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LABORATORY DIAGNOSIS OF ACUTE TOXOPLASMOSIS BY IMMUNOSORBENT AGGLUTINATION ASSAY (IgM ISA)

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Abstract. A group of 401 patients suspected for toxoplasmosis was examined by the indirect immunofluorescent antibody test (IFAT). All patients positive in IFAT (176) were examined by the immunosorbent agglutination assay (IgM ISA). In the IgM ISA 154 of them were negative, 10 temporarily and 12 high-positive. Some of high-positive patients were examined repeatedly; decrease of high levels of specific IgM antibodies occurred 2—9 months after the first examination. For the IgM ISA antigen prepared from peritoneal exudate of experimentally infected mice was used. The antigen was stable at 4 °C or in liquid nitrogen at least 1 year. The IgM ISA combined with IFAT and IgM IFAT was proved satisfactory for the diagnosis of acquired acute toxoplasmosis and can be recommended for laboratories with lower capacity.

The laboratory diagnosis of acute toxoplasmosis is usually based on the determination of specific IgM antibodies or the determination of circulating antigen in the sera of patients. There are some difficulties with the reliable determination of specific IgM antibodies, therefore, recently techniques based on the capture principle, excluding or considerably limiting alteration of results with other immunoglobulins, have been preferred. For this purpose double sandwich ELISA (Naot and Remington 1980), IgM ISA (Desmonts et al. 1981). SPIHA (Hermentin et al. 1983) and determination of antibodies after their separation on the column (Pyndiah et al. 1979) have been used. In this paper results of the determination of toxoplasmic IgM antibodies by the IgM ISA using antigen prepared in our laboratory are reported.

MATERIALS AND METHODS

Basically, the IgM ISA was carried out according to Desmonts et al. (1981). In brief: wells of U-shaped microtitre plates (Koh-i-noor, Dalečín) are coated with 0.1 ml of anti-human IgM antibody (Q-SwAHu/IgM, Sevac Prague) 1% solution in carbonate buffer pH 9.1 overnight at 4 °C. The wells are washed three times in phosphate-buffered saline containing 0.05% Tween 20 (PBS-T), postcoated with 1% bovine serum albumin in PBS-T for 1 hour at 37 °C, again three times washed in PBS-T, the sera of patients diluted in PBS are added, incubated for 1 hour at 37 °C, washed twice in PBS-T and twice in PBS. After removing of PBS, antigen in the form of purified toxoplasma suspension is added, plate is incubated in moist chamber overnight at 37 °C. The agglutination is estimated using a reader mirror.

In point reading the sera of patients are diluted 1 : 100 (in neonates 1 : 20) and are dropped to 3 consecutive wells (0.1 ml each). After washing, 0.1 ml, 0.15 ml, 0.20 ml of antigen are added to the first, second and third wells, respectively. The agglutination is estimated using points: the suspension of toxoplasmas covers the whole bottom of the well — 4 points, the suspension covers the whole bottom of the well with touch of sediment in the middle — 3 points, sediment in the middle of the well is clearly visible — 2 points, button-shaped sediment with thin margin on the bottom — 1 point, only button-shaped sediment — 0 points are recorded. The points of all three wells are summed. For convenience the point readings are called titres in the following text. It means that a high-positive serum can reach the titre 12 as a maximum (usually 10—12), in temporary increased values of specific IgM 6—9, in low values the titre 5 and less can be recorded.

The antigen was prepared from peritoneal exudate of mice experimentally infected with *Toxoplasma gondii*, P strain (the strain was isolated from congenitally infected infant by Jíra and Kozojed

in 1963). The toxoplasma suspensions were purified according to the method described previously (Mirovský and Valkoun 1981). The purified suspension was fixed with 2% formalin in PBS for 24 hours at 4 °C. Formalin was removed by centrifugation (at 950 g, for 10 min), the suspension was washed in PBS and added PBS with 0.2% formalin. The number of zoites was 2×10^8/ml. The antigen was sealed in ampoules, 0.3 ml in each, and stored at 4 °C or after quick freeze in liquid nitrogen. For the test itself the antigen was diluted 1 : 20 with borate buffer pH 9.5 with 0.4% bovine albumin. Thus, the number of toxoplasmas per well is 10^6, 1.5×10^6, 2×10^6 in the first, second and third wells, respectively.

The sera of patients from the region of the Central Military Hospital, Prague were examined without selection in sequence as they were sent by clinical workers usually with a diagnosis of lymphadenitis. The patients were adults, most frequently 20—30 years old. Exceptionally 5 small children were also examined. The sera were stored at —20 °C, once a week examined by the indirect immunofluorescence antibody test (IFAT) and according to possibilities once within 2—8 weeks by IgM ISA. All patients were examined for total toxoplasma IFAT antibodies (Kramář et al. 1964) and most of them also for IgM antibodies (IgM IFAT). For immunofluorescent determination of antibodies pig conjugates SwAHu/FITC and SwAHu/IgM FITC (Sevac, Prague) were used.

