html.

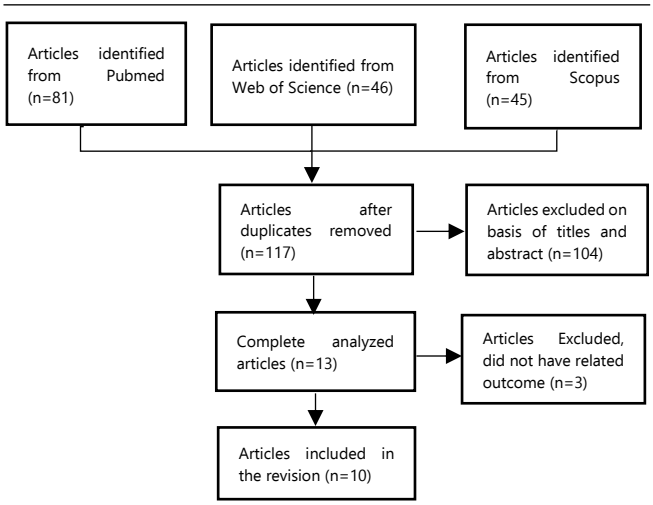
Search and Treatment of Heterogeneity

Heterogeneity was calculated by calculating I2, which provides an estimate of the proportion of variability in a meta-analysis due to heterogeneity rather than sampling error (chance) using the following formula: $I^2 = ((Q - df) / Q) \times 100\%$. Where Q is the result of the X2 test and df is the number of degrees of freedom. The interpretation was as follows: 0% to 40%, might not be important; 30% to 60%, may represent moderate heterogeneity*; 50% to 90%, may represent substantial heterogeneity*; and 75% to 100%, considerable heterogeneity*.

Results

Ten articles met the inclusion criteria (Figure 1). The pooled sensitivity (Figure 2) was 93.8 (95% CI: 79.2-98.4) and the pooled specificity (Figure 3) was 96.6% (95% CI: 89.8-98.9) for the diagnosis of congenital toxoplasmosis. A notable heterogeneity in the results was observed (Figures 2 and 3), with 83.7% sensitivity and 77.5% specificity, both of which were statistically significant (p<0.001). The DOR was higher than 300 in six studies (Figure 4). An average of 3.8 years of follow-up was found in six of the 10 articles.

Figure 1. Flowchart and Results of Article Selection Used in the Meta-analysis.

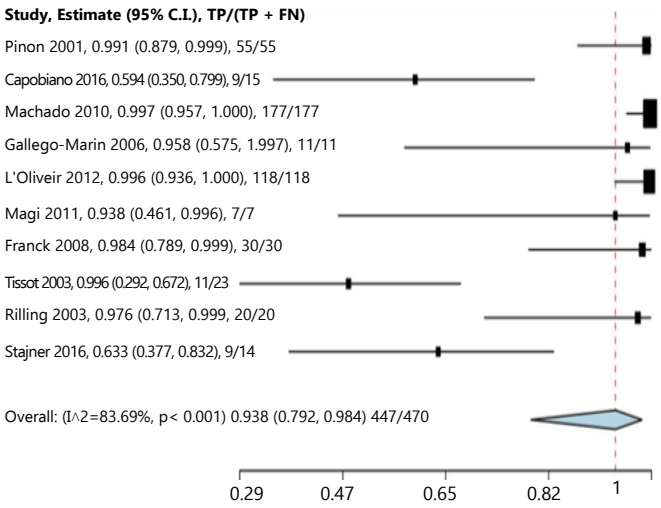


We examined whether there is a correlation between sample size and DOR values. The data showed normality in distribution according to the Kolmogorov Smirnov test (p=0.14 for DOR values and p=0.81 for sample size values). In consequence. We obtained a correlation coefficient of 0.66 with a p-value of 0.005 after the Pearson test, indicating a strong correlation between the DOR value and sample size. In contrast, when DOR mean values were compared between commercial and in-house tests (DOR in-house methods = 2380; DOR with commercial test= 2588), no significant differences were found (F Snedecor 0.004, p= 0.94).

