The effectiveness of GenoType MTBDRplus using in the diagnosis of tuberculosis in Zaporizhzhia region

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The method GenoType MTBDRplus, v.2 allows to study clinical material from positive and negative smears, to examine patients with new and previously treated cases of tuberculosis pulmonary and extrapulmonary localization. But now there is a small amount of researches concerning the use of this method in the diagnosis of tuberculosis.

Aim. To evaluate the effectiveness of GenoType MTBDRplus, v.2 method using in clinical material research due to the diagnosis of tuberculosis in the Zaporizhzhia region in comparison with standard methods of investigation.

Materials and methods. The analysis of 52 results of studies of clinical material using the GenoType MTBDRplus test system, v.2 from patients who were examined and treated in dispensaries of the Zaporizhzhia region in 2016 was carried out. There were 67.3 % of men and 32.7 % of women among the patients. The mean age of the patients was 46.0 ± 1.9 years. The study of the clinical material using the GenoType MTBDRplus test, v.2 was performed according to the standard instructions.

Results. The results were positive in 12.5 % of the cases when tested by GenoType MTBDRplus, v.2 (p < 0.05) among negative results of the microscopy of the clinical material. The proportion of coincidence between the cultural and molecular genetic methods was 90 % for sputum and 100 % for other clinical material. In the presence of multiresistance, both methods of investigation coincided in the number of detected cases (50 % for the GenoType MTBDRplus method, v.2 and 42.9 % for the cultural method, respectively, p < 0.05). In comparison with the results of the cultural method, the GenoType MTBDRplus test, v.2 made possible to establish resistance to rifampicin in 35.7 % (p < 0.05) of patients whose resistance was not determined. False-positive and false-negative results are due, however, to the heterogeneity of strains of mycobacterium tuberculosis in clinical specimens, the absence of viable mycobacteria, the absence of phenotypic manifestations of mycobacterium tuberculosis genetic mutations, and errors in the investigations. The sensitivity of the GenoType MTBDRplus test, v.2 is about 78 %, but the specificity reaches 97 %. At the same time, the sensitivity of the test for multidrug resistance determining reaches 100 %, the specificity is about 89 %. The greater positive predictive value for cases in which the DNA of mycobacteria is determined and the negative predictive value is for cases in which multidrug resistance is determined.

Conclusions. This indicates that the use of the GenoType MTBDRplus test, v.2, together with the cultural method of investigation, has a high diagnostic value, high sensitivity (100 %) and specificity (89 %) for the definition of multiresistant, and makes it possible to obtain a result in a short time compared to culture.
The aim

To evaluate the effectiveness of GenoType MTBDRplus, v.2 method using in clinical material research due to the diagnosis of tuberculosis in the Zaporizhzhia region in comparison with standard methods of investigation.
Table 1. The results of investigation by cultural and molecular genetic methods compared with the results of microscopy of clinical material

| The method          | Result | The results of microscopy |
|---------------------|--------|--------------------------|
|                      |        | negative | 1–9 AFB | 1+ | 2+ | 3+ |
| Cultural            | Positive | 7* | 1 | 3 | 2 | 5 |
|                     | Negative | 33 | 1 | 0 | 0 | 0 |
| Geno Type MTBDRplus, v.2 | Positive | 5* | 0 | 3 | 2 | 5 |
|                     | Negative | 35 | 2 | 0 | 0 | 0 |
| In total, n = 52    |        | 40 | 2 | 3 | 2 | 5 |

Table 1: The results of investigation by cultural and molecular genetic methods compared with the results of microscopy of clinical material

