

IMMUNOHISTOCHEMICAL METHOD IN TUBERCULAR LYMPHADENITIS DIAGNOSTICS

Makarova U.E, Loskutova K.S., Kirillina M. P.

The Yakut centre of science of complex medical problems SB RAMS, Yakutsk

The authors studied possibilities of application of immunohistochemical method in diagnostics of peripheral lymph nodes tuberculosis, and it was found out that the method allowed to increase frequency of mycobacterium tuberculosis revealing in comparison with the other methods in 2 times.

Keywords: peripheral lymph nodes tuberculosis, mycobacterium tuberculosis, diagnostics, pathomorphological pattern, immunohistochemical method.

Topicality. The most widespread extrathoracal localization of a tubercular infection contamination is the lesion of peripheral lymph nodes (LNTB), which in the structure of extrapulmonary tuberculosis occupies 1-3 place and with it to 80 % of patients are detected in the late stages of disease, therefore questions of its early diagnostics remain the most actual till now [1,3].

Difficulties of LNTB diagnostics in early stages of development are connected with the disease belonging to the big group of granulomatous illnesses (more than 70 nosologies). It is known that granulomas, unlike a vulgar inflammation are displayed by initially productive reaction and consequently at this stage verification of hyperplasia genesis of lymphoid tissue becomes more complex or even not resolved diagnostic problem. In similar cases the basic diagnostic criterion of tubercular disease is revealing of mycobacterium tuberculosis (MTB) in an investigated material. At LNTB diagnostics these are the extracted lymph node or its punctate, purulent secrete of fistulas.

Nowadays MTB in the investigated material is revealed by a microscopic method by means of classical Ziehl–Neelsen stain, cultivation of mycobacterium culture, luminescent microscopy with auramine stain and by a polymerase chain reaction method (PCR). Modern immunohistochemical mycobacterium detection method has been applied at cavernous and disseminated forms of tuberculosis of lungs, skin and becomes the most effective, raises quality of histological diagnostics of tuberculosis [6]. Thus in the accessible domestic and foreign literature practically there are no works mentioning application of the given method in tubercular lymphadenitis diagnostics.

It is necessary to note that last years reduction of cases of MTB revealing from material of lymph nodes takes place. If in 1953 in caseous lymph nodes bioptates by a method of direct bacterioscopy and culture MTB have been revealed in 100 % cases [2], then in 1977 they are revealed only in 35,8 % [4]. Our researches have confirmed a similar tendency - MTB have been revealed to 26 % of cases [5].

In this connection, the research **objective** was to assess results of immunohistochemical method application in peripheral lymph nodes TB diagnostics.

Patients and methods. 30 patients with suspicion on LNTB which have undergone to diagnostic surgical intervention have been chosen among patients with lymphadenopathy, surveyed in various treatment-and-prophylactic institutions. The pathomorphological description of lymph nodes bioptates and MTB definition by application of immunohistochemical method was made in laboratory of morphological researches of the Republican hospital №2 – National Medical centre Ministry of Healthcare Republic Sakha (Yakutia).

Immunohistochemical MTB revealing is done with application of system of visualization PolyVue Mouse/Rabbit HRP Kit (Diagnostic BioSystems, USA) according to firm-manufacturer instructions. Antigen unmasking was made within 2 minutes in cytrate buffer pH 6.0. Primary MTB antibodies NCL-MT (clone 1.1/3/1) («Novocastra», Great Britain) were incubated at 37 °C within one hour. Reaction result was visualized by diaminobenzidine (DAB kit «Pharmingen», USA).

Results and discussion. In 16 (53.33 %) from 30 cases pathomorphological research of lymph nodes biotates has shown presence of tubercular process (Fig.1), in 14 (46.67 %) - hyperplasia, proliferation of a lymphoid tissue without clear signs of granuloma formation. According from that, immunohistochemical research was made on two basic groups of patients. 16 patients with pathomorphological pattern characteristic for caseous LN tuberculosis have been included in the first group. The second group - 14 patients at routine histological research with the nonspecific lesion of a lymph node (lymphadenitis, hyperplasia), without granuloma formation.

