ДИФФЕРЕНЦИАЛЬНО-ДИАГНОСТИЧЕСКАЯ ЦЕННОСТЬ КОМБИНАЦИЙ ИММУНОФЛОУОРЕНСЦЕНТНОГО ТЕСТА НА АНТИТЕЛА К ОСТРОВКОВЫМ КЛЕТКАМ, РАДИОИММУННОГО ТЕСТА НА АНТИТЕЛА К ГЛУТАМАТДЕКАРБОКСИЛАЗЕ И ИММУНОФЕРМЕНТНОГО ТЕСТА НА АНТИТЕЛА К ТИРОЗИНФОСФАТАЗЕ

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ОБОСНОВАНИЕ. Тесты на антитела к антигенам β-клеток (АТ) – важнейший инструмент дифференциальной диагностики сахарного диабета (СД), позволяющий отличить СД 1 типа (СД1) от неаутоиммунных форм СД. Для правильного выбора тестов надо знать, насколько убедительно они доказывают наличие или отсутствие СД1. Главным индикато- ром доказательности тестов служат коэффициенты правдоподобия (КП) их результатов. Чем больше КП положитель- ного результата (КП+), тем убедительнее тест подтверждает СД1, чем меньше КП отрицательного результата (КП−), тем убедительнее тест исключает СД1.

ЦЕЛЬ. Сравнить доказательность одиночных и комбинированных тестов на антитела к островковым клеткам (ICA), глутаматдекарбоксилазе (GADA) и тирозинфосфатазе (IA-2A) и оценить посттестовые вероятности СД1 при его разных претестовых вероятностях.

МЕТОДЫ. Одновременные тесты на ICA, GADA и IA-2A провели у 169 детей и подростков с впервые выявленным СД1 и у 169 людей без этого заболевания. ICA определяли иммунофлюоресцентным методом, GADA и IA-2A – радиоиммун- ным и иммуноферментным методами соответственно. КП рассчитывали с помощью программы MedCalc, посттесто- вые вероятности СД1 – по формуле, основанной на теореме Байеса.

РЕЗУЛЬТАТЫ. Среди одиночных тестов наиболее доказательным как при подтверждении, так и при исключении СД1 является тест на ICA, поскольку он имеет наибольший КП+ и наименьший КП−. Среди комбинаций тестов при под-тверждении СД1 наиболее доказательна комбинация ICA и GADA, поскольку ее КП+ больше, чем у всех других комби- наций. При исключении СД1 наиболее доказательна комбинация из трех тестов, поскольку ее КП− меньше, чем у всех других тестов.

ЗАКЛЮЧЕНИЕ. При дифференциальной диагностике СД для подтверждения СД1 рекомендуется применять комби- нацию ICA и GADA. При обоих положительных результатах эта комбинация обеспечивает наибольшие посттестовые вероятности СД1. Для исключения СД1 лучше всего подходит комбинация ICA, GADA и IA-2A. При всех отрицательных результатах эта комбинация обеспечивает наименьшие посттестовые вероятности СД1.

КЛЮЧЕВЫЕ СЛОВА: сахарный диабет; дифференциальный диагноз; антитела к островковым клеткам; антитела к глутаматдекар- боксилазе; антитела к тирозинфосфатазе; операционные параметры диагностических тестов; коэффициент правдоподобия

DIFFERENTIAL DIAGNOSTIC UTILITIES OF COMBINED TESTING FOR ISLET CELL ANTIBODY, GLUTAMIC ACID DECARBOXYLASE ANTIBODY, AND TYROSINE PHOSPHATASE ANTIBODY

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BACKGROUND. Beta-cell antibody tests are used for the differential diagnosis of diabetes mellitus. They permit to discrimi- nate between the type 1 diabetes (T1D) and non-autoimmune diabetes types. To choose an appropriate test for ruling in or
ruling out the T1D a physician needs to know how conclusive test results are. The most powerful estimate of test conclusiveness is its likelihood ratio (LHR). The higher LHR of a positive result (LHR^+), the more posttest probability of T1D; the lower LHR of a negative result (LHR^-), the less posttest probability of T1D.

