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Evaluation of a commercial IgE ELISA in comparison with IgA and IgM ELISAs, IgG avidity assay and complement fixation for the diagnosis of acute toxoplasmosis

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ABSTRACT

A panel of sera from patients with known case histories representative of acute toxoplasmosis (primarily lymphadenopathy, $n = 106$), latent toxoplasmosis (asymptomatic, $n = 368$) and negative samples ($n = 54$) was used to evaluate the capacity of five serological tests to differentiate among patients with acute or latent toxoplasmosis and non-infected individuals. Positive IgA, IgE and IgM ELISA results and low IgG avidity and complement fixation test (CFT) titres of ≥256 were considered to be indicative of acute toxoplasmosis. The most sensitive methods were IgM ELISA (98.1%) and CFT (97.1%), albeit with low specificity (65.0% and 64.5%, respectively) and positive predictive values (43.3% and 42.7%, respectively). IgG avidity assay and IgE ELISA had the highest specificity (97.7% and 91.7%, respectively) and the highest positive predictive values (89.4% and 75.6%, respectively). The best association between serological results and clinical findings was obtained with IgE ELISA (86%, as expressed via Youden's index). In a subset of 259 samples categorised by the period between the onset of clinical symptoms and sampling, >50% of patients had enlarged lymph nodes for <4 months, despite a broad range of differences. However, IgM remained positive for 12–18 months, IgA for 6–9 months and IgE for 4–6 months. IgG avidity remained low for a maximum of 4 months, after which avidity increased despite the persistence of enlarged lymph nodes and a positive IgE assay. Detection of IgE appears to be a highly specific test for confirming the acute nature of *Toxoplasma* infections that have been detected by other sensitive methods.

Keywords Acute infection, diagnosis, IgE ELISA, lymphadenopathy, serology, toxoplasmosis

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INTRODUCTION

Toxoplasmosis in post-natally infected immunocompetent individuals, caused by the protozoan *Toxoplasma gondii*, usually occurs in two phases, acute and latent. Differentiation of these phases is both clinically and diagnostically decisive. Recent infection has an acute character and is often accompanied by clinical symptoms, most frequently lymphadenopathy, low-grade fever and malaise. In pregnant women, the infection can be transmitted to the embryo during the acute phase. In comparison, the subsequent latent phase runs a course free of clinical signs, and transmission of the infection to the embryo does not occur in pregnant women with latent toxoplasmosis. Permanent antigen stimulation means that individuals with latent toxoplasmosis usually have life-long anti-*Toxoplasma* antibodies [1–3].

Toxoplasma seroprevalence in the general population of the Czech Republic is *c.* 30% [4], but in certain seropositive individuals, primarily pregnant women, it is difficult to determine whether the toxoplasmosis is acute or latent [5]. The most reliable indicator of acute toxoplasmosis is seroconversion from a negative to a positive titre, or a four-fold rise in titre over a 3-week interval [1]; however, at least a preliminary diagnosis is

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usually required upon first examination, and monitoring trends of antibody response is, in many cases, not possible. Anti-*Toxoplasma* IgM antibodies are considered to be the main marker of acute toxoplasmosis [6], but the limitations of tests for IgM, e.g., the persistence of positive values [7], lack of specificity associated with rheumatoid factor or other immunopathological responses [7–9] and innate anti-*Toxoplasma* IgM [10], necessitate the use of additional methods, such as IgA tests or IgG avidity assays. However, even these methods are not without specific problems, and it is still impossible to interpret results with absolute certainty.

Although considered to be a useful marker of acute infection, detection of anti-*Toxoplasma* IgE has been used only rarely for routine diagnostic purposes. Since 2001, a commercially available IgE ELISA has been incorporated into the diagnostic scheme at the National Reference Laboratory for Toxoplasmosis (Prague, Czech Republic). The present study evaluated the capability of this test to confirm acute toxoplasmosis and compared its performance with that of other diagnostic methods.

