IgM triplet in neonatal diagnosis by immunoblotting and its potential use as a diagnostic marker for congenital toxoplasmosis

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Abstract – Primary infection during pregnancy by the protozoan Toxoplasma gondii can be worrisome because transmission to the fetus may lead to congenital toxoplasmosis (CT). Neonatal diagnosis is usually performed by serological profile comparison of the mother and newborn. As previously reported in 2012 by C. L’Ollivier et al., three IgM bands at 75, 90 and 100 kDa called the “IgM triplet” has caught our attention and seems to be pathognomonic of CT. This retrospective multicenter study involved nine reference laboratories included in the French National Reference Center for Toxoplasmosis network and concerned determining the specificity and sensitivity of this IgM triplet. On this basis, we were able to propose a new read of the comparison of IgG and IgM immunoblot profiles of mother and infant to increase the sensitivity of this diagnostic marker. The effect of the trimester of pregnancy at the time of infection, but also of maternal treatment with pyrimethamine/sulfadiazine/folic acid on the presence of this IgM triplet in the infant, could be studied. The presence of the triplet appears pathognomonic for the diagnosis of CT, and it increased the sensitivity of the immunoblot assay from 55.04% to 72.48%. As a result, it would be wise to enhance conventional immunoblot reading by adding the presence of the three IgM bands in the infant pattern for neonatal diagnosis of CT.

Key words: Toxoplasma gondii, Congenital toxoplasmosis, Neonatal diagnosis, Immunoblotting, IgM triplet.

Résumé – La triplette IgM dans le diagnostic néonatal par immunoblot et son utilisation potentielle comme marqueur diagnostique de la toxoplasmose congénitale. La primo-infection pendant la grossesse par le protozoaire Toxoplasma gondii peut se révéler préoccupante car la transmission au fœtus peut conduire à une toxoplasmose congénitale (TC). Un diagnostic néonatal est généralement réalisé par comparaison des profils sérologiques de la mère et du nouveau-né. Comme précédemment rapporté en 2012 par C. L’Ollivier et al., l’association de trois bandes d’IgM à 75, 90, et 100 kDa appelée la « triplette IgM » a retenu notre attention et semble être pathognomonique de la TC. Cette étude rétrospective multicentrique impliquant neuf laboratoires de
Introduction

Le parasite *Toxoplasma gondii* est un intracellulaire protozoaire qui infecte nombre de personnes dans le monde. Bien que les infections humaines soient asymptomatiques, ce parasite entraîne de graves conséquences pour le fœtus, notamment l’infection congénitale toxoplasmique (CT). L’incidence est variable et peut être modifiée, entre autres, par des soins maternels par pyriméthamine/sulfadiazine/acidé folinique sur la présence de la triplette IgM chez l’enfant a pu être analysée. La présence de cette triplette semble pathognomonique pour le diagnostic de TC et elle permet d’augmenter la sensibilité du test immunoblot de 55,04 % à 72,48 %. Ainsi, il pourrait être judicieux d’améliorer la lecture conventionnelle de l’immunoblot en ajoutant la présence des trois bandes IgM dans le schéma du nourrisson pour le diagnostic néonatal de TC.

**Materials and methods**

**Ethics**

Le but de l’étude était d’analyser des spécificités du test d’immunoblot et de proposer une nouvelle lecture de la bande IgM. Celle-ci permet d’améliorer la sensibilité du test et de minimiser les effets secondaires neurologiques ou oculaires [7]. Donc, la lecture de la triplette IgM peut être faite dès le plus jeune âge possible pour commencer le traitement antiparasitaire.

**Collection of data**

Ce travail multicentrique à caractère prospectif a inclus dans le Centre National de Référence pour la Toxoplasmose à Angers, Grenoble, Lille, Marseille, Nice, Paris Bichat, Paris Pitié Salpêtrière, Reims et Strasbourg.

Les tests d’immunoblot ont été réalisés dans neuf centres référents (NRCT) de 2006 à 2020. Les données recueillies comprenaient les informations suivantes : le diagnostic prénatal, le traitement antiparasitaire maternel par pyriméthamine/sulfadiazine/acidé folinique, ainsi que les résultats d’amniocentèse et de cordocentèse.

