

LETTER TO THE EDITOR

DIAGNOSIS OF ACUTE TOXOPLASMOSIS IN AN IMMUNOCOMPETENT PATIENT BY ISOLATION OF THE AGENT AND IMMUNOHISTOCHEMISTRY FROM LYMPH NODE BIOPSY.

Sir,

Toxoplasmosis is a zoonotic disease highly prevalent in our country⁷, usually diagnosed in its acute form only by serology, through the detection of specific IgM antibodies. The disease, caused by *Toxoplasma gondii*, presents usually with few symptoms, but in some patients a more aggressive clinical picture can be seen, with lymph node enlargement, pneumonitis and hepatosplenomegaly². In these patients, the differential diagnosis includes several other infectious diseases, as cytomegalovirus, Epstein-Barr virus and HIV acute infections, all of which may present with similar clinical findings⁸. The histopathologic changes in toxoplasmic lymphadenitis are characterized by reactive follicular hyperplasia, with aberrant shapes, presence of clusters of epithelioid histiocytes mainly in the borders of germinal centers and distention of paracortical sinus by monocytoïd cells or immunoblasts. Trophozoites or tissue cysts are rarely seen¹⁰. Serologic diagnosis in patients with acute disease frequently requires testing of multiple and serial samples, in order to detect the presence and elevation of specific IgM or IgG antibodies, which usually appear and rise in titers after the second week of the disease³. The other available techniques for the diagnosis of acute toxoplasmosis are time consuming and/or restricted to specialized research centers, including *T.gondii* antigen detection or isolation and nucleic acid detection by DNA recombinant technology^{5,6,9}. We describe an approach to the diagnosis of toxoplasmosis in an immunocompetent patient, based on lymph node biopsy, which proved to be quick and precise, before or at the time of serological detection. A 45 year old male patient presented to the Instituto de Infectologia Emilio Ribas, with a 30 day history of fever, low back pain and an alleged weight loss of 20 kg. He reported ingestion of meat from hunted armadillos, during a two week trip to a rural area in Southern Brazil and Paraguay, where he also had contact with domestic animals

in a poor sanitation house. Physical examination on admission disclosed painful generalized lymphadenopathy with modest hepatosplenomegaly. Abdominal ultrasound disclosed hepatosplenomegaly, without signs of lymph node enlargement. Computadorized tomography of the brain and X-ray film of the chest were normal. Hematological and biochemical tests were unrevealing and a lymph node biopsy was performed on the third day after admission. The node was cut in several pieces which were sent to histopathological analysis and cultured for viruses, fungi and injected intraperitoneally(ip) in checked albino Swiss mice, to identify specific pathogens like *T.gondii*. The routine histopathological analysis revealed hypertrophy of lymphoid follicles, tentatively diagnosed as lymphoid reactional hyperplasia. The classical picture of aberrant follicle forms and proliferation of histioid and monocytoïd cells was not found⁴. Four days after inoculation, the peritoneal exudate of infected mice yielded crescentic forms, consistent with *T.gondii* tachyzoites, as seen on phase contrast microscopy. Giemsa stained smear of the exudate revealed macrophages harboring nucleated crescentic forms in their cytoplasm, with size compatible with *T.gondii* tachyzoites. A sample of the exudate was also seeded onto a monolayer of HeLa cells, with the development of a cytolytic effect after 4 days. A myriad of crescentic forms could be seen free in the medium. This medium was injected ip into mice and on other HeLa cells' monolayer, again reproducing the disease. These cell culture tachyzoites showed a positive membrane immunofluorescence when tested with fluorescein conjugated monoclonal antibody (kindly furnished by Dr.M.E.Camargo). This mAb is directed against a major surface protein of *T.gondii*, p30, therefore identifying the coccidian forms seen on microscopy as *T.gondii*. A retrospective immunohistochemical analysis of the paraffin embedded lymph node material, using new slices attached to glue coated slides, revealed one macrophage with a pseudocyst, with posi-

tive stained tachyzoites. The immunohistochemical technique employed involved a primary IgG antibody against RH strain of *T.gondii* produced in rabbits and purified in Protein A Sepharose, a secondary antibody against rabbit IgG induced in goats, a complex of peroxidase anti-peroxidase(PAP) produced in rabbits and its binding revealed by H₂O₂ and diaminobenzidine as peroxidase reagent⁴. All these results were available within four days after the biopsy. The specific antibody response, measured 15 days after admission, included elevated titers of both IgG(1/2048) and IgM(1/16). Treatment with pyrimetamine and sulfadiazine was instituted with good clinical response. The patient became afebrile, started to gain weight and was discharged in good health 20 days after admission.

The diagnosis of toxoplasmosis by isolation of *T.gondii* in lymph nodes has been described in an anecdotal report¹¹. In another recent report, a 10% yield was found with this technique, in a relatively large, 5 of 50, patients series¹. In both reports, no parasites could be seen in tissue sections, probably due to their low numbers and to the lack of immunohistochemistry test. The absence of some classical histopathological signs in the lymph node described in our report can be explained by the fact that the biopsy was done early in the course of the disease, before the establishment of a more characteristic histologic pattern. When lymph node biopsy is performed earlier in the disease course, this non-diagnostic histologic pattern is usually seen, which can only be clarified by the use of additional techniques, such as the isolation and immunohistochemistry techniques, as described in this report, avoiding confusion with other clinically similar diseases⁸.

