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Identification of Biomarkers for Diagnosis and Prognosis of Congenital and Acute Toxoplasmosis

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Background. The diagnosis of congenital toxoplasmosis can be inconclusive in many cases. Despite the several serological tests developed, the literature on biomarkers that can assist in the diagnosis of congenital an acute toxoplasmosis is limited. The objective of this study was to analyze the immunoreactive profile of *Toxoplasma gondii* protein bands with the potential to be biomarkers for diagnosis and prognosis of congenital and acute toxoplasmosis.

Methods. Peripheral blood samples from women of childbearing age and/or pregnant women diagnosed with acquired toxoplasmosis as well as from congenitally infected children were selected and submitted to immunoblotting for analysis of the immunoreactive bands profile by immunoglobulin G (IgG) antibodies.

Results. When comparing the immunoreactive bands profile for antibodies present in samples from different groups and subgroups, the 150, 18.5, and 16.96-kDa bands were more immunoreactive with the antibodies present in serum samples from the acquired infection group. The 343, 189, 150, 75, and 42-kDa bands showed more chance to be detected by the symptomatic congenital infection subgroup samples, while the 61, 50, and 16.96-kDa bands were significantly immunoreactive with the acute infection subgroup samples.

Conclusions. The identification of these potential biomarkers can assist in early diagnosis and treatment of congenital toxoplasmosis.

Keywords. immunoblotting; congenital toxoplasmosis; prognosis.

Congenital toxoplasmosis is one of the most important forms of infection among the diseases that affect fetuses. The infection is caused by *Toxoplasma gondii* and can result in fetal infeasibility or clinical manifestations at any stage of the child's life. In addition, there is an estimated global incidence of 1.5 cases per 1000 live births [1], and toxoplasmosis is characterized as a public health problem.

The early detection of toxoplasmosis during prenatal and/or neonatal screening in conjunction with the immediate initiation of adequate treatment leads to a better prognosis in congenital cases [2]. The prognosis has been mainly related to the period of gestational infection. In the first trimester, the infection can lead to fetal death. Clinical signs of Sabin's tetrad are common in the second trimester and third trimester of gestation, where usually the fetuses are asymptomatic in 80% of cases; however, clinical signs can manifest days, months, or years after birth [3].

The laboratory diagnosis of toxoplasmosis is based mainly on the detection of anti-*T. gondii* immunoglobulin M (IgM) and immunoglobulin G (IgG) antibodies, which determine the stage of infection [4, 5].

IgM antibodies are present in the acute phase of the infection and reach high levels within a month; however, due to the half-life of antibodies, these become undetectable after a few weeks [6]. In some cases, anti-*T. gondii* IgM can be detected for more than a year, which makes diagnosis difficult, especially in cases with suspected infection in the first trimester of pregnancy [7].

IgM immunoglobulins do not cross the placental barrier; therefore, they are markers of congenital infection when detected in the serum of newborns [8]. However, it is known that up to 55% of infected neonates do not have these antibodies or can present them at undetectable levels for most of the available tests [9]. On the other hand, anti-*T. gondii* IgG antibodies decrease to a medium residual level after the third month of infection, determining the chronic phase of the disease [8]. Maternal IgG antibodies are circulating in the child's blood, so their presence suggests congenital infection, **as long as** the title of this class of immunoglobulins remains stable or ascending until the child's first year of life [10], making the diagnosis late.

The immunoblotting technique has been used to confirm the diagnosis of congenital toxoplasmosis; however, this and other techniques do not determine whether the infection is due to

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congenital or acquired transmission when performed after the first months of the child's life [11].

In this context, there is a need to identify biomarkers that assist in the diagnosis of inconclusive cases of toxoplasmosis in pregnant woman and in congenitally infected children that are effective and enable early treatment to prevent or minimize the clinical signs that can appear months or years after birth.

The aim of this study was to analyze the immunoreactive profile of *T. gondii* protein bands with the potential to be biomarkers for the diagnosis of acute acquired toxoplasmosis, as well for the diagnosis and prognosis of congenital toxoplasmosis.

METHODS

Biological Samples

This was a cohort study and was carried out from June 2016 to June 2020. Serological samples from children and women of childbearing age and/or pregnant women were obtained from the serum bank of the Laboratório de Estudos da Relação Parasito Hospedeiro na Universidade Federal de Goiás. In addition, peripheral blood samples were collected from patients with suspected toxoplasmosis seen in the pediatrics and gynecology/obstetrics departments at Hospital das Clínicas da Universidade Federal de Goiás (HC-UFG). The biological samples (peripheral blood) were collected after the consent of the patients or their guardians with signing of the informed consent form.