AIMS. To compare conclusiveness of single and combined tests for antibodies to islet cells (ICA), glutamate decarboxylase (GADA), and tyrosine phosphatase IA-2 (IA-2A), and to estimate posttest probabilities of T1D at various pretest probabilities.

METHODS. All antibodies were tested in parallel in 169 children and adolescents with a new-onset T1D, and in 169 persons without this disease. ICA, GADA, and IA-2A were determined by indirect immunofluorescence, radioimmunoe assay, and ELLISA, respectively. LHR^+ and LHR^- were calculated with the MedCalc Statistical Software. Posttest T1D probabilities were calculated from Bayes theorem-based equation.

RESULTS. Among single tests, an ICA test had the greatest LHR^+ and the smallest LHR^-, and consequently was the most reliable either for ruling in or ruling out the T1D. Among test combinations, an ICA&GADA combination had the greatest LHR^+ and was the most suitable for T1D confirmation. The triple combination ICA&GADA&IA-2A had the smallest LHR^- and was the most suitable for T1D exclusion.

CONCLUSIONS. In the differential diagnosis of diabetes, the most appropriate test for ruling in the T1D is the double combination ICA&GADA. With both antibodies positive, this combination provides the highest posttest T1D probabilities at any pretest probability. The most appropriate test for ruling out the T1D is the triple combination ICA&GADA&IA-2A. With all three antibodies negative, this combination provides the lowest posttest T1D probabilities.

KEYWORDS: diabetes mellitus; differential diagnosis; islet cell antibody; ICA512 antibody; glutamate decarboxylase antibody; operational characteristics; likelihood ratio

Differential diagnosis between type 1 diabetes mellitus (DM1) and other types and variants of DM is essential for patients with DM. In patients with clear clinical and laboratory signs of DM1, this is not a problem. For example, ketosis, high levels of glucose and glycated haemoglobin (HbA1c), and a low C-peptide level are diagnostic in a patient with classic DM1 symptoms, and no additional examination is required.

However, in a patient without classical DM1 symptoms and ketosis, with moderate hyperglycaemia and a borderline C-peptide level, not only DM1 but also type 2 DM (DM2) or some variant of monogenic DM are likely to occur. In such cases, the differential diagnosis becomes more complicated, and antibodies to β-cell antigens must be determined to confirm or rule out DM1.

Russian endocrinologists have long used tests to detect islet cell antibodies (ICAs), glutamic acid decarboxylase antibodies (GADAs), tyrosine phosphatase (cytoplasmic islet cell antibody 512, IA-2A) and insulin antibodies (IAs) in their clinical practice and scientific medical studies [1–4]. The test for antibodies to the zinc transporter 8 (ZnT8A) has also been used for scientific purposes [5]. However, no one test has been proven to be the most effective for DM1 diagnosis.

The validity of laboratory tests is typically assessed by its diagnostic sensitivity (DSens), diagnostic specificity (DSpec) and diagnostic accuracy (DA). However, the likelihood coefficients (LCs) of test results are crucial indicators [6, 7], because they clearly indicate how convincingly a positive test result confirms and a negative result rules out the disease. In addition, LCs are useful when comparing different tests with close DSens and DSpec. Finally, LCs enable quick assessments of posttest (a posteriori) and pre-test (a priori) probabilities.

We determined DSens, DSpec, DA and other operational parameters for the immunofluorescence test for ICA, the radioimmune test for GADAs and the enzyme immunoassay for IA-2A [8]. However, we did not calculate LCs (and neither have others); therefore, the evaluation of such tests and their combinations, on the basis of LCs, has not been carried out.

AIM

The scientific aims of this study were assessment of validity of single tests for ICA, GADA and IA-2A as well as their combinations on the basis of calculations of DSens, DSpec, DA and LC as well as evaluation of post-test probabilities of DM1 at different pre-test probabilities and different results of single tests and their combinations.