Immunohistochemical analysis and *T.gondii* isolation from biopsied lymph nodes may allow for a quick and definitive diagnosis of acute toxoplasmosis.

A.C.S.GUIMARÃES Instituto Adolfo Lutz (Parasitoses sistêmicas). São Paulo, SP, Brasil.
A.A.B.PORTELA Inst.Infectologia E.Ribas. São Paulo, SP, Brasil.
M.KAWARABAYASHI Instituto Adolfo Lutz (Parasitoses sistêmicas). São Paulo, SP, Brasil.
M.F.ARAÚJO Inst.Infectologia E. Ribas. São Paulo, SP, Brasil.
C.PAGLIARI Lab. Patol. Mol. Infeciosas FMUSP. São Paulo, SP, Brasil

J.E.TOLEZANO Instituto Adolfo Lutz (Parasitoses sistêmicas). São Paulo, SP, Brasil.
H.F.ANDRADJR. Lab.Protozoologia Inst. Med. Tropical S. Paulo(LIM-49). São Paulo, SP, Brasil.

Correspondence to: Prof.Dr. Heitor Franco de Andrade Jr.,Av. Dr.Enéas de Carvalho Aguiar, 470. 05403-000 São Paulo, SP., Brazil.

ACKNOWLEDGEMENT

This work was partially supported by LIM-49/HCFMUSP. We are indebted to M.Wolff by comments and criticism to the final work and to P.J.Spencer for computer work.

REFERENCES

1. ARROYO, R. & TROPER, L.- Aislamento de *Toxoplasma gondii* de ganglios linfáticos humanos. **Rev. costarric. Cienc. med.**, 12: 19-25, 1991.
2. BEAMAN, M.H.; McCABE, R.E.; WONG, S. & REMINGTON, J.S. - Toxoplasmosis. In: MANDELL, G.L.; BENNET, J.E. & DOLIN, R., ed. **Principles and practice of infectious diseases**. 4.ed. New York, Churchill-Livingstone, 1995. p. 2455-2475.
3. CAMARGO, M.E. & LESER, P.G. - Diagnostic information from serological tests in human toxoplasmosis. II - Evolutive study of antibodies and serological patterns in acquired toxoplasmosis, as detected by hemagglutination, complement fixation, IgG- and IgM-immunofluorescence tests. **Rev. Inst. Med. trop. S. Paulo**, 18: 227-238, 1976.
4. CONLEY, F.K.; JENKINS, K.A. & REMINGTON, J.S. - *Toxoplasma gondii* infection of central nervous system. Use of the peroxidase-antiperoxidase method to demonstrate toxoplasma in formalin fixed paraffin embedded tissue sections. **Hum. Path.**, 12: 690-698, 1981.
5. FACHADO, A.; FONTE, L.; ALBERTI, E. et al. - Usefulness of the detection of *Toxoplasma gondii* antigens in AIDS patients. **Rev. Inst. Med. trop. S. Paulo**, 36: 525-529, 1994.
6. GUIMARÃES, A.C.S.; KAWARABAYASHI, M.; BORGES, M.M.; ANDRADE JR., H.F. & TOLEZANO, J.E. - Utilização do cultivo celular para proliferação de *Toxoplasma gondii*. **Mem. Inst. Oswaldo Cruz**, 87(supl.2):254, 1992.
7. GUIMARÃES, A.C.S.; KAWARABAYASHI, M.; BORGES, M.M.; TOLEZANO, J.E. & ANDRADE JR., H.F. -Regional variation in toxoplasmosis seronegativity in the São Paulo Metropolitan region. **Rev. Inst. Med. trop. S. Paulo**, 35:479-483, 1993.
8. McCABE, R.E.; BROOKS, R.G.; DORFMAN, R.F. & REMINGTON, J.S. - Clinical spectrum in 107 cases of *Toxoplasma* lymphadenopathy. **Rev. infect. Dis.**, 9:754-774,1987.
9. ROTH, A.; ROTH, B.; HAFFKEN, G. et al. - Application of polymerase chain reaction in the diagnosis of pulmonary

- toxoplasmosis in immunocompromized patients. **Europ. J. clin. Microbiol. infect. Dis.**, **11**:1177-1181, 1993.
10. SAXÉN, E.; SAXÉN, L. & GRÖOMROOS, P. - Glandular toxoplasmosis: a report on 23 histologically diagnosed cases. **Acta path. microbiol. scand.**, **44**:319-328, 1958.
11. SHEAGREN, J.N.; LUNDE, M.N. & SIMON, H.B. - Chronic lymphadenopathic toxoplasmosis. A case with marked hyperglobulinemia and impaired delayed hypersensitivity responses during active infection. **Amer. J. Med.**, **60**:300-305, 1976.

Recebido para publicação em 30/11/1995.

Aceito para publicação em 07/02/1996.