Materials and methods

The analysis of 52 results of studies of clinical material using the GenoType MTBDRplus test system, v.2 from patients who were examined and treated in dispensaries of the Zaporizhzhia region in 2016 was carried out. There were 67.3% of men and 32.7% of women among the patients. The mean age of the patients was 46.0 ± 1.9 years. Comparison of the received results by using the GenoType MTBDRplus, v.2 test was performed according to standard methods of MTB identification: Ziehl-Neelsen staining method, microbiological method using an automated system BACTEC MGIT 960, seeding on solid culture media Lowenstein-Jensen and Finn II (the data of BACTEC MGIT 960 results in all cases coincide with the results on solid nutrient media, so their total results regarded as a cultural investigation results) with a further determination of resistance to drugs [4]. Results of smear microscopy were evaluated as negative – in cases of absence of acid-fast bacilli (AFB), 1–9 AFB – the presence of the appropriate number of AFB in smear microscopy, “1+” – in the presence of 10–99 AFB in smear, “2+” – in the presence of 1–10 AFB in each vision’s field of smear, “3+” – in the presence of more than 10 AFB in each vision’s field of smear [8].

In order to master the technique and for permission to perform this technique, physician-bacteriologist from Community institution “Zaporizhzhia regional clinical tuberculosis dispensary” was trained in the laboratory of new pathogens, Fondation Merieux (Lyon, France) from 12.01.2014 to 12.12.2014 in topic: “The identification of Mycobacterium tuberculosis complex (MTBC) and determine its resistance to rifampicin and/or isoniazid using GenoTypeMTBDRplus” and received the certificate.

The study of the clinical material using the GenoType MTBDRplus test, v.2 was performed according to the standard instructions [2,5].

Statistical analysis was performed by using “Statistica® for Windows 6.0” (StatSoft Inc., № AXXT71D833214- FAN5). Statistical significance of differences between groups in qualitative indicators was determined by the one-tailed Fisher’s exact criterion. For the levels of statistical significance values of the probability of the difference between the groups (p) levels of less than 0.05 were taken. We determined the sensitivity, specificity, predictive value of positive and negative results and their confidence intervals.

Results and discussion

The results of the Geno Type MTBDRplus, v.2 test data with culture investigation data of clinical material comparatively with the microscopy results were compared (Table 1).

We determined that the results of these tests coincided with the microscopy result “3+”, “2+” and “1+” in 100 % of cases. If the result of microscopy was “1–9 AFB” there was a coincidence of results with cultural test in 50 % of cases. The results were positive in 17.5 % of the cases when tested by cultural method and in 12.5 % of the cases when tested by GenoType MTBDRplus, v.2 (p < 0.05) among negative results of the microscopy of the clinical material. Thus, in negative results of smear microscopy of clinical material GenoType MTBDRplus, v.2 test and the cultural method have significant diagnostic value.

We found that presence of MTB DNA in clinical material was not detected by cultural test in 1 case, MTB were diagnosed only by using test system GenoType MTBDRplus, v.2 when comparing the results of diagnostic methods. In 4 cases MTB were found only by cultural method, and MG test system GenoType MTBDRplus, v.2 showed negative results (Fig. 1).

The proportion of coincidence between the culture and molecular genetic methods was 90 % for sputum and 100 % for other clinical material. False-positive and false-negative results are due, perhaps, to the heterogeneity of strains of mycobacterium tuberculosis in clinical specimens, the absence of viable mycobacteria, the absence of phenotypic manifestations of mycobacterium tuberculosis genetic mutations, and errors in the investigations.

When analyzing coincidence of positive results between test GenoType MTBDRplus, v.2 and cultural method we found that resistance to anti-tuberculosis drugs (H/R) by the cultural method was determined in 64 % of patients (Table 2). Mutations in the genotype of MTB that are responsible for resistance to H/R were determined in 93 % of patients by the GenoType MTBDRplus, v.2 method.

In the presence of drug resistance HR (multiresistance), both methods of investigation coincided in the number of detected cases (50 % for the GenoType MTBDRplus method, v.2 and 42.9 % for the cultural method, respectively, p > 0.05). But the use of MG test allows you to receive the result within two days, which proves its diagnostic value.
In comparison with the results of the cultural method, the GenoType MTBDRplus test, v.2 made it possible to establish resistance to rifampicin in 35.7 % (p < 0.05) of patients whose resistance was not determined.

