blockers, provided that the expected benefit to treatment of the inflammatory disease is significant. To our knowledge, this is the first study to describe the long-term surveillance of patients who developed TB while being treated with TNF-α blocker therapy, and who then resumed TNF-α treatment after being treated successfully for TB. The data suggest that TB is not a contraindication to recommencing anti-TNF-α therapy if this would be beneficial for the underlying inflammatory disease.

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RESEARCH NOTE

Seroprevalence and incidence of *Toxoplasma gondii* infection in the Legnano area of Italy

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ABSTRACT

The decreasing prevalence of anti-*Toxoplasma* antibodies in Europe has re-opened the question of the appropriateness of serological screening during pregnancy. A study of 3426 pregnant women, resident in the Legnano area of Italy, revealed that the IgG seroprevalence according to ELISA was 21.5%, and that of IgM according to ELISA and enzyme-linked fluorescent assay was 1.2% and 0.9%, respectively. The incidence of infection, estimated on the basis of IgG avidity, was 0.9%. These results confirm a decrease in the prevalence of IgG, but indicate a high incidence of infection, thus suggesting that screening for anti-*Toxoplasma* antibodies during pregnancy should be maintained.

Keywords Antibodies, incidence, pregnancy, screening, seroprevalence, *Toxoplasma*

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Screening tests for pregnant and pre-pregnant women have been developed to prevent congenital *Toxoplasma gondii* infection. These tests are applied in various ways in France, Belgium, Switzerland and Austria [1,2], but are not recommended for routine use in the UK, The Netherlands or Norway [3,4]. In Italy, serological tests for toxoplasmosis are performed as a routine check during pregnancy. However, the decreasing incidence of *Toxoplasma* infection in Europe [5,6] has changed the epidemiological picture and re-opened the debate concerning the appropriateness of serological screening during pregnancy. This has raised the question of how to calculate the true incidence of this infection. Some of the proposed mathematical models use data concerning the seroprevalence of IgG- and IgM-specific antibodies [7]. In particular, IgM antibodies seem to be useful for detecting acute or recent infections; however, their early disappearance or persistence over time limits their significance [8], thus making it important to assess the avidity of anti-*Toxoplasma* IgG antibodies. A high level of IgG avidity usually excludes an infection during the previous 3–5 months [9,10], so that, in the absence of other clinical data, high titres of IgM without IgG may indicate an acute infection, while the presence of IgM and IgG with low or borderline IgG avidity could indicate recent infection. Thus, measuring avidity could provide more useful data for calculating incidence. The aim of the present study was to investigate the prevalence and incidence of *Toxoplasma* infection among pregnant women resident in the area of Legnano (Milan, Italy) in order to evaluate the appropriateness of maintaining *Toxoplasma* screening during pregnancy.

In 2004 and 2005, 3426 pregnant women (aged 15–44 years) were referred to the Microbiology Unit of Legnano Hospital for serological screening; 45.2% were in the first trimester of pregnancy, 29.3% were in the second, and 25.5% were in the third.

IgG and IgM anti-*Toxoplasma* antibodies were detected using ELISAs (ETI-TOXOK-G-PLUS and ETI-TOXOK-M-reverse-PLUS; DiaSorin, Saluggia, Italy). The IgG cut-off value was 15 IU/mL; for IgM, samples with an absorbance at least as great as the value for the 'cut-off control' contained in the kit were considered to be positive. The IgM-positive samples were also tested using an enzyme-linked fluorescent assay (ELFA) (VIDAS Toxo IgM; bioMérieux, Marcy l'Etoile, France), and were considered to be positive when the index was ≥0.65, and as borderline when it was between 0.55 and 0.64. IgG anti-*Toxoplasma* avidity (VIDAS Toxo IgG Avidity; bioMérieux) was assessed for the ELISA IgM-positive samples, and was classified as low if the index was <0.2, borderline if it was ≥0.2 to <0.3, and high if it was ≥0.3. Samples that were rheumatoid factor-positive (Arthri-Slidex; bioMérieux) were not included in the analysis.