MATERIALS AND METHODS

Patients and sera

In total, 439 patients (97 males aged 0–85 years, median 19 years, and 342 females aged 1–79 years, median 28 years) of known clinical status were examined. Anti-*Toxoplasma*-positive patients were tested repeatedly, but results obtained before the introduction of the IgE ELISA were not included. The total number of serum samples analysed was 545. The reasons given for submitting sera to the National Reference Laboratory for Toxoplasmosis were: lymphadenopathy (29.3%); follow-up after clinical toxoplasmosis (25.5%); screening during pregnancy (20.8%); differential diagnosis in patients with other diagnoses (7.3%); a previous finding of anti-*Toxoplasma* antibodies (5.6%); screening before planned pregnancy (4.7%); febrile and sub-febrile states and fatigue (2.6%); abortion (1.1%); and others (3.1%). In a subset of 259 samples, the time after onset of clinical symptoms (hypertrophy of lymphatic nodes), as indicated by the patient at the first examination, was also available; the longest follow-up period was 14 years. The samples were tested with all currently used serological tests and were sorted into one of three groups according to the clinical status reported by the clinician and the serological results obtained.

Group A: Sera from patients with acute toxoplasmosis. Samples included in group A were collected from patients with symptoms of clinical toxoplasmosis, e.g., lymphadenopathy, sometimes accompanied by febrile temperatures and fatigue. Acute toxoplasmosis was also confirmed by serological and clinical follow-up of patients; most tests yielded results indicative of acute toxoplasmosis (see 'Serological interpretation criteria'); in general, the time after onset of clinical symptoms did not exceed 5 months.

Group L: Sera from patients with latent (non-acute) toxoplasmosis. This group included samples from patients with a known history of toxoplasmosis, who were asymptomatic at the time of sampling, or whose records were annotated by the attending clinician as 'check-up testing', 'marked improvement', or 'marked decrease in size of lymph nodes'. Characteristically, the results of most tests were indicative of latent toxoplasmosis (see 'Serological interpretation criteria'), and the time after onset of clinical symptoms was >6 months.

Group N: Sera from Toxoplasma-negative individuals. Samples in group N were taken from patients who, at the time of serum collection, were not infected with *T. gondii* and did not have anti-*Toxoplasma* antibodies. Sera that generated equivocal results were not included in the study.

Serological tests

Toxoplasma antigen. The complement fixation test (CFT), IgG ELISA, IgG avidity assay and Test-Line ELISA kits used a commercially available antigen (Sevapharma, Prague, Czech Republic), produced by the Tween–ether preparation method [11] from whole tachyzoites of the *T. gondii* P-Cz strain [12].

Complement fixation test. A commercially available CFT (Sevapharma) [13] was performed according to the manufacturer's recommendations [14].

Triplet IgG avidity assay. An in-house IgG avidity assay [15] was based on an anti-*Toxoplasma* IgG ELISA [16], using porcine anti-human IgG conjugated with horseradish peroxidase (Sevapharma). In brief, the assay was performed using three dilutions of each sera (1:1, 1:5 and 1:25), which were pipetted, in parallel, on to two antigen-coated microplates (A and B). Before the conjugate was pipetted, plate A was treated with 6 M urea for 20 min, while plate B was not. By measuring the absorbance of each serum on plates A and B, the avidity index (AI) at the optimal serum dilution (i.e., when the absorbance on plate B was closest to 1.0) was calculated using the formula

$$\mathrm{AI} = \frac{\text{Absorbance A}}{\text{Absorbance B}}$$

with an AI of ≤0.3 considered to indicate low avidity, and an AI of >0.3 considered to indicate high avidity.

IgA, IgE and IgM ELISA. Specific IgA, IgE and IgM antibodies were measured using commercial ELISA kits (Test-Line Clinical Diagnostics, Brno, Czech Republic), based on the principle of ELISA capture [17], used according to the manufacturer's instructions. In order to evaluate the test results, a positivity index (PI) was calculated using the formula

$$\mathrm{PI} = \frac{\text{Absorbance of tested sample}}{\text{Absorbance of cut - off control}}$$

with PI >1.1 considered positive, PI 0.9–1.1 equivocal, and PI <0.9 negative.

Serological interpretation criteria

Negative CFT results (titres <8) were considered to indicate anti-*Toxoplasma*-negative samples. The following results were regarded as indicative of acute toxoplasmosis: positive IgA, IgE and IgM (PI >1.1); low IgG avidity (AI ≤0.3); and CFT titres ≥256. Other results were considered to be indicative of latent infection.