Discussion

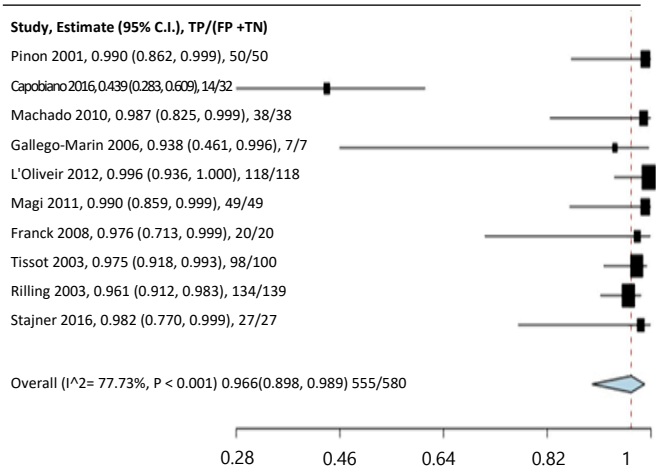
Out of the ten articles examined, seven displayed a sensitivity of 100%, while only three showed a sensitivity of less than 70%.[5,11,13] Additionally, 60% of the evaluated articles exhibited a sensitivity

Figure 2. Sensitivities and Confidence Intervals (CI 95%) and Pooled Sensitivity (Diamond) of Western Blot.



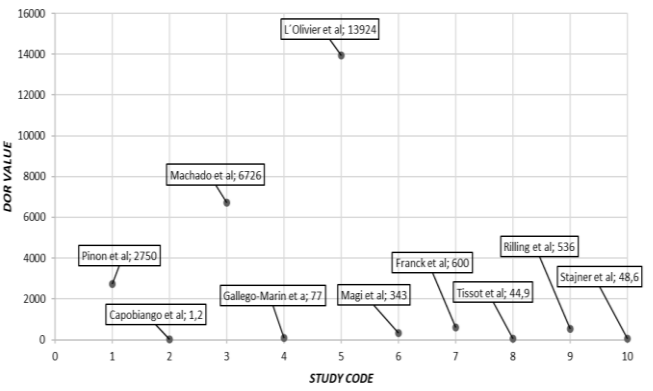
Legend: Techniques for the diagnosis of congenital toxoplasmosis. TP: True positive. FN: False negative.

Figure 3. Specificity and Confidence Intervals (CI 95%) and Pooled Specificity (Diamond) of the Western Blot Technique for the Diagnosis of Congenital Toxoplasmosis.



Legend: TN: True Negative. FP: False Positive.

Figure 4. Diagnostic Odds Ratios (DOR) of Western Blot Technique for the Diagnosis of Congenital Toxoplasmosis.



and specificity of 100%.[11,13] The specificity of the articles was 100% with the exception of Capobiango5 (43.7%), which had the lowest specificity.[11,13] These findings corroborate the superior performance of most western blot assays in congenital toxoplasmosis and the low variability among the studies.[5,11,13] The lower performance of Capobiango5 can be attributed to the usage of an in-house test, which is not extensively standardized but only validated within the laboratory where it was developed.[14] The use of in-house tests is often necessary when there are no commercially available tests, as is the case in Latin-American countries where the cost of importing such tests can be prohibitive.[15] The utilization of in-house tests likely leads to an increase in false negatives.[14] Overall, these results emphasize the importance of employing well-standardized western blot assays to ensure diagnostic accuracy and in this way implement early treatment and avoid long-term sequelae such as chorioretinitis, brain calcifications

