ART diagnosis of mycobacterium tuberculosis DNA

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The epidemiological situation of tuberculosis in the Russian Federation is one of the important problems of public health and society as a whole. Tuberculosis today ranks second among infectious diseases in terms of the frequency of deaths, second only to AIDS. The high prevalence of multiple and extensively drug resistance (MDR / XDR) of mycobacterium tuberculosis poses a particular epidemic danger. The most unfavorable tuberculosis situation has developed in the Siberian (Siberian Federal District) and Far Eastern Federal Districts. One of the main reasons for the spread of M. tuberculosis with multidrug resistance is the late determination of drug resistance (Balabanova Ya.M., 2005; Chaulet R. eí al., 1995). It was found that the use of molecular genetic methods that determine mutations in the genes of M. tuberculosis in patients living in epidemiologically difficult regional conditions and having limited opportunities for verifying the pathogen using traditional cultural methods, allows prescribing an adequate treatment regimen at the early stages and achieving good indicators of the effectiveness of treatment on an outpatient basis. The detection of M. tuberculosis DNA in the diagnostic material by molecular genetic methods allows us to consider the result obtained as an objective and reliable criterion in favor of the specific etiology of the process in the diagnosis of pulmonary tuberculosis. At the same time, the sensitivity of detecting the pathogen by PCR method significantly exceeds the sensitivity of detecting M. tuberculosis by traditional microbiological methods (microscopy and inoculation). Pulmonary tuberculosis with negative sputum microscopy results is detected in more than 2/3 of cases (77, 4%) during prophylactic fluorographic examinations, and there is a low frequency of detection of M. tuberculosis by culture methods (30.2%), while the level of primary drug resistance was 51.7%, including MDR - 18.3%. The inclusion of molecular genetic research methods in the mandatory diagnostic minimum for examination of patients with pulmonary tuberculosis with negative sputum smear results for acid-fast mycobacteria (AFM) increases the detection rate.

WHO is developing its response to drug resistance in mycobacteria. But not always the methods of diagnosis and treatment recommended by this respected and authoritative international organization can be safely extended to Russian practice. It is no coincidence that domestic specialists in phthisiology at one time reacted very coolly to the DOTS strategy imposed on our country. The WHO Regional Office is currently adapting the Global TB Strategy for the European Region 2016–2020. The main goal of this five-year plan is to prevent the transmission of drug-susceptible and drug-resistant TB by ensuring universal access to TB and MDR / XDR-TB prevention, diagnosis and treatment services in all Member States of the WHO European Region. This Plan is aligned with the Health-2020 ". The following objectives must be achieved,adapted from the End TB Strategy:

- reduction of mortality from TB by 35%;
- reduction of TB incidence by 25%;
- the success rate of treatment of MDR-TB patients at the level of at least 75%. PCR diagnostics. In practical work, test systems are used,

amplifying (increasing the number exponentially to a level that allows detection by existing methods) fragments of repeated sequences IS6110, specific for mycobacterium tuberculosis complex. The use of repeated sequences provides amplification with greater sensitivity, since the number of copies of these sequences in the chromosome of most mycobacteria ranges from 5 to 20. Genodiagnostics at the proper level is available only to large clinics and research centers. The use of PCR for screening surveys of the population is inappropriate. This method should be used in combination with other laboratory tests, in difficult diagnostic cases, especially with extrapulmonary localization of the process. To date, there is information about 11 genes, involved in the formation of resistance of mycobacteria to the main anti-tuberculosis drugs. This resistance is due to the occurrence of mutations in genes, the products of which are targets for drugs or are involved in their activation. Resistant mycobacterial strains carrying different mutations can be widespread in different geographic regions, therefore, in order to create and use diagnostic test systems, a preliminary study of the nature of resistance of "local" mycobacteria is necessary.

The PCR results are assessed using various methods of hybridization of the product with specific complementary labeled DNA probes. Provided the optimal choice of the gene for amplification or the nucleotide sequence of the gene, this method allows with high specificity to identify the pathogen. Amplification tests are fast and safe. The advantages of the method are its high specificity (98–100%), speed (result in 1.5–4.5 h), high sensitivity in patients with a positive sputum smear (more than 92%), the ability to study any biological materials. The disadvantages of the method include the complexity of the interpretation of the results (requiring special training), high cost, low sensitivity in patients with negative sputum smear (60–70%).