We also set out practical tasks, such as providing doctors with recommendations on the use of tests for antibodies in differential diagnostics of DM and helping them to correctly interpret results of the tests.

METHODS

Design of the study

We tested ICA, GADA and IA-2A one time using serum samples of 169 children and adolescents with newly diagnosed DM1 (DM1 group) and 169 samples of control subjects without this disease (control group, C).

Acceptance criteria

The DM1 group included patients with the maximum probability of having DM1, while the C group included subjects with a maximum probability of not having DM1. We applied the strict criteria listed in online application 1 to classify the subjects into the DM1 or C group.

Conditions of performing

The N.I. Pirogov Russian National Medical Research University (Moscow, Russia), the Sechenov University (Moscow, Russia), the Russian Medical Academy of Continuous Professional Training (Moscow, Russia), National Medical Research Centre of Endocrinology (Moscow, Russia), Morozov Children’s City Clinical Hospital (Moscow, Russia), Z.A. Bashlyaeva Children’s City Hospital (Moscow, Russia),...
Clinical Hospital (Moscow, Russia) and other treatment and prevention institutions of Moscow and the Moscow region participated in the study.

Duration of the study
The study was conducted over a period of 6.5 years (from 2011 to 2017).

Description of medical intervention
The study did not include any medical interventions. Serum samples for antibody testing were obtained from blood samples taken during planned clinical and laboratory examinations of subjects, conducted in inpatient or outpatient settings.

Primary study outcomes
The primary study outcomes included the result values of tests for antibodies and their combinations, including DSens, DSpec, DA, values of LC tests and values of post-test probabilities of DM1 with positive and negative results of antibody tests.

Outcome registration methods

Methods for antibody testing
ICA, method of indirect immunofluorescence: The results were considered positive for ICA titers ≥ 10 units of the Juvenile Diabetes Foundation International (JDF) [8].

GADA, radioimmune method, Immunotech IRMA Anti-GAD test system: The results were considered positive for GADA titers > 1 IU/ml [9].

IA-2A, immunoassay method, Medizym Anti-IA2 test system: The results were considered positive for IA-2A titers ≥ 10 IU/ml [10].

Methods for calculating operational parameters and diagnostic accuracy (DA)
We calculated DSens, DSpec and the predictive value of the positive and negative results from the contingency tables, and the DA by obtaining area under receiver-operating curves (AUC). We used the MedCalc medical-statistical program [11] for these calculations.

Theoretical foundations of LC calculations
In general, LC represents the ratio the (probability of obtaining a definite test result in a subject with a disease)/(probability of obtaining the same result in a subject without a disease).

The more the LC deviates from the unity, the more convincingly this test proves the presence or absence of the disease.

LC can be calculated for both positive and negative test results. In our case, the LC of the positive result (LC+) is calculated as the ratio of the probability of a truly positive test result for antibodies (i.e. positive result in a DM1 patient) to the probability of a false positive result (i.e. a positive result in a subject without DM1):

$$LC^+ = \frac{DSens}{100 - DSpec}$$ (1)

The larger the LC+ value, the better this test confirms DM1. For example, a test with an LC+ = 30 confirms DM1 10 times more convincingly than a test with an LC+ = 3.

An LC of a negative result (LC-) is calculated as the ratio of the probability of a false negative result of the test for antibodies (i.e. negative result in a DM1 patient) to the probability of a truly negative result (i.e. a negative result in a subject without DM1):

$$LC^- = \frac{100 - DSens}{DSpec}$$ (2)

The more the LC deviates from the unity towards zero, the better this test rules out DM1. For example, the test with LC- = 0.1 rules out DM1 5 times more convincingly than the test with LC- = 0.5.

It is obvious from formulas (1) and (2) that the LCs depend on both the DSens and the DSpec. The higher the numerical values of these parameters are, the more LC+ and LC- deviate from unity.

