SEROLOGICAL STATUS FOR TORCH IN WOMEN OF CHILDBEARING AGE: A DECADE-LONG SURVEILLANCE (2012-2022) IN ITALY

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Abstract

Introduction. Serological screening and seroprevalence data of TORCH infections represents a key instrument to estimate immunity and vaccination levels, exposure rates to prevent and treat TORCH congenital infections.

Hypothesis Serology allow to identify women susceptible to primary infection.

Aim. Assess the prevalence of women at risk of primary infections by TORCH pathogens in Palermo Palermo, South of Italy, in the decade 2012-2022.

Methodology. A retrospective study was performed to evaluate the serological status (IgG and/or IgM) of 2359 women of childbearing age (WCBA), ranging from 16 to 46 years, attending the AOUP "P. Giaccone" University Hospital of Palermo.

Results. The results showed an overall prevalence of anti-TORCH IgG of 90.5% for herpesvirus (HSV), 81.2% for Rubella Virus (RV), 72.1% for cytomegalovirus (CMV), 20.9% for *Toxoplasma gondii* (TOX), and 4.8% for *Treponema pallidum* (TP). IgM positivity was 16.9% for HSV2, 10.3% for TOX, 4% for CMV and, 2% for RV. A recent/active infection by TP was confirmed in 28.3% of the seropositive women. Our results indicate that only a small percentage of WCBA was subjected to a comprehensive TORCH serological screening whilst most WCBA were tested only for a single pathogen. In addition, no significant differences were found in terms of the overall TORCH IgG seroprevalence among different age groups ($p>0.05$).

Conclusion. Identifying WCBA at risk of exposure during pregnancy allows to prevent and reduce possible congenital infections, providing detailed guidelines and instructions. The results of this study showed that in Italy the risks of acquiring primary infection by a TORCH agent is still high, therefore effective prevention strategies including serologic screening should be implemented.

Keywords: TORCH, seroprevalence, women, childbearing age, Italy

Impact statement

TORCH agents are recognized as major pathogens during pregnancy. The research of immunoglobulins (IgGs/IgMs) is the mainstay of diagnosis of TORCH infections. To date, no systematic exploration of TORCH set seroprevalence trends at country-level is available in Italy. Only a few regional studies investigating single TORCH agents have been published. The aim of this retrospective study is to provide a picture of the prevalence of anti-TORCH IgG in women of childbearing age, over almost 10 years. The results allowed to evaluate the adherence to TORCH

serological screening and to define the extent of women susceptible to primary infection. The availability of these data is essential to design the most effective prevention strategies to reduce the risk of congenital infections.

Introduction

The acronym TORCH classically refers to a group of well-known bacteria, parasites and viruses which have been related to pregnancy complications, congenital anomalies, and permanent sequelae due to intrauterine/perinatal infections [1–4]. TORCH set includes *Toxoplasma gondii* (TOX), Rubella Virus (RV), Cytomegalovirus (CMV), Herpes Simplex Virus (HSV), and a broad "other" category of microbial agents, including *Treponema pallidum* (TP). The high rate of asymptomatic TORCH infections and the absence of effective preventive prophylaxis, except for the anti-RV live attenuate vaccine, increase the risk of vertical transmission in new-borns and infants. The overall rate of transmission of TOX, RV and CMV primary infection to the fetus ranges between approximately 30-80% [1, 5].

Congenital infections with CMV and TOX are more commonly linked with long-term sequelae that may not be apparent at birth, such as sensory deficits, developmental delay/mental retardation, and central nervous system lesions [4].

Rubella is a mild viral disease, in most cases asymptomatic. Infection with RV causes the most severe damage especially if acquired during the first trimester [2].

The majority of neonatal HSV infections are caused by HSV type 2. Maternal primary infection during the third trimester has the highest percentage of neonatal infection. It has been estimated that HSV2 risk of vertical transmission is 30% higher compared to HSV1. Secondary reactivation of HSV is 10 to 30 times less likely to result in transmission to the infant [1,2].