All samples came from projects approved by the ethics committee of HC-UFG, via Plataforma Brasil.

Laboratory Tests for the Diagnosis of Toxoplasmosis

All analyzed samples were subjected to immunoenzymatic testing for toxoplasmosis with the Bioelisa-Bioclin commercial kit (Quibasa) according to the manufacturer's instructions.

The presence of the parasite was investigated in peripheral blood samples using the polymerase chain reaction (PCR) technique according to the protocol described by Burg et al [12].

Serological and/or molecular confirmation was used as a criterion for inclusion of samples for analysis by immunoblotting.

Analysis of Medical Records

The clinical and laboratory data of the patients included in the study were obtained by analyzing the medical records at HC-UFG. In the case of women of childbearing age and/or pregnant women, the medical records were analyzed for confirmation of seroconversion of toxoplasmosis and to determine the patients' health status. Ophthalmological, neurological, auditory, and behavioral examinations to classify the clinical condition of the children included in the study were analyzed.

Selection of Biological Samples

The inclusion criteria of this study encompassed patients who presented positive serology (anti-*T. gondii* IgM and/or IgG) for toxoplasmosis and/or the presence of parasite DNA in biological samples. Exclusion criteria were samples from patients who presented negative results for infection and/or diagnosed with autoimmune diseases and human immunodeficiency virus.

The groups and subgroups analyzed were as follows.

Congenital toxoplasmosis group: Samples from children congenitally infected with *T. gondii* who presented reagent serology anti-*T. gondii* IgM and IgG; or concentration of anti-*T. gondii* IgG antibodies greater than that of their mothers; or persistence of anti-*T. gondii* IgG antibodies until the first year of life; and/or positive molecular diagnosis for toxoplasmosis.

Symptomatic congenital toxoplasmosis subgroup: Samples from children congenitally infected with *T. gondii* who had characteristic clinical manifestations of toxoplasmosis after birth.

Asymptomatic congenital toxoplasmosis subgroup: Samples from children congenitally infected with asymptomatic *T. gondii* until the time of blood collection.

Acquired toxoplasmosis group: Samples from healthy women of childbearing age and/or pregnant women who presented seroconversion (IgM and IgG or only anti-*T. gondii* IgG) and/or positive molecular diagnosis for toxoplasmosis.

Acute acquired toxoplasmosis subgroup: Samples from women of childbearing age and/or pregnant women with acquired toxoplasmosis who showed serological reagent for anti-*T. gondii* IgM and IgG. Most of the samples referring to this subgroup were collected within 4 months after the diagnosis of seroconversion, while the rest varied between 6 and 7 months.

Chronic acquired toxoplasmosis subgroup: Samples from women of childbearing age and/or pregnant women with acquired toxoplasmosis who presented serology reactive for anti-*T. gondii* IgG.

Toxoplasma gondii Crude Antigens Preparation

To obtain crude antigens, the RH strain of *T. gondii* was used, obtained by peritoneal washing of Balb/c mice, which was centrifuged and suspended in phosphate-buffered saline ×5, pH 7.2, in 3 stages according to Machado et al [13]. The sediment was transferred to microtubes containing glass beads and subjected to the Mini-BeadBeater shaker (BioSpec Products) for 6 cycles of 1 minute to obtain crude antigen composed of surface and cytoplasmic antigens from the lysis of the parasite [14]. The concentration of proteins present in the antigen was determined using the Bradford method [15].

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis and Electrotransfer

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to Laemmli [16], with some modifications. After the preparation of stacking gel (Tris-HCl 1 M,

SDS 10%, pH 6.8) and separation gel (Tris-HCl 1.5 M, SDS 10%, pH 8.8) with 4% and 12% polyacrylamide, respectively, 30 µg of *T. gondii* proteins was diluted in sample buffer (2% SDS, 10% glycerol, 5% 2-mercaptoethanol, 60 mM Tris-HCl, pH 6.8, and 0.002% bromophenol blue) and applied to the gel.

The proteins were separated on the SDS-PAGE by an electric current at 70 V and 60 mA in running buffer (250 mM Tris-HCl, 2.5M glycine, 1% SDS) in the Mini Protean II system (Bio-Rad) [16].

The electrotransfer of proteins to 0.45 µm nitrocellulose membrane (Bio-Rad) was performed according to the protocol described by Towbin et al, with modifications [17]. The gel was dipped in transfer buffer (20% methanol, 25 mM Tris, 192 mM glycine, pH 8.0) for the assembly of the immunotransfer structure, which was placed and maintained in a Minitrans-Blot device (Bio-Rad) for 2 hours at 150 V and 150 mA.