The incidence of toxoplasmosis was calculated by adapting Janssen's mathematical model for calculating the incidence of human immunodeficiency virus infections [11]:

$$I\mathrm{d}t = (n\mathrm{d}t/N)(365/T)(100)$$

where Idt is incidence, $n$dt is the number of individuals with low or borderline avidity, N is the number of IgG-negative individuals plus individuals with low or borderline avidity, and T is the estimated mean number of days of low or borderline avidity (=120) [10].

In total, 737 (21.5%) of the 3426 pregnant women were positive for IgG, and 42 (1.2%) were positive for IgM anti-*Toxoplasma* antibodies according to ELISA (Table 1); 31 (73.8%) of the 42 IgM-positive samples were also positive or borderline according to ELFA.

Avidity was measured in 37 samples, and was low or borderline in eight (26.7%) of 30 ELFA IgM-positive or borderline samples from five women in the first, one in the second and two in the third trimester, and in two (28.6%) of seven ELFA IgM-negative samples, both from women in the first trimester (Table 2). It was not possible to investigate whether these were women with persistently low IgG avidity, because they could not be followed-up (these women were not

Table 1. Prevalence of IgG anti-*Toxoplasma* antibodies (ELISA), IgM (ELISA and ELFA), and IgG avidity and incidence in 3426 pregnant women during 2004–2005

	Pregnant women		
	No.	[redacted]	95% CI
ELISA IgG-positive	737	21.5	20.13–22.89
ELISA IgM-positive	42	1.2	0.86–1.60
ELFA IgM-positive	31	0.9	0.58–1.22
Low or borderline IgG avidity	10	0.3	0.11–0.47
Incidence		0.9	0.58–1.22

ELFA, enzyme-linked fluorescent assay.

Table 2. ELFA IgM anti-*Toxoplasma* antibodies and IgG avidity in 42 ELISA IgM-positive pregnant women during 2004–2005

ELFA IgM-positive			IgG avidity	
Result	**No.**	[redacted]	**High**	**Low**/borderline
Positive or borderline	31	73.8	22/30 (73.3%)	8/30 (26.7%)
Negative	11	26.2	5/7 (71.4%)	2/7 (28.6%)
Total	42	100	27/37 (73.0%)	10/37 (27.0%)

ELFA, enzyme-linked fluorescent assay.

considered in the incidence calculation). IgG avidity was not measured in five subjects because they were IgG-negative. Each of these subjects was weakly IgM-positive according to ELISA (the ratio of sample absorbance to the 'cut-off control' absorbance was between 1 and 1.75). The women who could be followed-up (all ELFA IgM-negative) showed the disappearance of ELISA IgM after 2–7 months, but IgG was not detected in any of these cases. Patient records documented the presence of IgM 4–7 years previously for four subjects with weakly positive IgM according to ELISA and ELFA, and positive IgG and high IgG avidity. The incidence estimated on the basis of ELISA and ELFA IgM positivity with low or borderline IgG avidity was 0.9% (Table 1).

Overall, the data indicated a prevalence of anti-*Toxoplasma* IgG of 21.5% (95% CI 20.13–22.89), which is similar to the figure reported in an Italian study during 2000 [12], but is only about half the prevalence recorded in Italy during the 1980s [13,14]. The figure of 21.5% is also similar to that reported in other European countries [5,6,15]. The prevalence of anti-*Toxoplasma* IgM was 1.2% (95% CI 0.86–1.60) according to ELISA, and 0.9% (95% CI 0.58–1.22) according to ELFA, but, given the persistence of IgM over time, it is difficult to use only IgM to estimate the incidence of infection. Furthermore, non-specific phenomena may make evaluation difficult; thus, in cases with weak IgM positivity without IgG, the low IgM titres remained constant or disappeared without detection of IgG.

Considering low or borderline avidity in the presence of IgM positivity according to both ELISA and ELFA (actual presence of IgM) as the means of identifying recent infections, the estimated incidence (using Janssen's mathematical model for human immunodeficiency virus infection) was 0.9% (95% CI 0.58–1.22), which is higher than incidences reported in northern Europe [16,17], but similar to French data [18] and lower than the incidence of 3.5% reported recently for Italy [19]. However, these differences may be related to the different mathematical models used.

In conclusion, despite the decrease in toxoplasmosis, the incidence in the area studied is still one of the highest in Europe (*c.* 9/1000 pregnant women), which suggests that, at least in this area, it is appropriate to maintain active screening for toxoplasmosis during pregnancy.

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