Serological screening of TORCH infections provides information on the immunological status of women of childbearing age (WCBA), allowing the identification of women susceptible to primary infection. Thus, the research of immunoglobulins M (IgMs) and/or immunoglobulins G (IgGs) is the mainstay of diagnosis of TORCH infections [6]. The epidemiology of TORCH agents is variable across countries and may also change according to ethnical groups and socio-economic classes within a single country [5, 7, 8]. To date, no systematic exploration of TORCH set seroprevalence trends is available in Italy, but only few regional studies investigating single TORCH agents have been published [9–11]. Therefore, the aim of this study was to retrospectively evaluate the serological status (IgG and/or IgM) of WCBA, attending the AOUP "P. Giaccone" University Hospital of Palermo, South of Italy, over almost 10 years, in order to estimate the adherence to TORCH serological screening and to assess the prevalence of women at risk of primary infections.

Methods

Patient enrollment and specimen collection

A retrospective analysis of the data routinely acquired during patient care at the University Hospital AOUP "P. Giaccone" of Palermo, Sicily was performed. The results of 5643 serological screening collected from 2359 patients were analyzed. The patients selected were all women aged 16-46 years (WCBA) tested for at least one TORCH agent according to medical prescription of care services (National Health Care) or the Gynecology wards of the University Hospital, from 10 November 2012 to 1 April 2022. For each of the 2359 patients, only the result of the first serologic test performed for each individual TORCH agent was included in the analysis. Patients with an unspecific or borderline test result were excluded.

Serologic screening

TORCH screening tests were performed on sera samples according to manufacturer's instructions after blood centrifugation at 1200 × g for 10 min. The presence of specific IgG and IgM reactivity was tested using an Enzymatic Immuno Assay (EIA) (Euroimmun Sprinter, Italia Diagnostica MedicaS.r.l) or Chemiluminescence immunoassays (CLIA) (LIAISON® XL, Diasorin, Saluggia, Italy or Vitros 3600 Ortho Clinical Diagnostics, US). Serological results were analysed according to the manufacturer's instructions. IgM positivity, irrespective of IgG antibodies (Abs) results, was considered indicative of acute or recent infection. For TP screening, a non-Treponemal test (SIFILIDE RPR, LTA s.r.l, Itaonline, Bussero, Milano, Italy) and a Treponemal test (ELISA IgM and IgG, VIRCLIA, Alifax S.r.l, Italia) were performed simultaneously. The TP samples were considered seronegative if both tests gave a negative result; seropositive and indicative of a recent infection, when positive specific antibodies and a non-Treponemal RPR titer >8 were found; or seropositive and indicative of a past infection, when positive specific antibodies and a RPR test negative or with titers <8 were found.

Statistical analysis

Univariate binomial and beta-binomial regression models, with variables "years" and "age (0:16-25, 1:26-35, 2:36-46)" used as continuous regressor, were fitted to detect significant trends in percentages of positives. Chi-square and Fisher's exact tests were used to compare differences between the

groups. All analyses were performed with R software version 4.1.2 (https://www.r-project.org/) and two-tailed p-values < 0.05 were considered statistically significant.

Results

TORCH seroprevalence

The sera included in this study were collected from 2359 WCBA, whose age ranged from 16 to 46 years, with a mean age of 29 ± 6. Most of the patients had been screened for Abs against a single TORCH pathogen, 20.9% had been tested for two pathogens (TOX and CMV), while 14% were screened for three agents (TOX, CMV and RV). In particular, 61.2% of the WCBA included in the study had been tested for TOX, 58.6% for CMV, 46.9% for TP, 45.1% for RV and 5% for HSV1-2. HSV2 IgGs were researched in 2.1% of WCBA. The serological results of IgG and/or IgM assays performed against each TORCH agent are shown in Table 1.