RESULTS

A total of 401 patients were examined for toxoplasmosis by the IFAT, 176 of them were positive (titre ≧ 16). All positive patients were examined by the IgM ISA, 154 of them were negative (≦ 5), 10 temporarily (6—9) and 12 high-positive (10—12). The correlation between IgM ISA and IFAT titres is apparent in Table 1. With increasing IFAT titre the probability of positive IgM ISA is increasing. However, exceptions are possible, in low IFAT titres IgM ISA can be high-positive and in contrast, in high IFAT titres IgM ISA can be negative. Furthermore, the correlation

Table 1. Correlation between IFAT and IgM ISA in 176 patients positive in the first IFAT examination

		IgM ISA titres			Total of patients
		0—5	6—9	10—12	
IFAT titres	16—64	113	1	1	115
	128—512	37	7	6	50
	≧ 1,000	4	2	5	11
Total of patients		154	10	12	176

Table 2. Correlation between IgM IFAT and IgM ISA in 86 patients positive in the first IFAT examination

		IgM ISA titres			Total of patients
		0—5	6—9	10—12	
IgM IFAT titres	neg.	60	1	0	61
	16	13	1	0	14
	32	5	2	0	7
	64	0	0	3	3
	128	0	0	1	1
Total of patients		78	4	4	86

of IgM IFAT and IgM ISA is given in 86 IFAT positive patients, simultaneously examined by both assays (Table 2). The correlation is very good, the sera of patients with IgM IFAT titres ≧ 64 were always high-positive in the IgM ISA. Fifty selected patients negative in IFAT were also negative in IgM ISA.

Serological values of patients examined repeatedly are shown in Table 3. According to this table it is evident that high levels of IgM antibodies usually decrease after 2—9 months after the first examination. The acute toxoplasmosis was confirmed by laboratory tests in 3% of the patients examined.

Table 3. Comparison of IgM ISA, IFAT and IgM IFAT antibody titres in 8 high-positive toxoplasma patients. Patient No. 3a is an eight-months old child, No. 3b is its mother. Patient No. 5 is positive for rheumatoid factor.

Patient No.	Months after onset	IFAT	IgM IFAT	IgM ISA
1	0	512	64	12
	2	128	16	4
	4	32	16	2
	12	1,000	16	0
2	0	4,000	128	12
	17	256	neg.	0
3a	0	2,000	64	12
3b	0	256	16	0
4	0	256	64	12
	4	4,000	64	12
	9	1,000	32	0
5	0	128	64	12
	7	256	64	5
6	0	256	64	12
	2	1,000	128	12
	5	256	32	6
7	0	1,000	64	12
	3	2,000	128	11
	8	1 000	16	3
8	0	256	64	8
	0.5	256	64	12
	4	2,000	64	6
	9	1,000	neg.	5

Stability of the undiluted antigen was at least 1 year both at 4 °C and in liquid nitrogen. The antigen diluted with borate buffer was stable at least one week at 4 °C. It was also possible to freeze it quickly in vapours of liquid nitrogen (—150 °C) and store at least 3 months.

DISCUSSION

Initially, due to technical reasons, only patients with increased IFAT titres (≧ 64) or patients positive in the IgM ISA were examined by IgM IFAT. This method showed

to be incorrect. We found out that the conventional assays determining total antibodies cannot be decisive for the diagnosis of acute toxoplasmosis. Generally accepted opinion that the acute toxoplasmosis is characterized by high titres of complement fixation test (CFT) and IFAT has only limited validity. In one third of patients with acute toxoplasmosis the first examination revealed medium (128—512) or low (16—64) IFAT titres. Nevertheless, both high IgM titres and clinical picture gave evidence of acute toxoplasmosis. Our results confirm those reported by van Knapen et al. (1986). In contrast, even high IFAT titres (≧ 1,000) cannot always give evidence of acute toxoplasmosis (Table 1).

Due to practical reasons we have withdrawn from CFT (unpublished data) and henceforth every patient is examined by IFAT with total and anti-IgM conjugate. Patients positive in IFAT with total conjugate are examined by IgM ISA regardless of the result of the IgM IFAT. Significant discrepancies between IgM IFAT and IgM ISA have not been recorded in this group. We consider the IgM ISA more accurate because it determines ratio between specific and total IgM concentrations. The IgM IFAT can be influenced by the presence of rheumatoid factor (RF), antinuclear antibodies (ANA) or high IgG levels (Naot and Remington 1980, Desmonts et al. 1981, Hermentin et al. 1983). Due to technical reasons we could devote to RF and ANA determination only exceptionally, mostly with negative results (unpublished data). Decrease of high levels of specific IgM antibodies after 2—9 months corresponds with experience of other authors (Brooks et al. 1987).

Patients with acute toxoplasmosis proved by laboratory tests and with corresponding clinical picture were cured with combination of Daraprim, Sulphamethoxidin and Rovamycin in the Department of infection diseases (dr. L. Kostrhun and dr. V. Nádvorník).