After transfer, the nitrocellulose membrane was stained with 1% Ponceau-S solution (Sigma) and 10% acetic acid to confirm protein transfer. The membrane was cut into strips approximately 5 mm wide and stored at −20°C until use [18].

Immunoblotting

The nitrocellulose membrane tape was blocked with skimmed milk powder in Tris-buffered saline (TBS)-Tween 20 (20 mM Tris-HCl, 500 mM NaCl, 0.05% Tween 20, pH 8.0) and then washed and incubated for 1 hour with patient's serum at a dilution of 1:100. After 3 washes with TBS-Tween 20, the nitrocellulose membrane was incubated for 1 hour with human anti-IgG conjugated to peroxidase (Sigma), which was diluted 1:3000 in buffer. The development was carried out with 0.2% of 3.3 diaminobenzidine tetrahydrochloride (Sigma) and hydrogen peroxide diluted in TBS [18].

Determination of the Antigenic Profile

The analysis of the profile of immunoreactive bands was performed by the OriginPro software, using a linear regression equation. From the standard molecular weight marker (Precision Plus Protein Standards; Bio-Rad) it was possible to determine the molecular masses of proteins present in the bands that were immunoreactive with anti-*T. gondii* IgG antibodies.

Statistical Analysis

To verify the association between the variables under study, Pearson χ^2 and Fisher exact tests were used when relevant. The measure used was odds ratio and for all statistical analyzes a significance level of 5% was considered. Thus, *P* values less than or equal to .05 were considered to be statistically significant. All analyzes were performed using Stata 14.0 software.

RESULTS

Groups Analyzed and Clinical Data

One hundred and sixteen serum samples from children and women of childbearing age and/or pregnant women infected with *T. gondii* were selected and divided according to the criteria previously described, that is 58 samples from the congenital toxoplasmosis group and 58 samples from the acquired toxoplasmosis group.

Of the 58 samples from women of childbearing age and/or pregnant women, 50% (29/58) of the samples were reagents for anti-*T. gondii* IgM and IgG, 50% (29/58) had anti-*T. gondii* IgG antibodies, and in 1.72% (1/58) it was possible to identify genetic material of the parasite (data not shown). The acquired toxoplasmosis group was separated into 2 subgroups, that is firstly 29 serum samples from women with acute acquired toxoplasmosis (anti-*T. gondii* IgM and IgG) and secondly 29 samples from women with chronic infection (anti-*T. gondii* IgG).

Of the 58 samples from children congenitally infected with *T. gondii*, 34.48% (20/58) presented positive serology for anti-*T. gondii* IgM and IgG, 65.51% (38/58) were reagents for anti-*T. gondii* IgG, 53.44% (31/58) presented concentrations of IgG antibodies higher than that of their mothers, in 15.51% (9/58) IgG antibodies were detected after the child's first year of life, and in 12.06% (7/58) it was possible to identify genetic material of the parasite (data not shown). The samples from the congenital toxoplasmosis group were separated into 2 subgroups according to the clinical information obtained from the medical records, that is 29 serum samples from children with symptomatic congenital toxoplasmosis and the other half of the samples belonging to the subgroup of children with asymptomatic congenital infection.

Of the children with symptomatic congenital toxoplasmosis, 51.72% (15/29) presented chorioretinitis and 48.27% (14/29) intracranial calcifications. In addition, the same percentage of children (17.24%) showed hydrocephalus (5/29) and microcephaly (5/29). Likewise, 6.89% of children presented visual impairment (2/29), liver disease (2/29), cortical dysfunction (2/29), and eye hemorrhage (2/29). It was also possible to identify 3.44% of children with lymphadenopathy (1/29), macular scar (1/29), and microphthalmia (1/29).

Analysis of the Profile of Immunoreactive Bands of the Congenital and Acquired Toxoplasmosis Groups

The *T. gondii* tachyzoite proteins were tested in 116 samples from patients with congenital and acquired toxoplasmosis.

The electrophoretic profile of protein extracts of *T. gondii* presented a molecular weight range of 343 to 8.3 kDa. The bands with high protein concentration were identified in the range of 75 to 50 kDa and below 10 kDa. However, the 30-kDa band was predominantly immunoreactive with anti-*T. gondii* IgG antibodies in serum samples from patients with congenital toxoplasmosis and acquired toxoplasmosis as 43.1% (25/58) and 46.55% (27/58) of the samples showed reactivity, respectively.