Theoretical foundations of DM1post-test probability calculations
Knowing LC, one can calculate the post-test probability (PtP) of the disease by considering its pre-test probability (PrP). This calculation is based on the Bayes theorem and is rather complicated; its detailed explanation is not included in our tasks, and we consider only its final stages.

PtP is expressed by the following formula:

$$PtP (%) = \frac{\text{Ratio of post-test chances}}{\text{Ratio of pre-test chances} \cdot LC + 1} \cdot 100$$ (3)

The ratio of chances in this case is taken as the ratio of PrP of presence and PrP of absence of DM1 in the patient. In turn, the ratio of post-test chances is calculated by the formula:

$$\text{Ratio of post-test chances} = \text{Ratio of pre-test chances} \cdot \text{LC}$$ (4)

Thus,

$$PtP (%) = \frac{\text{Ratio of pre-test chances} \cdot LC}{\text{Ratio of post-test chances} + 1} \cdot 100$$ (5)

Through formula (5), it is possible to calculate PtP for both positive and negative test results.

We give some examples of calculations of PtP. Suppose that a doctor examines a patient with an unspecified DM and estimates the probability of DM1 in this patient (PrP) to be 30%. Hence, the PrP of the absence of DM1 is 70%. Thus, the ratio of pre-test chances is 30:70 = 0.43.

To clarify the diagnosis, the doctor decides to perform the test for antibodies, in which LC+ = 10, and LC- = 0.5. In this case, with a positive test result,

$$PrP_{	ext{DM1}} = \frac{0.43 \cdot 10}{(0.43 \cdot 10) + 1} \cdot 100 = 81.1\%$$

and with a negative test result,

$$PrP_{	ext{DM1}} = \frac{0.43 \cdot 0.5}{(0.43 \cdot 0.5) + 1} \cdot 100 = 17.7\%$$.

In other words, with a positive test result, the probability of DM1 in a patient increases dramatically (from 30% to 81.1%), and if the result is negative, the probability decreases dramatically (from 70% to 17.7%).

In our study, we calculated PtP of DM1 for PrPs equal to 80%, 50%, 20% and 0.25%. Such PrPs correspond approximately to the following frequent diagnostic situations:
• The normal weight of a child or adolescent with DM symptoms, glucose and HbA1c levels are greatly increased, and the level of C-peptide is significantly lower than normal, but there is no ketosis. Due to the latter circumstance, the physician evaluates PrP of DM1 not as 100% but as 80%.

• For a slightly overweight child or adolescent with borderline fasting glucose level, the glucose levels after meals are slightly higher than 11 mmol/l, HbA1c slightly exceeds 6.5%, the C-peptide is slightly lower than normal, there are no DM symptoms, and there is no ketosis. The doctor believes the patient may have DM1 or another type of DM (DM2 or some variant of a monogenic DM) with equal probability (50%/50%).

• The normal weight of an adult with DM symptoms, glucose and HbA1c levels are greatly increased, the C-peptide level is normal but close to the lower limit, and there is no ketosis. Considering that this is an adult patient, the doctor is inclined to the diagnosis of DM2 and estimates that its probability as 80%, and PrP of DM1 as 20%.

• A child or adolescent with a one-time fasting hyperglycemia at 12 mmol/l, without DM symptoms and ketosis, the levels of glucose, HbA1c, and C-peptide are normal. The doctor assumes the hyperglycemia was accidental, or the laboratory made a mistake when measuring glucose. Nevertheless, the doctor wants to rule out concealed DM1. In this case, the doctor believes that the PrP of DM1 is equal to its prevalence in the population, which, according to the literature, is approximately 0.25% [12].

We used formula (5) for the calculation of the PtP of DM1.

Ethical review
The Ethics Committee of the I.M. Sechenov First Moscow State Medical University (119991, Moscow, Trubetskaya Street, 8) approved the publication of the results of the study without disclosing the personal data of the subjects (Protocol No. 08-15 of the meeting of 15.09.2015).