Statistical methods

To evaluate the discriminative ability of individual methods, the classification of samples as acute (group A) or latent (group L) toxoplasmosis was considered to be the reference standard. The sensitivity, specificity, positive and negative predictive values and the accuracy (proportion of correctly classified samples) of each test [18] were calculated (and expressed as percentages) by testing sera in groups L and A using each method. Youden's index (sum of sensitivity and specificity minus 100) was used as a measure of the overall diagnostic effectiveness of each test [19]. Robust locally weighted regression (lowess) with a bandwidth of 0.3 was used to obtain a smoothed curve, illustrating in scatterplots the trend in antibody levels/IgG avidity in terms of the period elapsed since seroconversion [20]. Statistical analysis was with the Stata software package v.7.0 (Stata Corporation, College Station, TX, USA).

RESULTS

Duration of symptomatic acute toxoplasmosis

The persistence of lymphadenopathy is shown in Table 1. After 2.5–4 months, the lymph nodes were swollen in 90% of cases, whereas lymphadenopathy was recorded in only 30% of examinations 4.5–6 months following the onset of symptoms. Only in four patients (five examinations) did the lymphadenopathy persist for a period of >6 months (at least 8.5 months in a girl aged 2 years; for 21 months in a woman aged 23 years with recurrent lymphadenitis connected with a defect in immunity; for 24 months in a girl aged 9 years with recurrent cervical lymphadenitis; and for 48 months in a boy aged 4 years with suspected reactivation of congenital toxoplasmosis). All patients showed positive values for anti-*Toxoplasma* IgA, IgG, IgE and IgM, high IgG avidity and, with the exception of a decline in the case of one female patient with recurrent lymphadenitis, a CFT titre of ≥256. All patients examined >6 months after the onset of lymphadenopathy were without clinical symptoms of toxoplasmosis.

Characteristics of serological tests

Results with the panel of sera from patients with acute toxoplasmosis (group A), patients with latent toxoplasmosis (group L) and anti-*Toxoplasma*-negative individuals (group N) are summarised in Table 2. All samples from group N gave negative results with all tests, with the exception of two non-specific positive results with the IgM test, one equivocal result with the IgM test and one equivocal result with the IgE test.

Sera in group A were positive for IgM, with the exception of two samples that yielded equivocal results. Negative and equivocal results occurred sporadically with tests for IgA and IgE. Two patients, both of whom were IgE-negative upon initial testing (month 2), gave positive results when re-examined after 2 months. Similarly, two patients with IgA-equivocal results in months 1 and 2 converted to positivity. The CFT titre was ≥256, with the exception of one sample from a patient examined in month 2.5 (titre 128), and two samples from the above-mentioned female patient with recurring lymphadenitis.

Table 3 compares the methods evaluated. The most sensitive methods for detection of acute

Table 1. Persistence of clinical symptoms and positive serological results following *Toxoplasma* infection

Duration of toxoplasmosis (months)	No. of samples tested	No. (%) of samples from patients with clinical symptoms	Distribution (%) results of serological tests[a]												
			IgA			IgE			IgM			IgG avidity		CFT	
			+	+/–	–	+	+/–	–	+	+/–	–	Low	High	≥256	8–128
0–2	41	41 (100)	97.6	2.4	0.0	95.1	2.4	2.4	97.6	2.4	0.0	95.1	4.9	100.0	0.0
2.5–4	30	27 (90.0)	90.0	3.3	6.7	90.0	0.0	10.0	90.0	6.7	3.3	70.0	30.0	93.3	6.7
4.5–6	40	12 (30.0)	70.0	12.5	17.5	55.0	25.0	20.0	85.0	5.0	10.0	12.5	87.5	97.5	2.5
6.5–9	35	1 (2.9)	48.6	14.3	37.1	25.7	22.9	51.4	54.3	28.6	17.1	0.0	100.0	74.3	25.7
9.5–12	31	1 (3.2)	38.7	9.7	51.6	16.1	16.1	67.7	54.8	29.0	16.1	0.0	100.0	54.8	45.2
12.5–18	29	0 (0.0)	17.2	6.9	75.9	6.9	20.7	72.4	41.4	20.7	37.9	0.0	100.0	31.0	69.0
18.5–36	25	2 (8.0)	16.0	4.0	80.0	16.0	4.0	80.0	24.0	16.0	60.0	0.0	100.0	32.0	68.0
36.5+	28	1 (3.6)	7.1	0.0	92.9	3.6	7.1	89.3	21.4	7.1	71.4	0.0	100.0	17.9	82.1
Total	259	85 (32.8)	52.1	7.0	40.9	42.1	12.7	45.2	62.2	13.9	23.9	25.1	74.9	66.8	33.2

