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SHORT COMMUNICATION

Toxoplasma gondii: Usefulness of ROP1 Recombinant Antigen in an Immunoglobulin G Avidity Assay for Diagnosis of Acute Toxoplasmosis in Humans

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Abstract

The results present in this study suggest that the *Toxoplasma gondii* recombinant ROP1 antigen in an IgG avidity test can be useful for detection of acute stage of infection. Specific antibodies of low avidity were detected in most of the sera from individuals with acute toxoplasmosis, while the absence or specific antibodies of high avidity were detected in sera from patients with chronic infection.

Key words: *Toxoplasma gondii*, ELISA, recombinant antigen, rhoptry protein 1, serology

Toxoplasma gondii is an obligate intracellular parasite that infects many warm-blooded animals. Although, human toxoplasmosis is generally asymptomatic, a serious disease can occur in the case of a congenital infection and immunocompromised individuals such as AIDS patients and transplant recipients (McAuley *et al.*, 1994; Sàfadi *et al*., 2003). For that reason, it is crucial to estimate the time of infection in pregnant women. Detection of specific IgM and IgG are important but inadequate steps in diagnosis of acute toxoplasmosis (Bertozzi *et al*., 1999; Bessieres *et al*., 1992; Brooks *et al*., 1987; Iqbal and Khalid, 2007; Liesenfeld *et al.*, 1997). The determination of the avidity index of IgG antibodies is considered very important in the diagnosis of an acute infection *vs* a chronic infection (Jenum *et al.*, 1997; Lappalainen *et al.*, 1993; Liesenfeld *et al.*, 2001; Montoya *et al*., 2002). In most cases, the currently available commercial tests are based on antigens obtained from a whole tachyzoites of *Toxoplasma* (TLA). The methods of producing tachyzoites as well as antigen(s) may vary significantly between laboratories and this means that the test is difficult to standardize. Furthermore, it was confirmed that low IgG avidity may be detected for a long time after infection (Ashburn *et al.*, 1998; Villavedra *et al*., 1999). That is why recombinant antigens are considered to replace the antigen obtained from lysed whole parasites. In this case the antigen composition of the test is precisely known and the method can be easily standardized. Several recent studies have reported the use of recombinant antigens (Beghetto *et al.*, 2003; Marcolino *et al*., 2000) or mixture of proteins (Pietkiewicz *et al.*, 2005) in determination of IgG avidity.

The aim of this study was to estimate the diagnostic value of r-ROP1 in IgG ELISA avidity for serodiagnosis of *T. gondii* infection in humans.

A total of 172 serum samples received from a routine toxoplasmosis screening were analyzed and divided into four groups according to the results obtained with the VIDAS Toxo-IgG, VIDAS Toxo-IgG avidity, and VIDAS Toxo-IgM commercial tests (bioMérieux, France): group I – 34 sera from patients suspected of an acute toxoplasmosis (positive IgM, positive IgG with low or borderline avidity); group II – 16 sera from patients with postacute toxoplasmosis (negative IgM, positive IgG with low or borderline avidity); group III – 92 sera from patients with chronic toxoplasmosis (negative IgM, positive IgG with high avidity); IV group – 30 sera from seronegative individuals. Furthermore, we analyzed the avidity maturation of specific antibodies in serum samples received from

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seven pregnant women classified as patients with acute or postacute phase of toxoplasmosis.

In this study, we used in-house IgG ELISA test with r-ROP1 expressed and purified as described in our previous paper (Holec-Gąsior *et al.*, 2009). Each serum sample was used in increasing serial dilutions (1:100–1:800) in separate plates coated with r-ROP1 and there were two different IgG ELISA procedures: normal and avidity ELISA washed with urea solution (PBS, 0.1% Triton X-100 with 6 M urea) three times for 10 min. All groups of human sera were examined by IgG ELISA and three groups (I–III) by IgG ELISA avidity test. Group IV was tested in order to calculate the cutoff which was set as the mean value of the negative serum samples plus two standard deviations.

The results were shown as avidity indexes (AIs) which were calculated as the ratio between optical density for the sample washed with urea solution and optical density for the sample washed with washing buffer (for the dilution which gave OD near 1.0). AI below 0.3 was considered as low avidity, 0.3–0.4 as borderline avidity and values above 0.4 as high avidity. This division was established on the basis of results obtain by Paul (1999) and applied by Pietkiewicz *et al.* (2007).

None of the negative serum samples reacted above cutoff, resulting in a specificity of 100% for ELISAs (Fig. 1). The sensitivity of IgG ELISA for r-ROP1 calculated for three groups of positive sera was at 43%. However, reactivity was different in each group (Fig. 1). Examination of group I sera showed that 29 out of 34 (85%) reacted with rROP1 and in 26 out of 29 cases (90%) reacted at low or borderline avidity. Only three sera (10%) from this group reacted at high avidity. In group II, 6 serum samples out of 16 (37,5%) had specific IgG antibodies against r-ROP1 and 4 of them reacted at high avidity, 2 at low or borderline avidity. Only 23 out of 92 sera (25%) from patients with chronic toxoplasmosis (group III) reacted with r-ROP1 and 22 cases (96%) were with high avidity. Only one serum sample from this group had low avidity (Fig. 2).