When comparing the immunoreactive bands profile between the groups, the samples from the group with acquired infection showed greater reactivity with the 150, 18.5, and 16.96-kDa bands when compared to samples from patients with congenital toxoplasmosis (Table 1 and Figure 1). For the other bands

Table 1. Comparison of the Immunoreactive Bands Profile Between Serum Samples From Patients With Congenital and Acquired Toxoplasmosis by Immunoblotting

Band, kDa	Congenital Toxoplasmosis Group, No.	Acquired Toxoplasmosis Group, No.	OR	CI 95%	*P* Value
150	7	16	0.36	.12–1.04	.036
18.5	4	14	0.23	.05–.82	.010
16.96	4	20	0.14	.03–.47	.000

Pearsons χ^2 and Fisher exact tests were used when relevant.
Abbreviations: CI, confidence interval; OR, odd ratio.

detected by the samples from both groups, there was no significant association.

Analysis of the Immunoreactive Band Profile of the Symptomatic and Asymptomatic Congenital Toxoplasmosis Subgroups

The 100-kDa band was immunoreactive with 44.82% (13/29) of serum samples from children with symptomatic congenital toxoplasmosis, while in the same percentage, 44.82% (13/29), the 30.99-kDa band was more immunoreactive with samples from the subgroup of asymptomatic congenital toxoplasmosis.

In the subgroups, samples from children with symptomatic congenital infection showed greater reactivity with the 343, 189, 150, 75, and 42-kDa bands. The 14.6-kDa band was detected mainly by samples belonging to the subgroup of asymptomatic congenital toxoplasmosis (Table 2 and Figure 2). For the other bands detected by the samples from both subgroups, there was no significant association.

Analysis of the Immunoreactive Band Profile in the Subgroups of Acute and Chronic Acquired Toxoplasmosis

The 50-kDa band was detected by 58.6% (17/29) of the samples belonging to the acute acquired toxoplasmosis subgroup; the 30.99 and 57-kDa bands were immunoreactive with 44.8% (13/29) of the patient samples with chronic acquired toxoplasmosis.

When comparing the immunoreactive bands profile between the subgroups, samples from women with acute infection were more reactive with the 61, 50, and 16.96-kDa bands (Table 3 and Figure 3), whereas no significant association was found for the other bands.

It was not possible to detect the 57-kDa band with samples from the subgroups of acute acquired toxoplasmosis, as shown in Table 3.

DISCUSSION

Early confirmation of congenital toxoplasmosis as well as the prognosis of infection are still major challenges. The most used methods for the diagnosis of toxoplasmosis are serological tests with soluble antigens of *T. gondii*. However, immunoblotting in conjunction with 2-dimensional electrophoresis and mass spectrometry have been used to detect new antigenic markers [19].

With the central objective of identifying biomarkers with potential for the diagnosis and prognosis of congenital toxoplasmosis, it was possible to identify that the 30-kDa band was predominantly immunoreactive with anti-*T. gondii* IgG antibodies in samples from congenital and acquired toxoplasmosis groups. These data demonstrate that there was no clear relationship with the high expression of proteins identified in the range