The overall anti-HSV1-2 IgG seroprevalence was 90.5%, 81.2% for RV, 72.5% for CMV, and 21.5% for TOX. TP serology indicative of syphilis infection was detected in 4.8% of women.

Seroprevalence by age

The study population was stratified into three age groups: 16–25, 26–35, and 36–46 years, with an age specific distribution of 28.1%, 53.6%, and 14.2%, respectively. The statistical analysis showed no significant changes in the overall TORCH IgG seroprevalence among the different age groups evaluated ($p>0.05$) (Table 2). No-significant trends were estimated for the overall TORCH IgG seroprevalence during the whole decade investigated, except for HSV1-2 for which a positive trend was observed ($p<0.05$). Significant erratic variations were detected by Fisher's exact test only for TP ($p=0.01$) and HSV2 ($p<0.001$) (Figure 1).

Detection of acute infection

Simultaneous IgM and IgG screening was available for 96.4% of WCBA tested for RV, 91.5% for CMV, 85.3% for HSV2 and 55.7% for TOX. IgM positivity was detected in 16.9% of WCBA tested for HSV2, 10.3% for TOX, 4% for CMV and 2% for RV (table 1). RPR assay confirmed a recent/active infection by TP in 28.3% of the women with an antibody positivity. Out of the 48 CMV IgM+IgG double-positive sera, 29.2% of samples had been tested to determine the IgG avidity index, allowing to detect a recent infection (low IgG avidity) in 50% of women tested. Unfortunately, IgG avidity tests for TOX and RV were not available, and no clinical information could be retrieved from the databases.

Discussion

Vertical transmission of TORCH agents, chiefly at primary infections, represents one of the main causes of symptomatic congenital infections, which can lead to devastating consequences in the developing fetus. This retrospective study provides a picture of TORCH seroprevalence of WCBA screened (for the first time) for a single or for multiple TORCH agents at the Microbiology Unit of the AOUP "P. Giaccone" University Hospital of Palermo, Sicily from 2011 to 2022. The availability of serological data, collected over almost 10 years, allowed to evaluate the adherence to TORCH serological screening and to define the extent of women susceptible to primary infection.

In Italy, serological tests (IgG and IgM) against TOX are free of charge and scheduled during the whole pregnancy. RV immunity and TP infections are screened before and/or during pregnancy, while CMV and HSV screening are only recommended (DPCM12 January 2017 1–3). Overall, the results of this study highlighted a limited access to a comprehensive TORCH screening as almost 50% of the WCBA was screened only for one or a few of the TORCH infectious agents. This implies that Italian WCBA may not be consistently advised to seek for serological testing before and during their pregnancies.

Congenital infections with CMV and TOX are more commonly linked with long-term sequelae that may not be apparent at birth, such as sensory deficits, developmental delay/mental retardation, and central nervous system lesions [12].

The overall seroprevalence rates of IgGs against TOX (21.5%) and CMV (72.5%) observed in this study were in accordance with other epidemiological European surveys which showed seroprevalence rates ranging between 19.4-43.8% for TOX and 30.4-89.7% for CMV [9, 11, 13, 14].

In Italy, anti-rubella vaccine was introduced in 1972 and is currently administered as combined measles-mumps-rubella (MMR) vaccine to all infants. According to the data of the Italian Ministry of Health, the vaccination coverage for rubella in Italy was 92.21% in 2020 (1 dose within 24 months of age, children born in 2018), increasing from 89.2% in 2012, and showing a minimum rate of 85.22% in 2015 and a maximum of 94.47% in 2019 (https://www.salute.gov.it/portale/documentazione/p6_2_8_3_1.jsp?lingua=italiano&id=20).

In Sicily during the same period rubella vaccine coverage was constantly lower respect to national average with a minimum rate of 79.18% in 2015 and a maximum of 92.2% in 2019.