That is, in a positive result of GenoType MTBDRplus, v.2 test its informativeness in determine resistance to drugs exceeded cultural method by almost 30 %. The difference between the results of MG and cultural methods, perhaps, due to the fact that not always the mutations that cause the occurrence of resistance to drugs appear phenotypically, as well as heterogeneity of strains of the pathogen.

Sensitivity, specificity and predictive value of Geno Type MTBDRplus, v.2 method was established compared to cultural results (Table 3).

According to the preliminary results of the investigation we found, that the sensitivity of the GenoType MTBDRplus test, v.2 is about 78 %, but the specificity reaches 97 %. At the same time, the sensitivity of the test for multidrug resistance determining reaches 100 %, the specificity is about 89 %. The greater positive predictive value for cases in which the DNA of MTB is determined and the negative predictive value is for cases in which multidrug resistance is determined.

Conclusions

1. According to the preliminary results of the investigation we found, that the use of GenoType MTBDRplus, v.2 test system allows determining additional 12.5 % of patients with bacterial excretion among patients with negative smears, p < 0.05. The proportion of coincidence between the cultural and molecular genetic methods is 90 % for sputum and 100 % for other clinical material.

2. Test system GenoType MTBDRplus, v.2 allows establishing resistance to rifampicin in 35.7 % (p < 0.05) patients, in comparison with the results of the cultural method. The proportion of multi-resistance detection is almost the same, but the diagnostic value of molecular genetic test is explained by much greater speed of results receiving (within two days versus to 2–3 weeks by use an automated system BACTEC MGIT 960 and 2–3 months – while use solid cultural media).

3. The specificity of positive result of GenoType MTBDRplus test, v.2 is 97 %, the sensitivity of the test for multidrug resistance determining reaches 100 %. The greater positive predictive value has MG method (93.3 %), and the negative predictive value – multidrug resistance determining (100 %).

Perspectives of further researches. Further investigation of clinical material from patients with pulmonary tuberculosis I and II categories of dispensary observation in Zaporizhzhia region by using a Geno Type MTBDRplus, v.2 test system according to the project Fondation Mérieux (France and ZSMU, Ukraine). It is planned to add this method with determination of resistance to second-line anti-tuberculosis drugs 2, identification of nontuberculous MTB.

Table 2. The distribution of investigation results of resistance to drugs by coincidence and differences between cultural and MG methods

| Positive results of cultural methods, N = 14 | Resistance | Positive results of Geno Type MTBDRplus, v.2 method, N = 14 |
|---------------------------------|------------|-------------------------------------------------------------|
|                                | abs. %     | abs. %                                                      |
| Positive test                  | 5          | 35.7 H +, R −                                           |
|                                | 0          | 0 H +, R +                                              |
|                                | 3          | 21.4 H +, R +                                           |
|                                | 6          | 42.9 H +, R +                                         |
| Negative test                  | 9          | 64.3 H −, R −                                          |
|                                | 11         | 35.7 H −, R +                                           |
|                                | 11         | 21.4 H −, R −                                           |
|                                | 8          | 57.1 H −, R +                                          |
|                                | 1          | 7.2 H −, R −                                           |

*: significant difference between the results of cultural and MG methods (p < 0.05).

Table 3. Sensitivity, specificity and predictive value of Geno Type MTBDRplus, v.2 method

| Parameters                                  | Positive test | Resistance to HR |
|---------------------------------------------|---------------|------------------|
| Sensitivity (confidence interval)           | 77.8 % (59.6–83.0 %) | 100.0 % (61.6–100.0 %) |
| Specificity (confidence interval)           | 97.1 % (97.4–99.8 %) | 88.9 % (63.3–88.9 %) |
| Predictive value of positive result         | 93.3 % (71.5–99.6 %) | 85.7 % (52.8–85.7 %) |
| Predictive value of negative result         | 89.2 % (80.3–91.7 %) | 100.0 % (71.2–100.0 %) |

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