+, positive; +/–, equivocal; –, negative. CFT, complement fixation test.
[a]Positive IgA, IgE, IgM, low IgG avidity and CFT titres ≥256 were perceived as indicative of acute toxoplasmosis.

Table 2. Serological results for patients with acute toxoplasmosis, latent toxoplasmosis, and for uninfected individuals

		Group A: acute toxoplasmosis (105 samples)		Group L: latent toxoplasmosis (386 samples)		Group N: negative sera (54 samples)	
Test	**Result**	**No.**	**%**	**No.**	**%**	**No.**	**%**
IgA ELISA	Negative	1	1.0	293	75.9	54	100
	Equivocal	3	2.8	26	6.7	0	0.0
	Positive	101	96.2	67	17.4	0	0.0
IgE ELISA	Negative	4	3.8	307	79.5	53	98.1
	Equivocal	2	1.9	47	12.2	1	1.9
	Positive	99	94.3	32	8.3	0	0.0
IgM ELISA	Negative	0	0.0	190	49.2	51	94.4
	Equivocal	2	1.9	61	15.8	1	1.9
	Positive	103	98.1	135	35.0	2	3.7
IgG avidity	Avidity low	76	72.4	9	2.3	–	–
assay	Avidity high	29	27.6	377	97.7	–	–
CFT	Negative	0	0.0	0	0.0	54	100.0
	Titres 8–128	3	2.9	249	64.5	0	0.0%
	Titres ≥256	102	97.1	137	35.5	0	0.0

CFT, complement fixation test.

toxoplasmosis were IgM ELISA and CFT, which also showed a high predictive value for the negative test. The lowest sensitivity was observed with the IgG avidity test, although 27 of 29 group A samples with high IgG avidity were also IgE-positive. On the other hand, the IgG avidity and IgE ELISA tests were highly specific. In terms of successful diagnosis, the IgE ELISA showed the highest overall test effectiveness (as expressed by Youden's index), and both IgE ELISA and the IgG avidity assay achieved the highest accuracy.

Duration of results indicating acute toxoplasmosis

Low IgG avidity prevailed in samples taken up to 4 months after the onset of symptoms, while IgE ELISA was predominantly positive until month 6, IgA ELISA until month 8, and IgM ELISA and CFT (titres ≥256) until month 12. However, results indicating acute toxoplasmosis dropped below 20% after month 4 when measuring IgG avidity, after month 9 with IgE ELISA, after month 12 with IgA ELISA, and after 3 years with CFT. In contrast, 21.4% of samples collected >3 years after the onset of symptoms remained positive by IgM ELISA (Table 1; Figs 1 and 2). If quantitative results are followed-up, a slight increase in the mean values of the positivity index (for IgE and IgM, but not IgA ELISA), or of CFT titres, was observed in month 1. The IgG avidity index was low in 84.5% and 12.5% of samples taken in months 1–4 and 5–6, respectively. In 92.3% of cases of low avidity, it was confirmed that ≤4 months had elapsed since the onset of clinical symptoms. The decline in CFT titres was very gradual during the first 6 months, but then declined sharply during a 2-year period. Negative values were not recorded for any of the patients, even after monitoring for several years (Fig. 2).