At the analysis of the research data it was found out that in the first group of patients MTB have been found by immunohistochemical method from 16 in 10 (62.5 %) cases, in 3 (18.75 %) - the result was doubtful, and in the others 3 (18,75 %) - negative. From the same 16 in 5 (31.25 %) cases MBT have been revealed by a microscopic method on Ziehl–Neelsen (Fig. 2), luminescent microscopy and culture. A little unexpected there was result of immunohistochemical research of biotates from patients of the second group. From 14 cases in 11 (78.57 %) by immunohistochemical method tuberculosis causative agents have been revealed (Fig. 3) and only in 3 - the result was negative. Thus by other methods of research (luminescent) MTB have been found in the lymph nodes biotates in one patient that compounds 7.14 %.

Thus, application of immunohistochemical method in caseous stage of a tubercular lymphadenitis has allowed a MTB revealing in 2 times more often, than all other methods of research, and at presence of a lymphoid tissue hyperplasia regarded as «the nonspecific lesion» category - more than on 71.43 %.

Conclusion. The authors consider that their research demonstrates possibilities of a modern method, its high efficacy and the importance in diagnostics, differential diagnostics of tuberculosis, and also is the most perspective direction of implementing of molecular-genetic methods in practical work. The received results of immunohistochemical research testify to complexities of diagnostics of a specific inflammation of lymph nodes in early stages that demands continuation of profound research of the problem.

References

1. Bellendir E.I. Pathogenesis of tuberculosis extrapulmonary localizations/ E.I. Bellendir //Coll. of SPbNIIT.-SPb.- 1997.- P. 23-25.
2. Belogurova V.P. Virulence and typicality of tubercular mycobacterium at some forms of tuberculosis in YASSR / V.P. Belogurova //Questions of regional epidemiology of tuberculosis. - Yakutsk, 1953. - P.115-134.
3. Extrapulmonary tuberculosis: the guide for doctors / under A.V.Vasilev ed. - SPb., 2000.
4. Djachkovskaja S.S. Clinico-cytological and bacteriological diagnostics of peripheral lymph nodes tuberculosis under modern epidemiological conditions of extrapulmonary tuberculosis: abstract of a thesis....PhD / S.S. Djachkovskaja. - M., 1977.
5. Makarova U.E. Pathomorphism of peripheral lymph nodes tuberculosis in modern socially-epidemiological conditions of Yakutia /U.E. Makarova //Coll. of YNIIT. - Yakutsk, 2002. - P. 85-91.
6. Ellinidi V. N., Ariel B.M., Samusenko I.A., Tugolukova L.V. Immunohistochemical method in tuberculosis diagnostics / V. N. Ellinidi, B.M. Ariel, I.A. Samusenko, L.V. Tugolukova //Arkh. Patologii. - 2007. -№5.-P.36-38.