The GeneXpert MTB / RIF test system has been recommended by WHO with the support of FIND for use in the diagnosis of tuberculosis only since 2010. The Gene Xpert MTB / RIF test system is a semi-quantitative nested PCR in real time in cartridges, and it is carried out in order to detect MBT DNA in sputum samples or concentrated sputum sediments; mutations in rifampicin resistance in samples obtained from patients at risk of developing resistance to this drug. GeneXpert MTB / RIF - closed-type test system; isolation and amplification are performed in a disposable cartridge, preliminary processing of the diagnostic material is reduced to minimal manipulations. This significantly reduces the possibility of material contamination. According to the WHO, rifampicin resistance correlates with isoniazid resistance. Resistance to rifampicin and isoniazid (MDR) usually indicates the need for simultaneous full testing for susceptibility to drugs of I and II lines, but this is possible only after isolation of the culture of mycobacteria. The result is processed on a computer and printed (positive / negative, rifampicin resistance present / not). The advantages of the method are speed (the whole process takes less than 2 hours), high specificity (100%), high sensitivity for patients with a positive sputum smear (92%), simplicity (required minimum skill level), no The result is processed on a computer and printed (positive / negative, rifampicin resistance present / not). The advantages of the method are speed (the whole process takes less than 2 hours), high specificity (100%), high sensitivity for patients with a positive sputum smear (92%), simplicity (required minimum skill level), no The result is processed on a computer and printed (positive / negative, rifampicin resistance present / not). The advantages of the method are speed (the whole process takes less than 2 hours), high specificity (100%), high sensitivity for patients with a positive sputum smear (92%), simplicity (required minimum skill level), no

the need for separate rooms for PCR. The disadvantages of the method include the ability to determine the resistance of the pathogen to only one anti-tuberculosis drug rifampicin, which predetermines the need for further studies to diagnose multidrug resistance; the possibility of using only native sputum and its concentrated samples (studies are continuing on the use of other biological samples); high cost of research. Considering the above limitations, the method is used as a screening method for examining populations with a high risk of multi-drug resistant tuberculosis (previously treated persons; those who have been in contact with MDR-TB patients; HIV-infected) in order to make a timely decision on isolating such patients and prescribing the correct treatment. The analysis result is not always accurate. A false positive result occurs only if all the requirements for the preparation and delivery of smears, etc. are not met. That is why both the patient and the doctors should strictly adhere to all the rules.

During 2015, I recorded the frequency-wave information using the devices of the Center "IMEDIS" with DNA of M. tuberculosis (sensitive and resistant to rifampicin) with different levels of DNA.

No.	Drug name DNA indicator	Additional information	Registration number
1	DNA is very low	sensitive	221
2	DNA is very low	R-resistant	1345
3	DNA low	sensitive	867
4	DNA low	R-resistant	227
5	DNA average	sensitive	230
6	DNA average	R-resistant	61
7	DNA high	sensitive	821/2
eight	DNA high	R-resistant	304

Screening for the diagnosis of tuberculosis according to the DNA of M. tuberculosis revealed:

- speed (the whole diagnostic process lasts less than 10-15 minutes);
- high specificity (100%);
- high sensitivity (98%);
- simplicity (required minimum skill level);
- no need for separate premises;
- minimum economic costs.

Disadvantage of the method:

- determination of the resistance of the pathogen to only one anti-tuberculosis drug rifampicin;
- there is no data on resistant mycobacterial strains carrying different mutations in different geographic areas.

Conclusions:

1. The ART method allows you to diagnose tuberculosis at the DNA level at all stages tuberculous process.

2. The ART method is competitive in comparison with the currently used

in medicine for the diagnosis of tuberculosis, both in terms of sensitivity and specificity, and in terms of economy and speed of diagnosis.

3. The use of ART in the diagnosis of M. tuberculosis DNA will make it possible to remove a number of existing problems in the field of diagnosis and treatment of tuberculosis.

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