Statistical analysis
We followed these principles to calculate the sample size; the sizes of DM1 and C groups were not preliminary decided. After the end of the enrolment period, 234 subjects were included in the DM1 group, and only 169 subjects were included in the C group. To ensure the legitimacy of our statistical analysis, we equalised the number of subjects in each group. For this purpose, we decided. After the end of the enrolment period, 234 subjects were included in the DM1 group, and only 169 subjects were included in the C group. To ensure the legitimacy of our statistical analysis, we equalised the number of subjects in each group. For this purpose, we randomly excluded 65 subjects from the DM1 group.

Thus, in the end, we had 169 subjects in each group, and only 169 subjects were included in the C group. To ensure the legitimacy of our statistical analysis, we equalised the number of subjects in each group. For this purpose, we randomly excluded 65 subjects from the DM1 group. To ensure the legitimacy of our statistical analysis, we equalised the number of subjects in each group. For this purpose, we randomly excluded 65 subjects from the DM1 group.

We used formula (5) for the calculation of the PtP of DM1.

Before one analyses the data in Table 2, it is necessary to explain that the calculated DSpec of the result “ICA+, GADA-, IA-2A+” amounted to 100%, since none of 169 subjects in the group C received such a result (see Table 1). However, the real DSpec of this result is most likely not 100%. First, the DSpec of all the components of this result (i.e. DSpec of single tests for ICA, GADA and IA-2A) is less than 100%. Second, it is likely that with increases in the size of group C, at least one result of “ICA+, GADA-, IA-2A+” will be found, i.e. DSpec will be less than 100%. For example, if one such result is found in group C increased twofold (338 people), the DSpec will be 99.7%, and with a triple increase in the C group, it will be 99.8%. Based on this reasoning, we took DSpec of the result of “ICA+, GADA-, IA-2A+” to be equal to 99.8%.

Based on the data in Table 2, we ranked the tests for their substantiation in the confirming and ruling out the presence of DM1.

When confirming DM1,
ICA and GADA > ICA, IA-2A > GADA, IA-2A > ICA, GADA and IA-2A > ICA.
When ruling out DM1:
ICA, GADA and IA-2A > ICA, GADA > ICA, IA-2A > ICA
GADA and IA-2A.

Our results made us wonder why all combinations of the two tests had greater validity for confirming DM1 than a combination of three tests. After all, it seems intuitive that three tests should be more accurate than two. The reason for this is that LC+ is directly proportional to DSens and DSpec (see formula (1)). In all combinations of two tests, the maximum DSpec is the same (99.4%), while in the combination of three tests, the maximum DSpec is 99.4% for the result of “ICA+, GADA+, IA-2A+” and 99.8% for the result of “ICA+, GADA-, IA-2A+”. However, the DSens of the combinations of the two tests (68.1, 64.5 and 53.3%) is greater than the DSens of the results of “ICA+, GADA+, IA-2A+” (52.1%) and the result of “ICA+, GADA-, IA-2A+” (12.4%). Therefore, LC+ for all combinations of two tests is higher. Similarly, when applying formula...
(2), the fact that a single ICA test has greater validity for ruling out DM1 than the combination of GADA and IA-2A is also explained. It should also be noted that the differences in validity of different tests are reliable. This was confirmed by the results of a comparative analysis of the characteristic curves (see Appendix 3 online).

Using formula (5), we calculated the PtP of DM1 for all possible results of single tests and their combinations. The results of these calculations without indication of confidence intervals are presented in Table 3, while those with the indication of confidence intervals are provided in the Appendix 4 online.