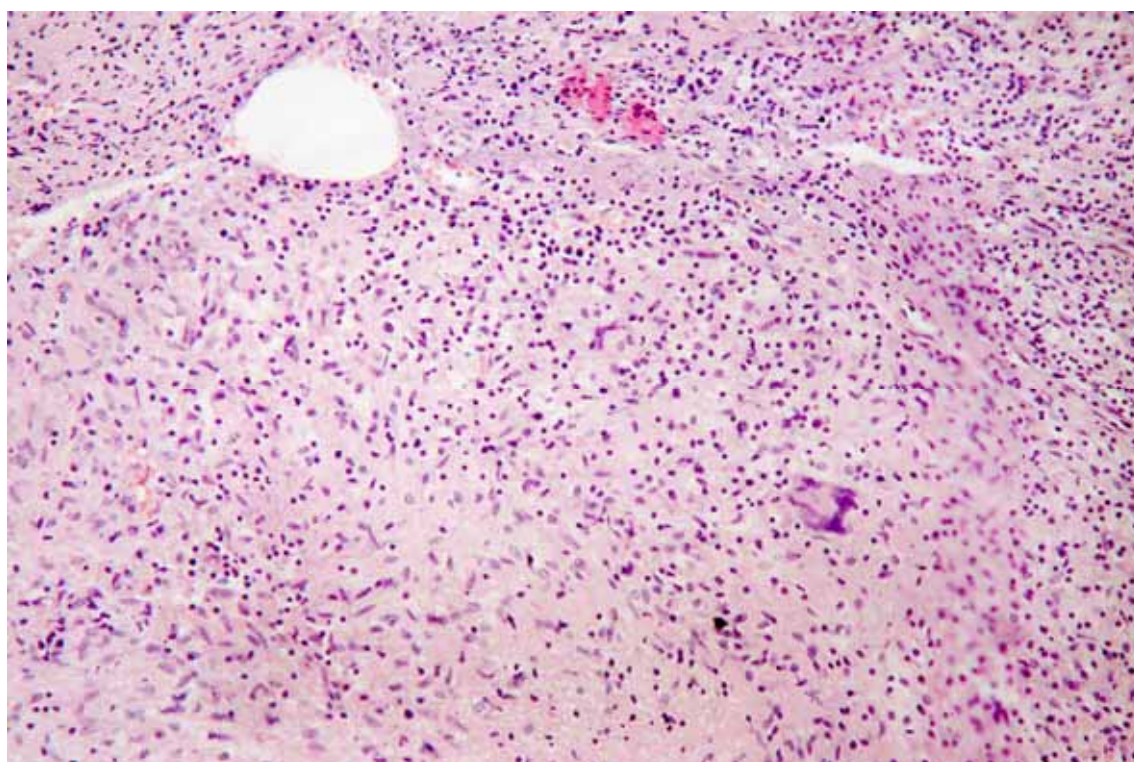


Fig. 1. A field of granulomatous inflammation in a lymph node, without a region of caseous necrosis, with Pirogov-Lanhsan giant cell (x100, hematoxylin and eosine stain).