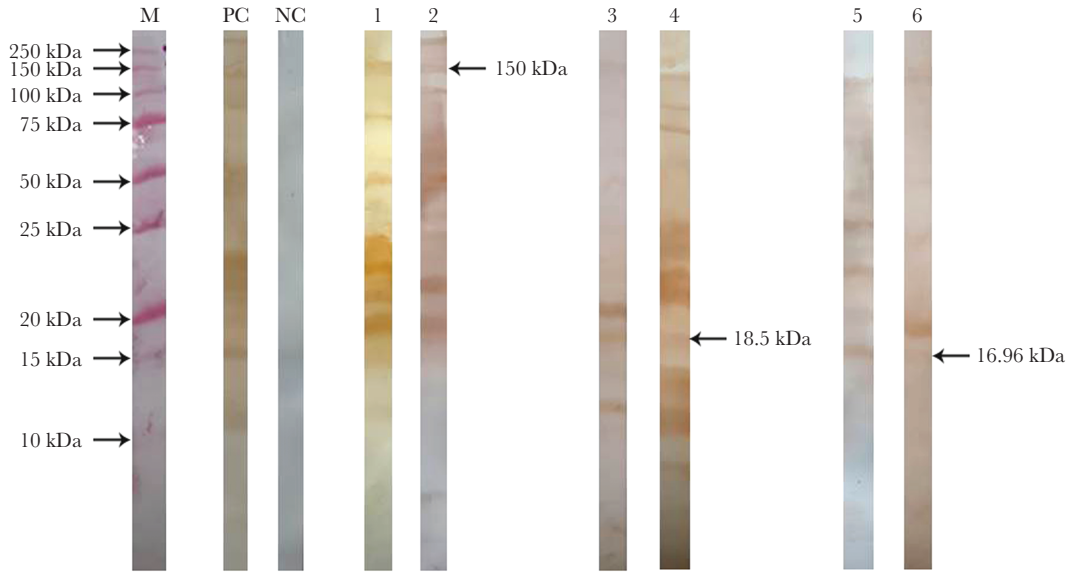

Figure 1. Antigenic profile of *Toxoplasma gondii* probed with antibodies from patients with acquired toxoplasmosis. Reactivity of anti-*T. gondii* IgG antibodies present in serum samples from the toxoplasmosis group with the 150, 18.5, and 16.96-kDa bands compared to the congenital toxoplasmosis group, as highlighted by samples 1, 2, 3, 4, 5, and 6. Molecular weight was calculated by linear regression equation. Abbreviations: M, molecular weight marker; NC, negative control; PC, positive control.

Table 2. Comparison of Immunoreactive Bands Profile Between Serum Samples From Children With Symptomatic and Asymptomatic Congenital Toxoplasmosis Using Immunoblotting

Band, kDa	Symptomatic Congenital Toxoplasmosis Subgroup, No.	Asymptomatic Congenital Toxoplasmosis Subgroup, No.	OR	CI 95%	*P* Value
343	10	1	17.50	1.93–794.47	.000
189	7	1	9.80	1.03–462.13	.020
150	6	1	7.30	.77–347.66	.044
75	7	1	8.91	.99–415.47	.022
42	11	3	5.30	1.14–32.79	.014
14.6	1	6	0.14	.00–1.29	.044

Pearsons χ^2 and Fisher exact tests were used when relevant.

Abbreviations: CI, confidence interval; OR, odd ratio.

75 to 50 kDa in the polyacrylamide gel with antigenicity compared to the tested serum samples.

The greater detection of the 30-kDa band coincides, in part, with the study by Luo et al, because 25 to 35-kDa protein bands, both in soluble tachyzoite antigens and in excreted and secreted antigens, were immunoreactive with all the serum samples from patients with toxoplasmosis they tested [19]. However, Machado et al demonstrated that the most prevalent antigens ranged from 66 to 45 kDa and 116 to 97 kDa in serum samples from children congenitally infected with *T. gondii* and their respective mothers [13].

Regarding the comparison of immunoreactive bands between the congenital and acquired toxoplasmosis groups, it was possible to verify that the 150, 18.5, and 16.96-kDa bands showed relevant statistical differences, suggesting that they are promising biomarkers for the diagnosis of acquired toxoplasmosis, and consequently for congenital toxoplasmosis. On the other hand, Capobiango et al reported that antibodies with higher frequency in congenitally infected children compared to children without infection detected proteins of molecular weight between 116 and 30 kDa [18].

The samples that identified bands 343, 189, 150, 75, and 42 kDa were more likely to belong to the symptomatic congenital toxoplasmosis subgroup than to the asymptomatic subgroup. This result is partially consistent with that reported by De Marco et al, in which only patients with active ocular toxoplasmosis had antibodies with specificity for bands greater than 110 kDa, which can be explained by the small number of samples analyzed in the study of children with retinochoroiditis associated with congenital toxoplasmosis [20].

Among the clinical manifestations of children with symptomatic congenital toxoplasmosis reported in this study, chorioretinitis (51.72%), brain calcifications (48.27%), and hydrocephaly and microcephaly (17.24%) were the most common, as in studies by Bischoff et al [21] and Olariu et al [22].

According to Garweg, ocular toxoplasmosis presents a more severe prognosis in cases occurring in South America when compared to Europe, due to the higher incidence of virulent strains [23]. In addition, the study found ocular inflammation is initially more pronounced in acquired cases than in congenital cases; however, the final visual acuity is generally better and the risk of recurrence is lower, demonstrating the importance of a prognostic marker for congenital toxoplasmosis [23].