**Assessment of the analytical performance of the IgM triplet**

Premièrement, la spécificité du test d’immunoblot a été évaluée dans le groupe CT (les enfants nés de mères infectées). Deuxièmement, la sensibilité de la triplette IgM a été évaluée dans la population générale de patients. Les résultats ont montré que la triplette IgM peut être utile dans le diagnostic de CT.

**Discussion**

La triplette IgM a été déterminée dans le contexte du diagnostic prénatal et postnatal de CT. Elle a permis d’améliorer la sensibilité du test immunoblot de 55,04 % à 72,48 %. Ainsi, il pourrait être judicieux d’améliorer la lecture conventionnelle de l’immunoblot en ajoutant la présence des trois bandes IgM dans le schéma du nourrisson pour le diagnostic néonatal de TC.
The sensitivity of the immunoblot assay in the diagnosis of CT was determined by adding the number of cases with different immunoblot profiles and the number of cases with a non-different immunoblot profile associated with the IgM triplet in the infant profile (Fig. 2 and Table 1). All immunoblots were originated from different rainbow patterns.

Other parameters related to the presence of the infant IgM triplet

The presence and absence of the IgM triplet was analyzed according to the trimester of pregnancy at the time of infection and positive parasite DNA detection by quantitative PCR (qPCR) on amniotic fluid when available.

Statistical analysis

Statistical analysis was performed using PRISM 5.0 and STATA 14.2. Detection rates of the IgM triplet in terms of the trimester of pregnancy at the time of infection and the positivity of the qPCR on amniotic fluid were compared across methods using a Chi² test and a Clopper–Pearson test.

Results

Specificity of the IgM triplet

In the NTC group \((n = 237)\), the IgM triplet was not found in any of the infant patterns. The presence of the IgM triplet in the mother’s profile appeared in 42 cases and was therefore not used for CT diagnosis. In addition, the specificity of the infant IgM triplet was 100%.

Sensitivities of the infant IgM triplet

In the CT group \((n = 258)\), the presence of neosynthesized IgM and/or IgG was detected in 142 infected newborns. A different immunologic profile from that of the mother was found in 122 and 78 newborns for the IgM and IgG patterns, respectively. The IgM triplet was detected in 140 newborns, 45 of whom had an identical IgG and IgM immunologic profile. The IgM triplet was detected in both mother and infant in 84 mother-child pairs. Taken together, the different immunologic profiles plus the infant IgM triplet led to detection of 187 CT cases in the newborns (Table 1 and Supplementary Table S1). Supplementary Table S1 provides detailed information for each newborn with CT, including the trimester of maternal infection, results of amniotic fluid qPCR, and the presence of the IgM triplet in both mother and infant profiles. The table also includes interpretations of conventional reading of IgG and IgM profiles, and conventional reading plus inclusion of the IgM triplet, and qualitative results of Platelia or ISAGA IgM. Finally, the sensitivity of the conventional reading of the mother–infant immunoblot profile, i.e., considering any supplementary well-defined band in infant serum, was 55.0%, whereas the association of the conventional reading plus the presence of the infant IgM triplet increased the sensitivity to 72.5% (Table 1).

Effect of pregnancy trimester at the time of infection on the IgM triplet

In all CT cases, the trimester of maternal infection was the first trimester in 16/258 cases (6.2%), second in 74/258 cases (28.7%), third in 133/258 cases (51.5%), and unknown in 35/258 cases (13.6%). Depending on the knowledge of the
trimester of infection, the percentages of the presence of the infant IgM triplet or its absence were: for the first trimester 43.8% (7/16) versus 56.2% (9/16), the second 37.8% (28/74) versus 62.2% (46/74), the third 67.7% (90/133) versus 32.3% (43/133), and when unknown 42.9% (15/35) versus 57.1% (20/35). The trimester of contamination had a significant influence on the occurrence of the IgM triplet (p < 0.0001) (Chi² test between second trimester group and third trimester group). The sample size for the first trimester was too limited. The proportion of IgM triplets for contamination of the third trimester of pregnancy [67.7%; 95% CI: 59.01–75.51] is significantly higher than in the second trimester [37.8%; 95% CI: 26.8–49.8].

Presence of the infant IgM triplet and maternal treatment with pyrimethamine/sulfadiazine/folic acid (PS)

In the CT group, the amniotic fluid qPCR test results were positive, negative, not performed and with missing data in 109, 13, 98 and 38 cases, respectively. A positive qPCR result on amniotic fluid triggered treatment with PS. The IgM triplet was observed in 50 cases among 109 cases with positive amniotic fluid qPCR, in three cases when the amniotic fluid qPCR was negative (13 cases), in 56 and 30 when qPCR has not been performed (98 cases) or data were missing (38 cases), respectively. Sampling in each category was too small to be compared statistically.