DISCUSSION

The concept of acute toxoplasmosis is perceived at two levels: in a temporal sense as a 'recently acquired' infection; and in a clinical sense as 'acquired toxoplasmosis with characteristic symptoms'. The present study took into account both

Table 3. Comparison of five assays evaluated for the diagnosis of acute toxoplasmosis

	IgA (positive)	IgE (positive)	IgM (positive)	IgG avidity (low)	CFT (titre ≥256)
Sensitivity (%)	96.2	94.3	98.1	72.4	97.1
Specificity (%)	82.6	91.7	65.0	97.7	64.5
Predictive value of a positive test (%)	60.1	75.6	43.3	89.4	42.7
Predictive value of a negative test (%)	98.8	98.3	99.2	92.9	98.8
Accuracy of the test (%)	85.5	92.3	72.1	92.3	71.5
Youden's index (%)	78.8	86.0	63.1	70.1	61.6

CFT, complement fixation test.

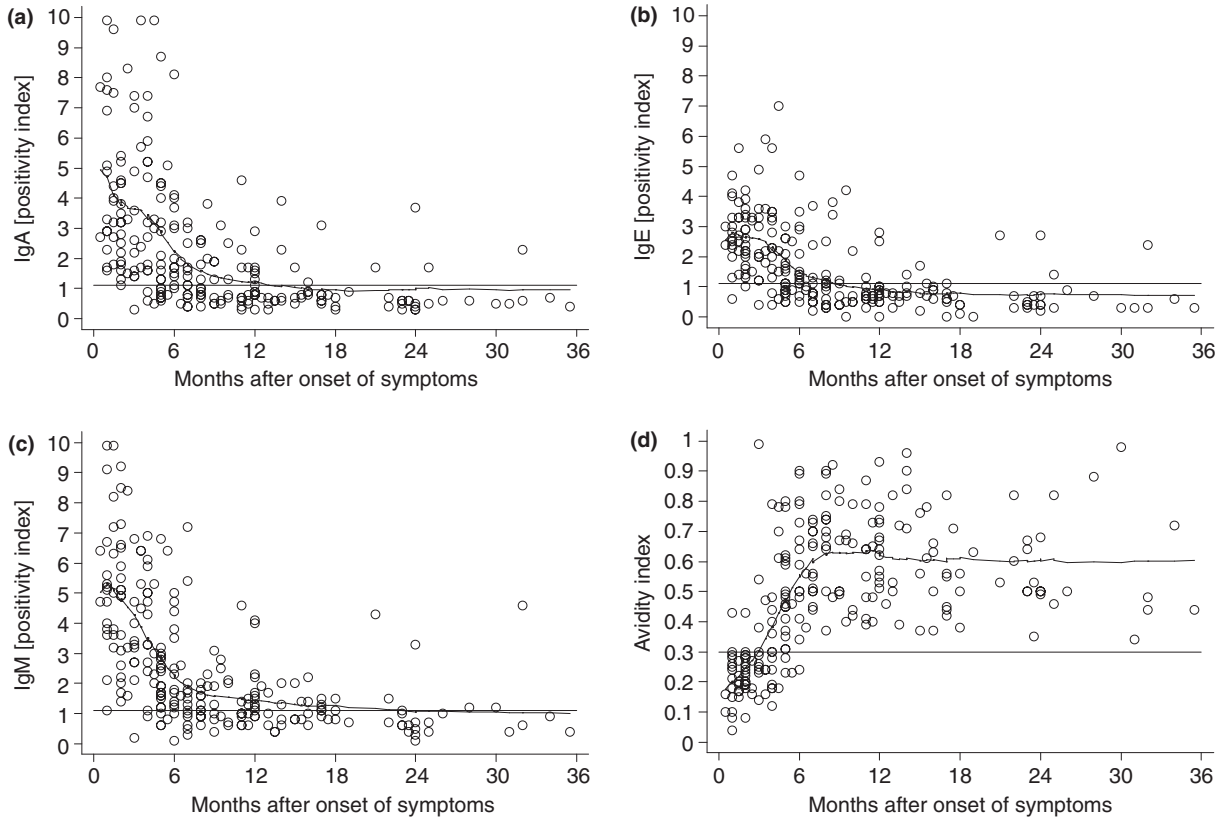

Fig. 1. Quantitative results obtained with four anti-*Toxoplasma* antibody tests—(a) IgA ELISA; (b) IgE ELISA; (c) IgM ELISA; and (d) IgG avidity test—performed with 231 samples for which the period between the onset of clinical symptoms and sampling was up to 36 months. ELISA tests were considered positive if the positivity index was >1.1; avidity was considered low if the avidity index was ≤0.3.