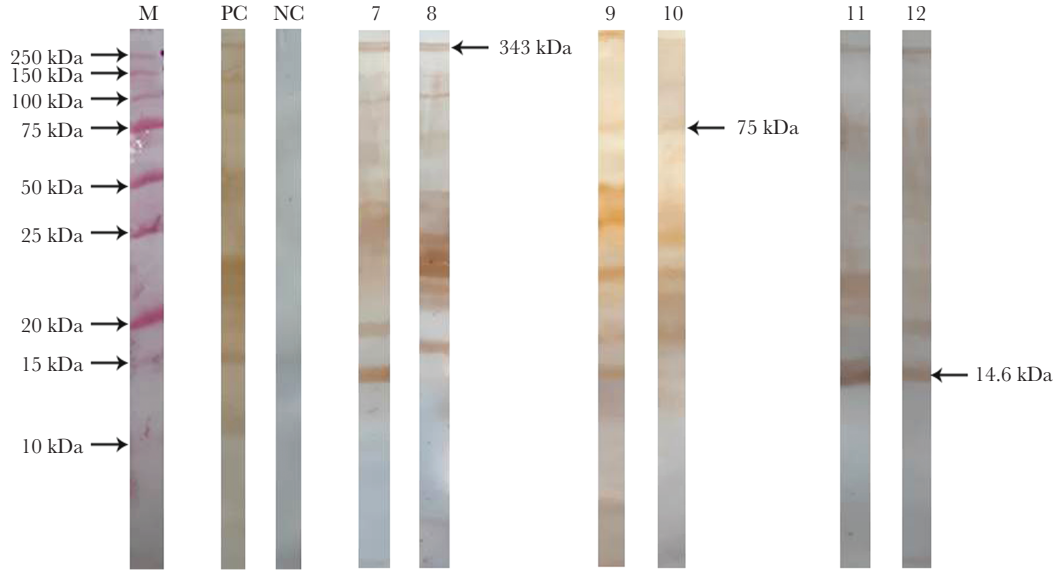

Figure 2. Comparison of the antigenic profile of *Toxoplasma gondii* probed with antibodies from patients with symptomatic and asymptomatic congenital toxoplasmosis. The 343 and 75-kDa bands are highlighted, represented by samples 7, 8, 9, and 10 from the symptomatic congenital toxoplasmosis subgroup and the 14.6-kDa band, represented by samples 11 and 12 from the asymptomatic subgroup. Molecular weight was calculated by linear regression equation. Abbreviations: M, molecular weight marker; NC, negative control; PC, positive control.

Table 3. Comparison of the Immunoreactive Bands Profile Between Serum Samples From Women With Acute and Chronic Acquired Toxoplasmosis by Immunoblotting

Band, kDa	Acute Acquired Toxoplasmosis Subgroup, No.	Chronic Acquired Toxoplasmosis Subgroup, No.	OR	CI 95%	*P* Value
61	13	1	22.75	2.78–1000.18	.00
57	0	13	…	…	…
50	17	2	19.13	3.46–185.57	.00
16.96	15	5	5.14	1.36–21.56	.00

Pearsons χ^2 and Fisher exact tests were used when relevant.

Abbreviations: CI, confidence interval; OR, odd ratio.

Regarding the specific antigenic profile associated with the different stages of *T. gondii* infection, there was greater recognition of the 61, 50, and 16.96-kDa bands by IgG antibodies present in the samples of the acute acquired toxoplasmosis subgroup, whereas in the study by Achar et al, antigenic proteins corresponding to 35, 43, 45, 56, and 107 kDa were immunoreactive with 20%–60% of the sera in patients with acute infection [24]. This is similar to the study by Costa et al, which demonstrated that proteins of 35 and 22 kDa could be used in a complementary way in the diagnosis of acute toxoplasmosis [25].

In addition, there was disagreement regarding the detection of bands by the antibodies present in the samples of the subgroup of chronic acquired toxoplasmosis, because in this study the 57-kDa band was more likely to be detected in the chronic phase in comparison to the acute phase of the infection, whereas in the study by Achar et al the 65, 95, 98, and 113-kDa bands were immunoreactive with antibodies from 17%–35% of patients with chronic toxoplasmosis [24].

It is worth mentioning that one of the difficulties in comparing the results obtained is due to the different values of molecular weights of proteins reported in the previously mentioned studies, which can be explained by methodological differences, such as the preparation of the *T. gondii* antigen, the preparation and running conditions of polyacrylamide gels, and dilutions of serum [9].

According to the PCR results, it was possible to detect genetic material in a sample from the group of acute acquired toxoplasmosis, because most women were under treatment during the period of sample collection. An effective diagnosis of toxoplasmosis during pregnancy, especially in cases of residual IgM, is essential for the initiation of treatment because it can reduce the rates of transmission of *T. gondii* to the fetus. A study conducted in Austria showed a significant decrease in the occurrence of vertical infection by *T. gondii* in mothers who were treated during prenatal care compared to the control group [26]. In addition, Hotop et al identified that maternal late treatment was considered a risk factor for symptomatic congenital toxoplasmosis [27].