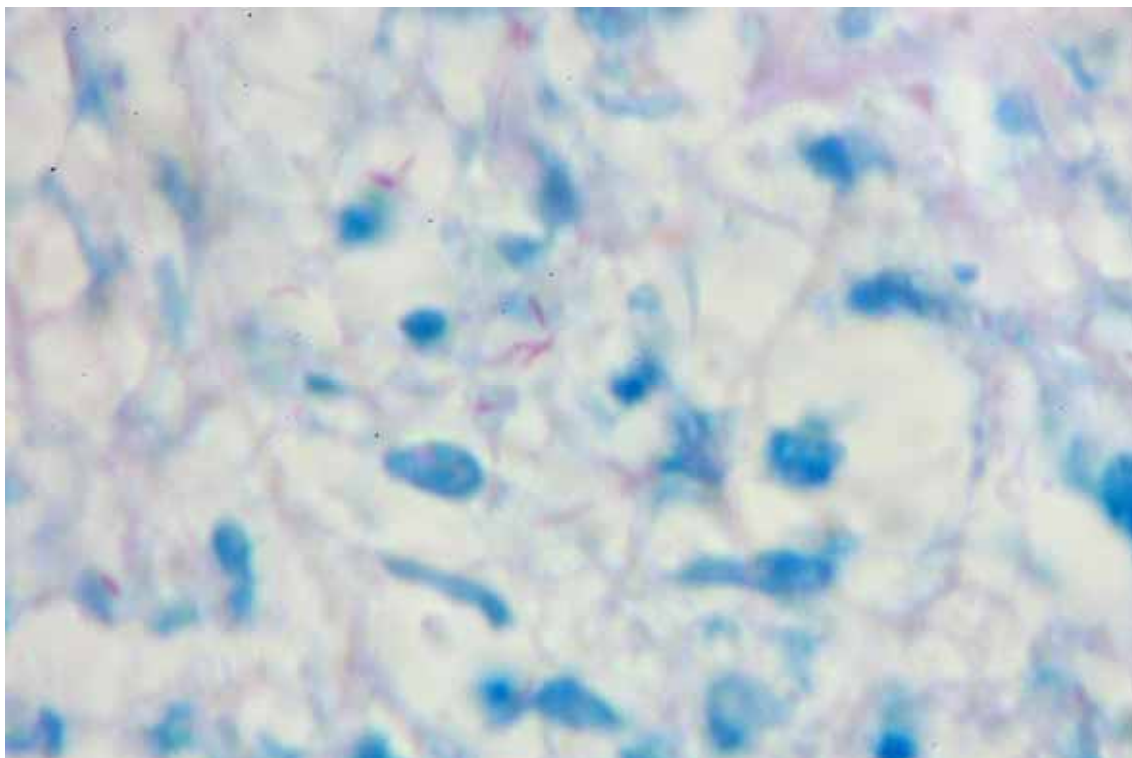


Fig. 2. MTB in a lymph node tissue, in area of granulomatous inflammation (x1000, Ziehl–Neelsen stain)

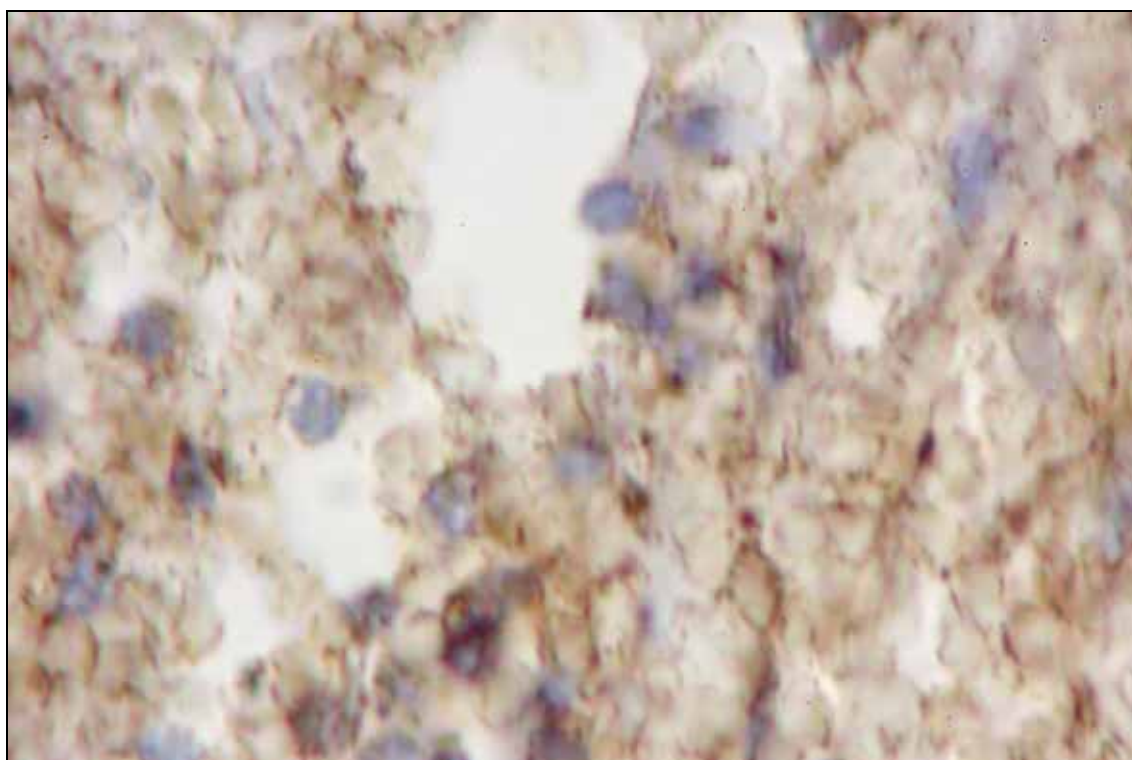


Fig. 3. MTB in a lymph node tissue, in area of granulomatous inflammation (x1000, immunohistochemical method, antibodies to MTB NCL-MT, clone 1.1/3/1).



Authors:

1. Makarova Uljana Egorovna, the senior researcher, PhD, YSC CMP SB RAMS
2. Loskutova Kjunnej Savvichna, the senior researcher, PhD, YSC CMP SB RAMS
3. Kirillina Maria Petrovna, the laboratorian-researcher, YSC CMP SB RAMS