In this study, the rate of IgG positivity against RV observed (81.8%) was comparable to previous studies conducted in Messina, Italy (85.8%) [15], and in UK [16], showing that 18% of WCBA tested susceptible to primary rubella infection. However, these percentages are suggestively lower compared to other European countries (≥95% anti-RV Abs seroprevalence) [14, 17, 18].

Although the incidence of congenital rubella was below the WHO target of 1/100,000 live births, the possibility of underreporting to the national surveillance system should be taken into account [19, 20]. Our data showed that the RV immunization coverage is not still optimal to guarantee the control of congenital infection, underlining the need to implement infants' vaccination and proper health surveillance in our geographic area.

Syphilis, one of the main sexually transmitted infections, is still a public health problem worldwide, being considered a re-emerging infection in several European countries, probably due to migration waves from areas where the infection is endemic [21, 22]. Although the transmission rate of Syphilis is more than 80% in recently infected mothers, congenital syphilis can be prevented with specific treatments. Thus, serological tests represent an important diagnostic instrument to identify infected mothers, potential source of congenital syphilis [22]. In this study, TP seroprevalence was higher (4.8%) compared to previous studies conducted on pregnant women in North of Italy and in other European countries which showed seroprevalence rates ranging from 0.16 to 2.9% [22–25]. Moreover, in this study, active syphilis was observed in 28.3% of the seropositive women. These data confirm the need to implement antenatal screening and sexual health campaigns in order to reduce the prevalence of syphilis.

HSV2 vertical transmission could occur during a primary infection but also as consequence of reactivation. Testing anti-HSV IgGs allows to detect seronegative women susceptible to infection as well as seropositive women at risk of reactivation. Previous studies showed higher prevalence of HSV2 infection in Northern Europe compared to Southern Europe (from 26.6 in Norway to 3.5% in Spain) [14]. In our study a surprisingly low number of WCBA had been screened for anti-HSV Abs (5% of the study population), with a seroprevalence of >90%. Anti-HSV2 Abs were detected in 20% of HSV IgG-positive WCBA.

The analysis of the anti-TORCH IgG seroprevalence trend during a 10-year period showed an almost stable seroprevalence for each infectious agent, with average values of 82.5% for RV, 82.3% for HSV (12.4% for HSV2), 72.2% for CMV and 21.1% for TOX infections. Analogous comprehensive studies in Italy are not available, although there are data obtained for specific TORCH agents,