Furthermore, due to the lack of adherence by women to all the examinations required during prenatal care and the fact that most newborns do not present symptoms and positive serology for anti-*T. gondii* IgM, the diagnosis and treatment is late in many cases [9, 28]. In a study by McLeod et al, newborns infected congenitally with *T. gondii* were treated for 1 year and monitored for clinical results. Of the children with severe

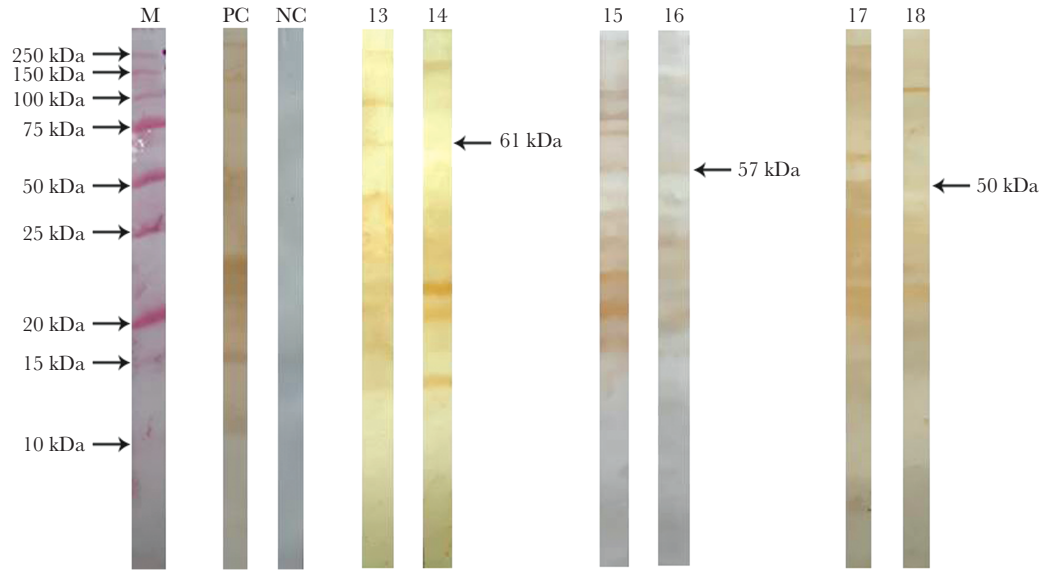

Figure 3. Comparison of the antigenic profile of *Toxoplasma gondii* probed with antibodies from patients with acute and chronic acquired toxoplasmosis. The 61 and 50-kDa bands are highlighted, represented by samples 13, 14, 17, and 18 of the acute acquired toxoplasmosis subgroup and the 57-kDa band, represented by samples 15 and 16 of the chronic subgroup. Molecular weight was calculated by linear regression equation. Abbreviations: M, molecular weight marker; NC, negative control; PC, positive control.

clinical manifestations after birth, 80% had normal motor function and 64% did not develop new eye injuries [29].

It was possible to identify that the 61, 50, and 16.96-kDa bands have the potential to be used as biomarkers in the diagnosis of acute toxoplasmosis, the 150, 18.5, and 16.96-kDa bands for acquired infection, and those of 343, 189, 150, 75, and 42 kDa for the prognosis of congenital infection. These biomarkers can help a faster diagnosis and treatment in inconclusive cases, preventing the appearance of sequelae throughout the life of congenitally infected children. We emphasize the need to identify proteins present in the bands of interest in order to obtain more accurate results that can be applied in serological tests in the future.