### Table 1. Comparison of frequency of test results for antibodies in the groups DM1 and C and evaluation of the DA of the tests

<table>
<thead>
<tr>
<th>Test</th>
<th>The test result</th>
<th>Groups</th>
<th>DM1</th>
<th>C</th>
<th>χ²</th>
<th>P_f</th>
<th>AUC</th>
<th>95% CI</th>
<th>P_AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICA</td>
<td>+</td>
<td></td>
<td>155</td>
<td>14</td>
<td>91.7</td>
<td>3</td>
<td>166</td>
<td>1.8</td>
<td>273.7</td>
</tr>
<tr>
<td>GADA</td>
<td>+</td>
<td></td>
<td>124</td>
<td>45</td>
<td>73.4</td>
<td>17</td>
<td>152</td>
<td>10.1</td>
<td>138.9</td>
</tr>
<tr>
<td>IA-2A</td>
<td>+</td>
<td></td>
<td>112</td>
<td>57</td>
<td>66.3</td>
<td>6</td>
<td>163</td>
<td>3.6</td>
<td>145.9</td>
</tr>
</tbody>
</table>

**Notes:** DA, diagnostic accuracy; “+” number of subjects with this test result; “−” number of subjects without this test result; F, frequency of this test result (%); χ², value of χ² calculated with the “N-1” method; P_f, probability of validity of the null hypothesis about the absence of differences between the frequencies of the results; AUC (area under curve), area under the receiver-operating curve of the test; 95% CI, 95% confidence interval for AUC; P_AUC, probability of the null hypothesis validity about the absence of differences between AUC and 0.5. Variants of tests with P_AUC > 0.05 are highlighted in grey.

aPositive result of at least one of several tests.
The analysis of the data in Table 3 indicates that among the single tests, the best for both confirmation and discarding of DM1 is the ICA test, since for all PrP, it provides the greatest FtP for a positive result and the lowest PtP for a negative result.

Among the combinations of tests, the combination of ICA and GADA tests is the best in the confirmation of DM1. When both results in this combination are positive, this combination provides the greatest PtP for all PrPs. When DM1 is ruled out, the combination of the three tests is most effective (in cases when all three results are negative).

### Table 2. Diagnostic sensitivity, diagnostic specificity and likelihood coefficients of tests for antibodies

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
<th>DSens. %</th>
<th>DSpec. %</th>
<th>LC+</th>
<th>LC−</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Single tests</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ICA</td>
<td>+</td>
<td>91.7</td>
<td>98.2</td>
<td>51.7</td>
<td>0.084</td>
</tr>
<tr>
<td>GADA</td>
<td>+</td>
<td>73.4</td>
<td>89.9</td>
<td>7.3</td>
<td>0.296</td>
</tr>
<tr>
<td>IA-2A</td>
<td>+</td>
<td>66.3</td>
<td>96.5</td>
<td>18.7</td>
<td>0.350</td>
</tr>
<tr>
<td><strong>Combination of two tests</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ICA and GADA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ICA+. GADA−</td>
<td>23.7</td>
<td>98.8</td>
<td>20.0</td>
<td>0.772</td>
<td></td>
</tr>
<tr>
<td>ICA+. GADA+</td>
<td>68.1</td>
<td>99.4</td>
<td>115.0</td>
<td>0.321</td>
<td></td>
</tr>
<tr>
<td>≥ 1 AT+a</td>
<td>97.0</td>
<td>88.8</td>
<td>8.6</td>
<td>0.033</td>
<td></td>
</tr>
<tr>
<td>ICA+. IA-2A−</td>
<td>27.2</td>
<td>98.8</td>
<td>23.0</td>
<td>0.737</td>
<td></td>
</tr>
<tr>
<td>ICA+. IA-2A+</td>
<td>64.5</td>
<td>99.4</td>
<td>109.0</td>
<td>0.357</td>
<td></td>
</tr>
<tr>
<td>≥ 1 AT+a</td>
<td>93.5</td>
<td>95.3</td>
<td>19.8</td>
<td>0.068</td>
<td></td>
</tr>
<tr>
<td>ICA+. IA-2A−</td>
<td>20.1</td>
<td>90.5</td>
<td>2.1</td>
<td>0.892</td>
<td></td>
</tr>
<tr>
<td>GADA−. IA-2A+</td>
<td>13.0</td>
<td>97.0</td>
<td>4.4</td>
<td>0.896</td>
<td></td>
</tr>
<tr>
<td>ICA+ IA-2A+</td>
<td>53.3</td>
<td>99.4</td>
<td>90.0</td>
<td>0.470</td>
<td></td>
</tr>
<tr>
<td>≥ 1 AT+a</td>
<td>86.4</td>
<td>87.0</td>
<td>6.6</td>
<td>0.156</td>
<td></td>
</tr>
<tr>
<td><strong>Combination of three tests</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ICA+. GADA−. IA-2A−</td>
<td>11.2</td>
<td>98.8</td>
<td>9.5</td>
<td>0.898</td>
<td></td>
</tr>
<tr>
<td>ICA+. GADA+. IA-2A−</td>
<td>16.0</td>
<td>99.4</td>
<td>27.0</td>
<td>0.845</td>
<td></td>
</tr>
<tr>
<td>ICA+ GADA−. IA-2A+</td>
<td>12.4</td>
<td>100.0a</td>
<td>99.8b</td>
<td>63.0c</td>
<td></td>
</tr>
<tr>
<td>≤ 1 AT+a</td>
<td>97.6</td>
<td>85.8</td>
<td>6.9</td>
<td>0.028</td>
<td></td>
</tr>
<tr>
<td>≤ 1 AT+a</td>
<td>97.6</td>
<td>85.8</td>
<td>6.9</td>
<td>0.028</td>
<td></td>
</tr>
<tr>
<td>ICA+. GADA+. IA-2A+</td>
<td>52.1</td>
<td>99.4</td>
<td>88.0</td>
<td>0.482</td>
<td></td>
</tr>
<tr>
<td>≥ 1 AT+a</td>
<td>97.6</td>
<td>85.8</td>
<td>6.9</td>
<td>0.028</td>
<td></td>
</tr>
</tbody>
</table>