definitions. The actual time at which a patient is infected is generally unknown. Studies of well-documented cases reveal that symptoms occur 2 weeks following infection by tissue cysts [21], or after 5–18 days following infection by *T. gondii* oocysts [22]. The first antibodies may be detected at the same time [1,23] or 1 week later [21]. The present results demonstrate that all the antibodies monitored are present at high levels in the sera of patients at 2–4 weeks after the onset of symptoms. The increase in antibody levels must therefore be very sudden. It appears that IgA levels peak in the second week following onset of symptoms, while maximum IgE and IgM values, as well as CFT titres, are reached after 2 weeks. However, there is enormous individual variability in antibody response kinetics. In the third and fourth months, IgA and IgE values revert to negative values in some patients. The increase in IgG avidity is marked, and most patients have high avidity after month 4. If ongoing lymphadenopathy is accepted as the decisive criterion, acute toxoplasmosis would generally not exceed a period of 4–6 months for the majority of patients. However, isolated cases in which lymphadenopathy and 'acute' antibody responses last for >1 year tend to be associated with a deficient immune response.

Assays for antibody detection tend to give positive results for a period longer than that of the actual symptoms. The level of IgE antibodies best follows the dynamics of clinical toxoplasmosis, in that levels drop below the cut-off for positivity within 2–3 months of the disappearance of symptoms. The decline in IgA levels takes 1–3 months longer. On the other hand, positive IgM and/or high CFT titres can be recorded for several years after symptoms have declined. Nevertheless, a high percentage of positive results after a number of years may be distorted by the fact that some patients monitored (for periods of up to 14 years) may have had a problematic decline in antibody levels following toxoplasmosis.

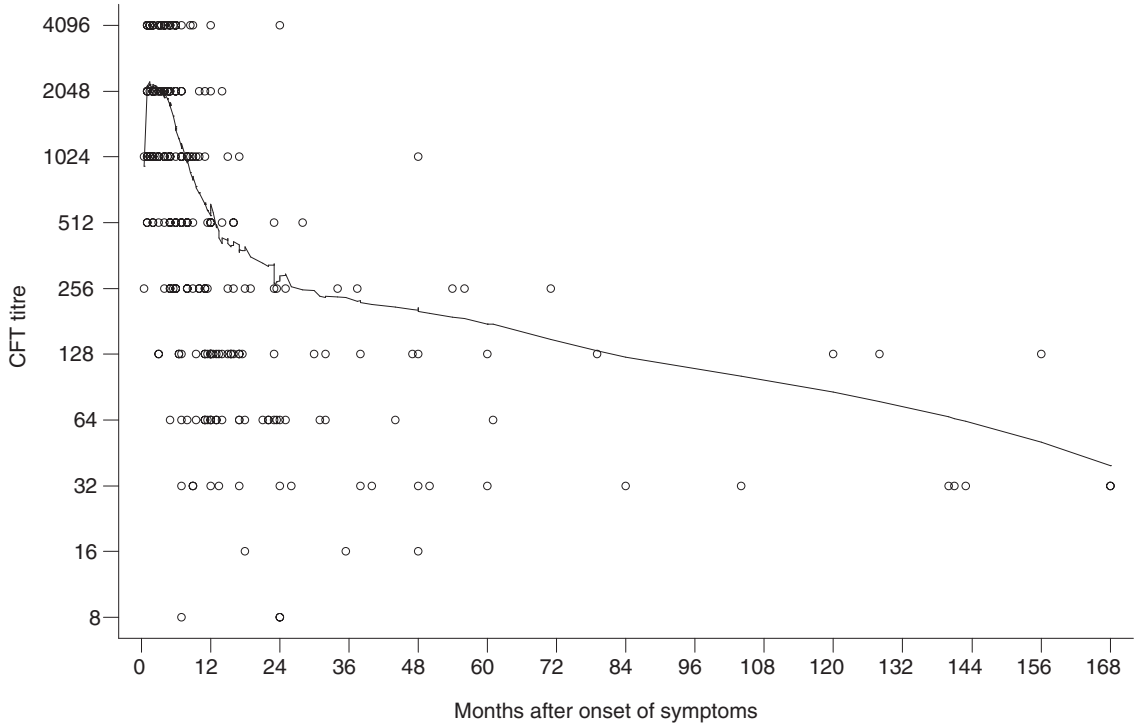

Fig. 2. Quantitative results of complement fixation tests (CFTs) for 259 samples with a known period between the onset of clinical symptoms and sampling.