Notes

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Potential conflicts of interest. All authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Torgerson PR, Mastroiacovo P. The global burden of congenital toxoplasmosis: a systematic review. Bull World Health Organ **2013**; 91:501–8.
2. Dard C, Fricker-Hidalgo H, Brenier-Pinchart MP, Pelloux H. Relevance of and new developments in serology for toxoplasmosis. Trends Parasitol **2016**; 32:492–506.
3. Montoya JG, Liesenfeld O. Toxoplasmosis. Lancet **2004**; 363:1965–76.
4. Murat JB, Hidalgo HF, Brenier-Pinchart MP, Pelloux H. Human toxoplasmosis: which biological diagnostic tests are best suited to which clinical situations? Expert Rev Anti Infect Ther **2013**; 11:943–56.
5. Nogareda F, Le Strat Y, Villena I, De Valk H, Goulet V. Incidence and prevalence of *Toxoplasma gondii* infection in women in France, 1980–2020: model based estimation. Epidemiol Infect **2014**; 142:1661–70.
6. Murat JB, Souvignet A, Fricker-Hidalgo H, Brenier-Pinchart MP, Bost-Bru C, Pelloux H. Assessment of the IgA immunosorbent agglutination assay for the diagnosis of congenital toxoplasmosis on a series of 145 toxoplasmic seroconversions. Clin Vaccine Immunol **2015**; 22:456–8.
7. Liu Q, Wang ZD, Huang SY, Zhu XQ. Diagnosis of toxoplasmosis and typing of *Toxoplasma gondii*. Parasit Vectors **2015**; 8:292.
8. Robert-Gangneux F, Dardé ML. Epidemiology of and diagnostic strategies for toxoplasmosis. Clin Microbiol Rev **2012**; 25:264–96.
9. Sensini A. *Toxoplasma gondii* infection in pregnancy: opportunities and pitfalls of serological diagnosis. Clin Microbiol Infect **2006**; 12:504–12.
10. Pomares C, Montoya JG. Laboratory diagnosis of congenital toxoplasmosis. J Clin Microbiol **2016**; 54:2448–54.
11. Rezende HHA, Storchilo HR, Lima JAS, et al. Nursing infant with acquired toxoplasmosis in the first months of life—a case report. Rev Inst Med Trop Sao Paulo **2017**; 59:e63.
12. Burg JL, Grover CM, Pouletty P, Boothroyd JC. Direct and sensitive detection of a pathogenic protozoan, *Toxoplasma gondii*, by polymerase chain reaction. J Clin Microbiol **1989**; 27:1787–92.
13. Machado AS, Andrade GM, Januário JN, et al. IgG and IgM western blot assay for diagnosis of congenital toxoplasmosis. Mem Inst Oswaldo Cruz **2010**; 105:757–61.
14. Hurley SS, Splitter GA, Welch RA. Rapid lysis technique for mycobacterial species. J Clin Microbiol **1987**; 25:2227–9.
15. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem **1976**; 72:248–54.
16. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature **1970**; 227:680–5.
17. Towbin H, Staehelin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc Natl Acad Sci U S A **1979**; 76:4350–4.
18. Capobiango JD, Monica TC, Ferreira FP, et al. Evaluation of the western blotting method for the diagnosis of congenital toxoplasmosis. J Pediatr (Rio J) **2016**; 92:616–23.
19. Luo J, Wan J, Tang Z, Shen S. Identification of novel antigens for serum IgG diagnosis of human toxoplasmosis. Exp Parasitol **2019**; 204:107722.
20. De Marco R, Ceccarelli R, Frulio R, Palmero C, Vittone P. Retinochoroiditis associated with congenital toxoplasmosis in children: IgG antibody profiles demonstrating the synthesis of local antibodies. Eur J Ophthalmol **2003**; 13:74–9.
21. Bischoff AR, Friedrich L, Cattan JM, Uberti FA. Incidence of symptomatic congenital toxoplasmosis during ten years in a Brazilian hospital. Pediatr Infect Dis J **2016**; 35:1313–6.
22. Olariu TR, Remington JS, McLeod R, Alam A, Montoya JG. Severe congenital toxoplasmosis in the United States:

clinical and serologic findings in untreated infants. Pediatr Infect Dis J **2011**; 30:1056–61.
23. Garweg JG. Ocular toxoplasmosis: an update. Klin Monbl Augenheilkd **2016**; 233:534–9.
24. Achar IG, Vatteone CG, Guillén I, et al. Perfil antigénico en fase aguda y crónica de toxoplasmosis en embarazadas por la técnica de western blot. Mem Inst Invest Cienc Salud **2018**; 16:35–43.
25. Costa JG, Peretti LE, García VS, et al. P35 and P22 *Toxoplasma gondii* antigens abbreviate regions to diagnose acquired toxoplasmosis during pregnancy: toward single-sample assays. Clin Chem Lab Med **2017**; 55:595–604.
26. Prusa AR, Kasper DC, Pollak A, Olischar M, Gleiss A, Hayde M. Amniocentesis for the detection of congenital toxoplasmosis: results from the nationwide Austrian prenatal screening program. Clin Microbiol Infect **2015**; 21:191.e1–e8.
27. Hotop A, Hlobil H, Gross U. Efficacy of rapid treatment initiation following primary *Toxoplasma gondii* infection during pregnancy. Clin Infect Dis **2012**; 54:1545–52.
28. Da Silva MG, Vinaud MC, De Castro AM. Prevalence of toxoplasmosis in pregnant women and vertical transmission of *Toxoplasma gondii* in patients from basic units of health from Gurupi, Tocantins, Brazil, from 2012 to 2014. PLoS One **2015**; 10:e0141700.
29. McLeod R, Boyer K, Karrison T, et al. Outcome of treatment for congenital toxoplasmosis, 1981–2004: the national collaborative Chicago-based, congenital toxoplasmosis study. Clin Infect Dis **2006**; 42:1383–94.