Heterogeneity within the studies can be attributed to differences in sample size. Upon examining the DOR values, we observed that most studies had a comparable DOR (> 300), whereas those with a DOR < 100 had smaller sample sizes. Despite these discrepancies, our meta-analysis demonstrated that western blotting provided consistent results in studies conducted in various geographic locations, with differences in population characteristics and local prevalence rates. Similarly, the use of various manufacturing methods, including commercial or in-house approaches, could have influenced the diagnostic performance of the test; however, our analyses suggest minimal impact considering these variations. Notably, only one of the ten studies had a low DOR. The present meta-analysis demonstrates that western blotting is a highly effective diagnostic tool for detecting congenital toxoplasmosis at an early stage and should be incorporated as a confirmatory test within evidence-based clinical practice guidelines.[3,16] Despite this, a significant obstacle to its widespread use is the high cost and limited availability of the western blot assay of the western blot assay and its limited availability. However, despite its relatively high cost, the use of western blotting was cost-effective for all different willingness-to-pay options according to a cost-benefit economic analysis considering the high cost of the sequela of congenital toxoplasmosis.[10] We believe that this issue could be addressed by expanding the market and reducing marginal use by pediatricians, which could ultimately lead to a decline in the prices of commercial western blot tests and easing its inclusion within the diagnostic protocols.[16]

The primary limitation of the current meta-analysis was the scarcity of studies that provided a comprehensive description of test performance, case-confirmation methods, and well-defined criteria for cases. Furthermore, it is essential to conduct long-term postnatal monitoring to detect new retinal lesions as they emerge. It is worth mentioning that this meta-analysis represents an initial endeavor to evaluate the diagnostic efficacy of western blot assays for congenital toxoplasmosis. Notably, the in-house methods yielded a lower DOR than the commercial tests. However, this difference was not statistically significant (F = 0.004, p = 0.94). These findings highlight the need to investigate the standardization and validation of in-house assays.

Summary – Accelerating Translation

El diagnóstico de toxoplasmosis congénita puede retrasarse si no hay IgM o IgA en el recién nacido. La técnica de Western Blot puede contribuir a un diagnóstico más temprano. El principal objetivo de este estudio fue analizar el rendimiento de la prueba Western blot para el diagnóstico precoz de la enfermedad. Para la recopilación de la información se recuperaron los artículos de las bases de datos de referencia bibliográfica de Internet (PubMed, Scopus y Web of Science) utilizando términos de búsqueda relacionados con nuestro objetivo (western blot, Immunoblot). Se continuó seleccionando artículos que cumplieran con los términos de elección e incluyeran los requisitos de criterios diagnósticos para casos y controles mediante el seguimiento serológico de niños después de los 10 meses de vida. Luego, cada estudio fue organizado en una matriz de Excel con las características relevantes para nuestra investigación, como la población estudiada, definición de casos y controles, criterios para determinar la positividad o negatividad de los casos (verdaderos positivos) y controles (verdaderos negativos), quienes fueron positivos en la prueba pero no estaban enfermos (falsos positivos), y aquellos que dieron negativo pero estaban enfermos (falso negativo). Luego se realizó un análisis estadístico calculando la sensibilidad, la especificidad y el índice de probabilidad de diagnóstico (DOR, por sus siglas en inglés) que establece las probabilidades de positividad en sujetos con enfermedad en relación con las probabilidades en sujetos sin enfermedad. Además, se examinó la heterogeneidad entre los estudios. Se identificaron y filtraron cuarenta y cuatro artículos relacionados con los términos de búsqueda utilizando los criterios de inclusión y exclusión y se seleccionaron diez artículos. La sensibilidad promedio de los estudios fue 93,8 (IC 95: 79,2-98,4) y la especificidad fue 96,6% (IC 95: 89,8-98,9). Los resultados mostraron que la prueba de Western blot es confiable para el diagnóstico seguro y oportuno de la toxoplasmosis congénita. Además, es importante considerar las diferencias entre unos estudios y otros en cuanto a su sensibilidad y especificidad cuando se usaron pruebas caseras las cuales no por definición faltan de mayor estandarización.

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Conflict of Interest Statement & Funding

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Author Contributions

Conceptualization: SSR, JEGM; Data Curation: SSR, MARD, JAAT, JDFL, LFMS; Formal Analysis: SSR, MARD, JAAT, JDFL, LFMS; Investigation: SSR, MARD, JAAT, JDFL, LFMS; Project Administration: JEGM; Supervision: JEGM; Visualization: JEGM; Writing – Original Draft: SSR, MARD, JAAT, JDFL, LFMS; Writing – Review & Editing: JEGM..

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