Discussion

Diagnosis of CT is based on biological tests performed during the prenatal and postnatal periods and mainly on serological tests in the neonatal period. The goal is to initiate treatment of the infected offspring as soon as possible to minimize clinical sequelae. It is recognized that comparing mother and infant IgG and IgM immunoblot profiles enables early neonatal diagnosis [13, 14]. To identify other diagnostic tests for early diagnosis of toxoplasmosis infection in newborns at risk of congenital toxoplasmosis, other approaches have been explored, such as evaluating specific T cell immunity to T. gondii antigens through measurement of lymphocyte proliferation and interferon-gamma production [2, 3]. Other authors have developed a multiplexed serology assay for detection of T. gondii IgG and IgM, rubella IgG, and CMV IgG, in serum, whole blood, and saliva using novel plasmonic gold (pGOLD) chips with promising results [6]. However, these two approaches are not yet widely used in the conventional diagnosis of CT. Postnatal screening and follow-up of neonates are essentially based on serological tests: detection of specific IgM and/or IgA using immunoanalysis, monitoring IgG kinetics during follow-up using immunoanalysis, and detecting supplemental IgG and/or IgM anti-Toxoplasma in the newborn using immunoblot.

Conventionally, supplementary band(s) in newborn patterns indicate specific antibody neosynthesis in the newborn’s serum, also confirming the CT diagnosis. The sensitivity of IgG and IgM immunoblot at birth (cord blood or J3 serum) is 65% to 79% [10], reaching as high as 95.8% in the combination of WB IgM with prenatal and serological neonatal tests during the first month of life [3]. Immunologic profile testing makes it possible to determine when individualized antibodies are synthesized by the newborn following toxoplasmosis infection. CT remains a continued challenge for pregnancy and due to the severe potential sequelae, it is still necessary to improve timely neonatal diagnosis. Our findings propose to supplement conventional reading of the immunoblot with the presence of the three IgM bands in the infant pattern: 75, 90 and 100 kDa. The IgM triplet appears to be pathognomonic for the diagnosis of CT. No IgM triplet was observed in the group of uninfected infants (n = 237). This enables us to increase sensitivity of the immunoblot assay appreciably from 55.0% to 72.5%. It is emphasized that the new reading comprises both the conventional reading and the presence of the infant’s IgM triplet, regardless of whether it is present or not in the mother’s pattern. This is the first time that a test has shown a specific marker of infection in the newborn at birth, using different immunologic patterns. For the IgM triplet, a different profile showing antibody neosynthesis should not be sought. This is a new concept because these three IgM bands do not reflect neosynthesis, but probably an immunologic response against proteins involved in mother-to-child transmission. We also emphasize that this new interpretation must be integrated into the overall approach to CT diagnosis and in no way replaces the IgM/IgA immunoanalysis assay or the monitoring of IgG kinetics during follow-up. The preferential occurrence of the IgM triplet when the time of infection is the third trimester is interesting because it is sometimes too late to program prenatal diagnosis at this term. The IgM triplet can then make up for this lack of information. A proteomics analysis should be undertaken to identify these specific proteins. Most of the studies on the pathogenesis of vertical T. gondii focus on the immune response to parasite antigen stimulation, but few data describe the proteins involved in transplacental invasion [1]. In fact, T. gondii is one of the few pathogens that can cross the placenta, which probably involves a strict specific invasive process. Moreover, based on French National Reference Center data, most CT diagnoses are made on post-natal diagnosis, which highlights the utility of the triplet [15]. The IgM triplet may be one of the avenues to explore to understand placental responses to T. gondii infection. Finally, a larger study could be performed among diagnostic laboratories in association with the NRCT to extend these data.

Conflicts of interest

The authors declare that they have no conflict of interest.
Supplementary information

The supplementary material of this article is available at https://www.parasite-journal.org/10.1051/parasite/2023020/olm.

Table S1. Detail of the retrospective reading of the immunoblot mother-child pairs in the CT group. POS: positive result; NEG: negative result; trim: trimester; ND: no data; NP: not performed.

References