In comparison with French antigen (Desmonts 1982) the antigen prepared in our laboratory has a little bit lower activity that may be explained by different method of preparation. Thus, as temporary IgM values we consider the titre 6—9 (French authors 6—8). Nearly all patients with proved acute toxoplasmosis had the titre 12 in the first examination by the IgM ISA. In fact, the stability of the antigen stored in liquid nitrogen is supposed to be more than 1 year.

Good quality of the antibody Q-SwAHu/IgM Sevac has been proved, no differences have been observed in comparison with antibody of the same firm labeled without Q (Valkoun 1986). For the coating lower concentration (to 50 times) can be used, however, due to practical reasons, we have used 1% solution of the antibody.

In adults, toxoplasmosis usually does not cause more serious difficulties if an immunosuppression has not occurred. We consider the proper diagnosis of acute toxoplasmosis to be important from the viewpoint of the differential diagnosis. The fact that on the basis of serological examinations originally supposed malignant disease was excluded in two patients from our group confirms it. We conclude with the statement that we consider the IgM ISA in the above described combination with IFAT and IgM IFAT to be very good for the diagnosis of acute acquired toxoplasmosis in laboratories with lower capacity (to 20 sera a week).

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ЛАБОРАТОРНАЯ ДИАГНОСТИКА ОСТРОГО ТОКСОПЛАЗМОЗА С ПОМОЩЬЮ ИММУНОСОБЩИИ АГГЛЮТИНАЦИИ (IgM ISA)

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Резюме. Обследовали группу 401 пациента с предполагаемым диагнозом токсоплазмоза с помощью непрямой иммунофлюоресценции (IFAT). Все пациенты положительно реагирующие в IFAT (176) обследовали с помощью метода иммуносорбции — агглютинации. (IgM ISA). В этом тесте 154 из них дало отрицательную реакцию, 10 частично и 12 высоко положительную реакцию. Некоторых из высоко реагирующих пациентов обследовали повторно, через 2—9 месяцев после первого обследования уровень специфических антител IgM снижалась. Для IgM ISA использовали антиген из перитонеального экссудата экспериментально зараженных мышей. Антиген сохранял свою активность в течение одного года при хранении при 4° С или в жидком азоте. IgM ISA в сочетании с IFAT и IgM IFAT дают достоверные результаты при диагнозе острого токсоплазмоза и могут быть рекомендованы для лабораторной диагностики меньших масштабов.

REFERENCES

BROOKS R. G., McCABE R. E., REMINGTON J. S., 1987: Role of serology in the diagnosis of toxoplasmic lymphadenopathy. Rev. infect. Dis. 9: 775—782.

DESMONTS G., 1982: Les recherches d'anticorps IgM spécifiques par immuno-adsorption (immunosorbent assay ou ISA) IgM immunosorbent agglutination assay (ISAgA). Lyon Méd. 248: 37—41.

—, NAOT Y., REMINGTON J. S., 1981: Immunoglobulin M-immunosorbent agglutination assay for diagnosis of infectious diseases: diagnosis of acute congenital and acquired *Toxoplasma* infections. J. clin. Microbiol. 14: 486—491.

HERMENTIN K., PICHER O., ASPÖCK H., AUER H., HASSL A., 1983: A solid phase indirect haemadsorption assay (SPIHA) for detection of immunoglobulin M antibodies to *Toxoplasma gondii*: application to diagnosis of acute acquired toxoplasmosis. Zbl. Bakt., I. Abt. Orig. 255: 380—391.

KRAMÁŘ J., ČERNÁ Z., CHALUPSKÝ J., 1964: Immunofluoreszenzreaktionen in der serologischen Diagnostik der Toxoplasmose. Zbl. Bakt., I. Abt. Orig. 193: 523—534.

MIROVSKÝ P., VALKOUN A., 1981: Preparation of purified *Toxoplasma gondii* suspensions. Folia parasitol. 28: 23—27.

NAOT Y., REMINGTON J. S., 1980: An enzyme-linked immunosorbent assay for detection of IgM antibodies to *Toxoplasma gondii*: use for diagnosis of acute acquired toxoplasmosis. J. infect. Dis. 142: 757—766.

PYNDIAH N., KRECH U., PRICE P., WILHELM J., 1979: Simplified chromatographic separation of immunoglobulin M from G and its application to *Toxoplasma* indirect immunofluorescence. J. clin. Microbiol. 9: 170—174.

VALKOUN A., 1986: Acute toxoplasmosis determination by immunoadsorption and agglutination method (IgM-ISA). Čas. Lék. čes. 125: 712—714. (In Czech.)

van KNAPEN F., PANGGABEAN S. O., van LEUSDEN J., 1986: Evaluation of laboratory diagnosis of toxoplasosis by means of an ELISA-triple test. Detection of class specific IgG, IgM and circulating antigen. Antonie Leeuwenhoek 52: 5—13.

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