253 scattered in terms of temporal and geographical coverage. A decreasing trend of TOX seroprevalence
254 in WCBA has been reported from 1995 to 2005 (48.5% to 21.5%), revealing, in parallel, a
255 geographical pattern, with TOX seroprevalence being lower in Northern Italy (Siena: 12.4%) than in
256 Southern Italy (Bari: 22.4%), probably due to different social-economic factors [10, 11]. By contrast,
257 a comparable prevalence of anti-CMV Abs in pregnant women has been observed in Sicily (Southern
258 Italy) and Lombardia (Northern Italy) [26]). Interestingly, an increasing seroprevalence trend (from
259 66.7% in 2012 to 73.7% in 2022) was observed over time for CMV in our study. This trend differs
260 from the negative trend observed in Germany in women from 1988 to 2018 (63.7% to 56.4%) [13].
261 In the attempt to spot possible age-related patterns and risk factors, we analysed the data considering
262 three age groups, 16–25, 26–35, and 36–46 years. No statistically significant differences were
263 observed in TORCH seroprevalence rate among the three age groups analysed. Likewise, a recent
264 seroepidemiological study in Croatia showed comparable RV and CMV seroprevalence rates between
265 different age groups of WCBA [14]. However, an age-related trend in terms of presence of CMV
266 IgGs has been determined in Central Italy, with a lower seroprevalence in younger people [27]. In
267 addition, a significant positive correlation with age groups was previously described for TOX
268 seropositivity, with a markedly increased reactivity in older people [10, 14]. Generally, the increasing
269 seroprevalence of TORCH pathogens with age could be explained as a result of cumulative exposure
270 to these pathogens throughout life. Our results indicate that almost 30% of WCBA were susceptible
271 to primary CMV infection. In addition, the seropositivity values for TOX (18.8%) and TP (4.8%)
272 suggested to maintain screening campaigns for these pathogens in order to promote the best
273 behavioural educational strategies. In this study, acute or recent TOX infections were detected by the
274 research of IgM antibodies in about 10% of the screened women. IgM-positive results were obtained
275 in 2% of WCBA tested for RV immunity, in a period between 2013 and 2019, mostly in immigrant
276 women. Unfortunately, no further investigations could be performed to confirm acute infections by
277 TOX. Likewise, we could not perform further analysis to understand whether anti-RV IgMs were
278 linked to a recent natural infection or to vaccination. However, recent CMV infection was verified by
279 an IgG avidity assay in 50% (7/14) of the IgG + IgM-positive women, altough the persistence of IgMs
280 over time may limit the significance of this screening. Nevertheless, an early diagnosis of acute
281 infection during pregnancy could be helpful for adoption of appropriate intervention and for proper
282 management. Unfortunately, we had only partial information on pregnancy and gestational week of
283 the WCBA included in this survey. Therefore, it was not possible to stratify the patient's cohort by
284 pregnancy and correlate the stage of pregnancy with the risks to transmit the infection to the fetus.
285 Early detection through maternal serological screening is crucial in the management of TORCH
286 congenital infections. Identifying women at risk of exposure during pregnancy allows to prevent and

reduce possible congenital infections, providing behaviour guidelines. Also, the detection of infections in pregnancy may allow paediatricians to identify high-risk infants for prompt and adequate treatement against congenital infections. The evolution of the epidemiological scenario of TORCH infection in Europe over the time, also due to migratory waves, has re-opened the discussion on the awareness of the risks related to TORCH infections and on the appropriateness of serological screening during pregnancy. The results of this retrospective study showed that in Italy the risks of acquiring primary infection by a TORCH agent is high. Increasing awareness of the risks posed by TORCH infections and implementing effective prevention strategies is a priority for the health bodies.

List of abbreviations

Toxoplasma gondii (TOX),
Rubella Virus (RV),
Cytomegalovirus (CMV),
Herpes Simplex Virus (HSV),
Treponema pallidum (TP)
Childbearing age women (WCBA)
Immunoglobulins M (IgMs)
Immunoglobulins G (IgGs)

Author contributions

SDG was responsible for the conceptualization, formal analysis, writing - review & editing - original draft. EP, FB, CC, GC, DP performed the analysis and wrote the original draft. CM e GM were responsible of data acquisition. DM was involved in data acquisition and revision of the manuscript. ME was responsible of statistical analyses. GMG and FD contributed to the review and editing. All authors read and approved the final manuscript.

Financial support

This work was supported by the Ministero dell'Istruzione, dell'Università e della Ricerca (Italian Ministry of Education, University and Research) Fondo Finalizzato alla Ricerca di ateneo (FFR) 2018-2020 and 2021.

Conflict of interest

The authors declare no conflict of interest.

Ethical standards

This work is part of the routine duties of the Microbiology Unit of the AOUP "P. Giaccone" University Hospital of Palermo, Italy. Therefore, institutional review and informed consent are not claimed. All analysed data are anonymous.

Data availability statement

Data supporting the conclusions of this article are included within the article.

Figure and Table legends

Figure 1. Observed vs predicted IgG seroprevalence variations for six screening tests over the years 2012-2022. Confidence intervals for predictions and p-values for trends are also reported. The size of the symbols is proportional to the sample sizes.

Table 1. Serological analyses results of TORCH immunoassays in 2359 women of childbearing age.

Table 2. Prevalence of anti-TORCH agents immunoglobulins in childbearing age women distributed by age-group.

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