In most published studies, an evaluation of diagnostic tests is based on a comparison with reference methods (e.g., the survey of 18 studies evaluating IgM ELISA, summarised by Liesenfeld *et al*. [9]). However, the true motivation for testing is not to determine whether patients have anti-*Toxoplasma* IgM, but whether they are suffering from acute toxoplasmosis. Moreover, no generally accepted methods are available as reference standards for the tests evaluated in the present study, especially for IgE ELISA. Therefore, a panel of sera with well-characterised case histories was used to make comparisons. The negative sera (group N) were not included in the calculations, as IgG avidity could not be determined. Many studies avoid inclusion of debatable sera taken during the period of transformation from acute to latent infection. Thus, Roberts *et al*. [24] did not include 'convalescence' sera, while Suzuki *et al*. [25] compared results with sera from the acute (1–3 months) and latent (13–38 months) stages. However, the greatest problems in diagnostic practice are posed by sera collected between months 4 and 12, and if the assays are compared solely using either problem-free samples from an unequivocally initial stage of infection, evident late stage latent toxoplasmosis, or wholly negative samples, all tests would have near perfect characteristics.

Test-Line kits are used currently by > 40% of Czech laboratories diagnosing toxoplasmosis. In regular external laboratory quality control surveys, they yield qualitative results comparable to products of other (mostly international) companies [26]. In the present study, IgM was the most sensitive assay, at the expense of low specificity [9] and a low positive predictive value caused by long-term endurance of IgM. Generally, IgM is still interpreted as being associated directly with acute toxoplasmosis, and even low-level IgM may provoke inappropriate interventions, especially in the case of pregnant women. The CFT results (assuming titres of 1:256 and over to be indicative of acute toxoplasmosis) were surprisingly similar to the IgM ELISA results. CFT, relying on a different principle than that of immunoassays, is unaffected by factors that provoke non-specific positivity in immunoassays. Furthermore, CFT is highly suitable for quantification of antibodies and monitoring antibody dynamics, and has been an important component of the diagnostic scheme [4]. The most specific method was the IgG avidity

assay, which, uniquely, provides relevant data concerning the duration of infection. For low avidity, the brief period from onset of infection is more important than ongoing clinical symptoms; avidity was low in only 72.4% of cases with lymphadenopathy. Unfortunately, there are few data available concerning the period post-infection, during which the disease can be transmitted from mother to foetus. IgA ELISA is less sensitive than IgM ELISA [23,27], but has a higher specificity and positive predictive value, and is a suitable joint method for detection of IgM [28]. IgE ELISA shows lower sensitivity than IgM assays [29–31], but the present study shows that, as with IgA, decreased sensitivity is not necessarily caused by failure to form these antibodies, and can also be caused by delayed or early cessation of antibody production, resulting in IgE antibodies being undetected during sample collection. Of all the antibody classes, IgE has the shortest duration, with positive results for up to 6 months following onset of symptoms, but this period can vary from 3 – 5 months to 11 months [31–34]. Foudrinier *et al*. [30] described two different kinetic patterns in asymptomatic seroconverters (short duration) and in seroconverters with overt toxoplasmosis, in which high IgE levels persist for a number of months. According to Gross *et al*. [33], IgE antibodies seem to be markers of disease activity.

The main advantages of the IgE ELISA are its high specificity and positive predictive value—the highest of all ELISA tests—with generally adequate sensitivity. Of the tests evaluated in the present study, the IgE ELISA yielded the highest proportion of qualitatively accurate results (i.e., the sum of false-negative and false-positive results was the lowest), as expressed by the highest value in Youden's coefficient. Furthermore, IgE positivity correlated closest with the persistence of clinical symptoms of toxoplasmosis. Thus, determination of IgE levels appears to be an excellent supplementary method with which to compensate for the low specificity and positive predictive value of other highly sensitive tests for the diagnosis of acute toxoplasmosis.

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