In this study we also demonstrated a comparison of avidity maturation of specific IgG antibodies against r-ROP1 and lysed, whole-cell antigen (TLA) in sera from seven pregnant women suspected of acute or postacute toxoplasmosis. Our results showed an increase in time of the avidity indexes (AI) in the IgG ELISA with TLA for all individuals tested (Table I). In the IgG ELISA with r-ROP1 antigen the increase of the avidity indexes was also observed for five individuals, however, there was no reactivity with specific IgG for three patients' serum samples (4 sera) obtained after 2, 8, 10 or 16 weeks from first time

Table I
Comparison of IgG avidity maturation in serum samples obtained from seven pregnant women suspected of acute or postacute toxoplasmosis.

Patient no	Number of weeks after the first sample was taken	IgM (VIDAS TOXO IgM)[a]	Lysed whole cell Assay (VIDAS TOXO IgG AVIDITY)		rROP1 assay	
			Avidity index (AI)	Interpretation[b]	Avidity index (AI)	Interpretation[c]
1	0	+	0.106	Low	0.19	Low
	24	+	0.117	Low	0.24	Low
2	0	+	0.03	Low	0.27	Low
	3	+	0.044	Low	0.37	Borderline
3	0	+	0.231	Borderline	0.66	High
	2	+	0.238	Borderline	–	–
4	0	–	0.121	Low	0.35	Borderline
	8	–	0.114	Low	–	–
	10	–	0.178	Low	–	–
5	0	+	0.108	Low	0.18	Low
	8	+	0.110	Low	0.22	Low
	12	–	0.111	Low	0.25	Low
	16	–	0.125	Low	–	–
6	0	+	0.217	Borderline	0.28	Low
	8	+	0.257	Borderline	0.31	Borderline
7	0	+	0.103	Low	0.22	Low
	4	+	0.12	Low	0.23	Low

[a] + means that patient has IgM, – means absence of IgM.
[b] AI below 0.2 means low avidity index, 0.2–0.3 – borderline avidity index, over 0.3 – high avidity index,
[c] AI below 0.3 means low avidity index, 0.3–0.4 – borderline avidity index, over 0.4 – high avidity index, – means lack of r-ROP1 antigen reactivity with IgG specific antibodies.

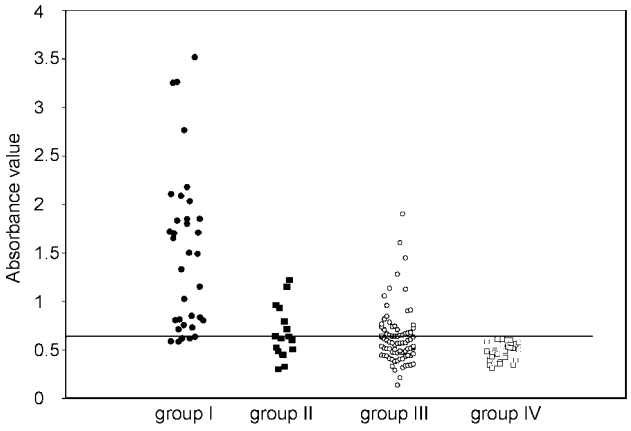

Fig. 1. Immunoreactivity of r-ROP1 protein with four groups of sera (diluted 1:100) from patients with acute (●), postacute (■), and chronic phase of toxoplasmosis (○) and from seronegative individuals (□).

Absorbance was measured at 492nm. Horizontal line represents the cutoff value (0.642).

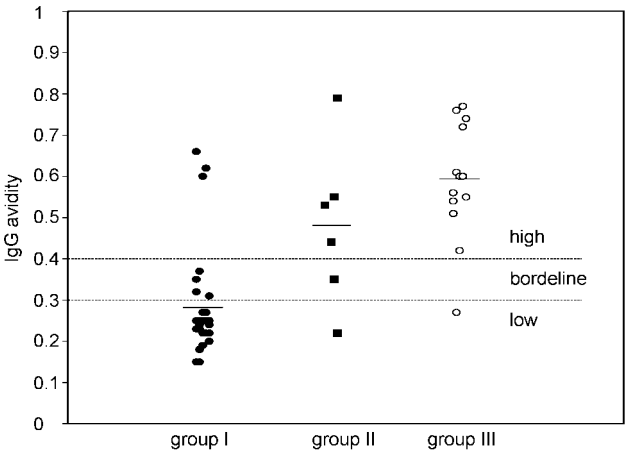

Fig. 2. IgG avidity indexes for r-ROP1 with seropositive sera from patients with an acute (●), postacute (■), and chronic toxoplasmosis (○).

The mean value is indicated for each group by horizontal line (0.282 for group I; 0.480 for group II; 0.594 for group III).

of serum delivery. These results together with those obtained for group III sera, where specific antibodies against r-ROP1 were not detected in 75% of serum samples from patients with chronic toxoplasmosis, confirmed our previously published data (Holec-Gąsior *et al.*, 2009) and Aubert *et al.* (2000) that IgG antibodies against r-ROP1 antigen are produced during the acute stage of toxoplasmosis but are uncommon in the chronic phase of the infection.

In conclusion, when specific IgG antibodies react with r-ROP1 both phases of toxoplasmosis can be suspected. Therefore, IgG avidity assay with r-ROP1 may indicate phase of infection, where low avidity suggests acute toxoplasmosis and high avidity or lack of reactivity may indicate chronic infection. However, further work is needed before an immunoassay with recombinant ROP1 antigen will be useful for clinical purposes.

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