Notes: DSens. diagnostic sensitivity; DSpec. diagnostic specificity; LC+: the likelihood coefficient of the positive result; LC−: the likelihood coefficient of the negative result

*aAt least one of several tests is positive
bFormal calculated values (explanation in the text)
cModified values (explanation in the text)

### Discussion of the primary results of the study

Physicians face the following questions when choosing a test for antibodies from several possible tests:
- Which test to choose for confirmation, and in which one to rule out DM1?
- How to evaluate the PtP of DM1 having obtained a test result?

The answers to these questions can be given by calculating the LC and PtP.

We found that the combination of ICA and GADA is best for confirming DM1, because it has the largest LC+ and provides the highest PtP of DM1 for any PrPs. If the GADA test is unavailable, the combination of ICA and IA-2A can be used, which is almost identical to the combination of ICA and GADA based on the LC+.

To rule out DM1, the combination of ICA, GADA and IA-2A is the best approach, since it has the smallest LC− and provides the lowest PtPs of DM1 for any PrP.

For quick assessment of PtP of DM1 for a specific test result, Table 3 can be used. In this case, one must chose the column with PrP of DM1, which is closest to the PrP in a patient examined. It is possible to determine the PtP of DM1 approximately for any PrP according to Fagan’s nomograph (13). This nomograph is illustrated in Figure 5 of online Appendix 4. Additionally, one can determine...
accurately the PtP by calculating values according to formula (5). The PtP of DM1 has to be estimated objectively in the patient, and LC values from Table 1 must be used to determine PtP according to Fagan’s nomograph and according to the formula (5).

When planning antibody tests, not only analytical but also economic aspects need to be considered. The fact is that in some regions of Russia, the system of compulsory medical insurance does not fully cover the cost of such tests, and patients have to pay for them independently. Since the cost of the tests is quite high, it is difficult for a patient with a small income to determine simultaneously several types of antibodies. At the same time, some doctors, aiming to get as much information as possible, prescribe the maximum number of tests for antibodies to all patients (for example, a combination of ICA, GADA and IA-2A). Our study indicates that in many cases, testing for antibodies can be minimised without compromising its differential diagnostic value. We found, in particular, that a combination of two tests (ICA and GADA or ICA and IA-2A) is sufficient to confirm the DM1 diagnosis. Moreover, to confirm DM1 in a low-income patient, a single test for ICA is sufficient, since it provides a sufficiently high PtP of DM1 for any PrP.

It often happens that the doctor is absolutely sure of the DM1 diagnosis, but the patient or his relatives cannot

Table 3. Post-test probabilities of DM1 with different results of antibody tests and for different pre-test probabilities of DM1

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
<th>80%</th>
<th>50%</th>
<th>20%</th>
<th>0.25%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>PtP</td>
<td>PtP</td>
<td>PtP</td>
<td>PtP</td>
</tr>
<tr>
<td></td>
<td></td>
<td>80%</td>
<td>50%</td>
<td>20%</td>
<td>0.25%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>80%</td>
<td>50%</td>
<td>20%</td>
<td>0.25%</td>
</tr>
</tbody>
</table>

Notes:

*aThese results are non-informative, as they do not have DA (AUC does not differ significantly from 0.5).

*bCalculations are performed for a DSpec equal to 99.8% (see explanation in the text).
accept this. This is especially true for parents of children with newly diagnosed DM1. In the hope of disproving the diagnosis of DM1, parents ask the doctor to conduct antibody tests. In this situation, a combination of two tests is also sufficient. For example, a combination of ICA and GADA can be prescribed, explaining to parents that with the result of “ICA+ GADA-”, DM1 is confirmed with a probability of 98.8%, and with the result of “ICA+, GADA+”, it is confirmed with almost 100% probability.

Our data are applicable for the differential diagnosis of newly diagnosed DM in children and adolescents. In addition, ICA is most often used in this setting, while GADA and IA-2A are used less often. For other categories of patients with autoimmune DM, for example, in patients with latent autoimmune diabetes of adults (LADA), the GADA is of greater diagnostic value [14, 15].

In the leading diabetes laboratories in Russia, other types of antibodies have been investigated, including the antibodies against the zinc transporter 8 (ZnT8A). These antibodies are detected in 4–8% of DM1 patients in the absence of all other antibodies [16], particularly in 5% of patients with LADA [5]. The addition of the ZnT8A test to the tests for ICA, GADA and IA-2A should increase the informative value of the serological differential diagnostics of DM.

Our data on the differential diagnostic value of single and combined antibody tests coincide well with those of other investigators [17–19].

Limitations of the study
Advantages
- We applied strict criteria for the inclusion of patients in the DM1 group, thus minimising the “contamination” of the DM1 group by patients with other types and variants of DM. This raises the reliability of estimates of operational parameters of tests for antibodies.- We calculated LCs and PtPs to be able to assess, for the first time, the information and validity of the results for these antibody tests.
Disadvantages
- Because the study was conducted over a long stretch of time, it is possible that the variability of the operational parameters of the antibody tests may have affected our results slightly (due to the heterogeneity of the reagents used in different years).

CONCLUSION

The results of this study can be directly used by practitioners for the differential diagnosis of DM. Physicians should keep in mind that all the data and practical recommendations given in the article refer only to the specific methods used to detect the antibodies and should not be generalised to other testing methods used in Russia and abroad.

ADDITIONAL INFORMATION

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Conflict of interest. None of the participants in this work has any connection with the manufacturers of testing systems or reagents used for antibody studies or other laboratory studies.
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ПРИЛОЖЕНИЯ [SUPPLEMENTS]

Приложения доступны на сайте журнала по URL: https://endojournals.ru/index.php/dia/article/view/9364

ПРИЛОЖЕНИЕ 1
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SUPPLEMENT 5
Fagan’s nomogram for the approximation of post-test probabilities of type 1 DM with known likelihood ratios for the antibody tests


СПИСОК ЛИТЕРАТУРЫ | REFERENCES


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