CLINICAL LABORATORY DIAGNOSTICS

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Analysis of the application of laboratory tests in the diagnosis of diseases of connective tissue

D. A. Batsatsa[1], T. V. Bulgakova[1], S. N. Bardakov[2]

[1] INVITRO-SPb LTD,
57, Dnepropetrovskaya ul., St. Petersburg, 196105, Russian Federation
[2] S. M. Kirov Military Medical Academy,
6, ul. Academika Lebedeva, St. Petersburg, 194044, Russian Federation

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The determination of antinuclear antibodies is included in the criteria for the diagnosis of connective tissue diseases, differential diagnosis and determines the prognosis and treatment tactics of the patient. The existing strategy for laboratory diagnosis of connective tissue diseases involves screening (ANA Hep-2 cell + ENA / ELISA), followed by the selection of refinement tests (ELISA, immunoblot). The assessment of the actually used algorithms, however, revealed that every third patient was assigned almost all available tests simultaneously to confirm a connective tissue disease. Rational screening was performed in 1/3 of the cases. Low frequency of application of ANA immunoblots significantly reduced the effectiveness of diagnosis. It is advisable to provide more information about the primary screening methods for connective tissue diseases of practicing doctors, namely therapists and general practitioners, who act as the primary diagnostic link for this category of patients.

Keywords: diffuse connective tissue diseases, screening, antinuclear antibodies.

Introduction

Connective tissue diseases (CTD) is a heterogeneous group of autoimmune diseases caused by the excessive production of antibodies towards nuclear and cytoplasmic antigens of connective tissue cells. The determination of antinuclear antibodies (ANA) is included in the criteria for the diagnosis of connective tissue diseases, is essential for the differential diagnosis and determines the prognosis and treatment tactics for the patient.

[1] Autoimmune pathology occurs in 20–25 % of cases of all diseases (i.e. every fourth patient). The overall ratio of men to women is 1:9 [2]. The existing strategy for laboratory diagnosis of connective tissue diseases involves screening (ANA Hep-2 cell + ENA / ELISA), followed by the selection of refinement tests (ELISA, immunoblot). Screening methods should be highly sensitive, and confirmatory tests should be highly specific [3].

Objective

The aim was to assess the actual algorithm for using laboratory tests in the diagnosis of connective tissue diseases and to determine if the diagnostic search matches established diagnostic algorithms.

Materials and methods

We examined 144 blood sera of patients with a presumptive diagnosis of connective tissue disease at the age of (*Me*): $_{32}$ 36 $_{38.2}$ years, among them women were 115/144 ($_{72}$ 79 $_{86}$ %), and men — 29/144 ($_{13}$ 20 $_{27}$ %) (Table 1).

Table 1. **Age groups of patients**

	M	F	TOTAL
Amount	29	115	144
Age			
0–25 (I)	11	25	36
26–50 (II)	15	63	78
≥ 51 (III)	3	27	30

Based on the order and set of prescribed tests, we identified 5 groups of patients as shown in table 2.

Table 2. **The analyzed patient groups**

Group	Criterion	Example
Screening only (1)	Screening of diffuse connective tissue diseases	ANA Hep-2 + ENA test
Completed algorithm (2)	ANA Hep-2 + ENA → immunoblot, ELISA	ANA Hep-2 "+" + ENA "–" → immunoblot ANA (RNP +), dsRNA "+"
Partial algorithm (3)	Incomplete Research	ANA Hep-2
Performing all tests at once (4)	Running consecutive tests at once	ANA Hep-2; ANCA, immunoblot, AMA, RF, anti-CCP
Difference sequence (5)	Inappropriate sequence	ANCA → ANA Hep-2

A comprehensive study of the samples included the determination of ANA Hep-2 cell, anti-citrullinated cyclic peptides (anti-CCP), anti-modified citrullinated vimentin (anti-MCV) — by ELISA; ANA immunoblot (ENA-screen produced by Euroimmun, Germany), rheumatoid factors (RF) and HLA-B_{27} (by PCR, DNA Technology).

Results

A positive ANA Hep-2 cell screening result was found in 51/144 ($_{28.3}$ 36.1 $_{44.5}$%). At the same time, $HLAB_{27}$ was performed both in the case of positive ($_{9.6}$ 19.6 $_{32.5}$%) and negative in case of ANA Hep-2 results ($_{10.3}$ 17.4 $_{26.7}$%). ENA was performed in 26/144 cases ($_{12.1}$ 18.1 $_{25.3}$%) with a positive ENA result 6/26 ($_{8.9}$ 23.1 $_{43.6}$%). In all cases with ANA Hep-2 the result was negative. In none of the patients ENA was included in the full screening of connective tissue diseases (ANA Hep-2 + ENA) (Fig. 1).

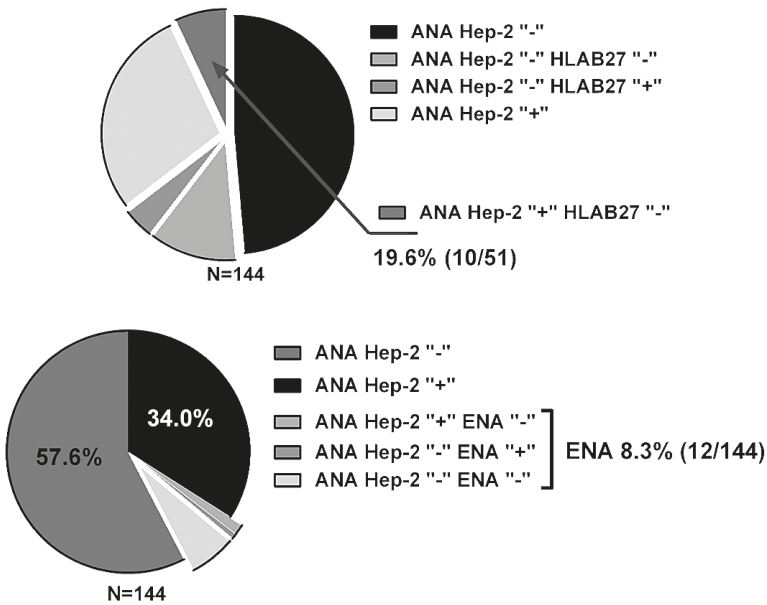

Fig. 1. Determination of ANA Hep-2 cell

Most of the patients were included in group 4, which is characterized by the simultaneous execution of all available tests for connective tissue diseases. An ANA immunoblot was performed 3/144 (2.1%), while 1/3 (33.3%) was positive. Immunoblot for systemic sclerosis (SSc) 6/144 (4.1%) was performed, while 1/6 (16.7%) was positive. The feasibility of performing the ANA immunoblot was justified in 2/3 of the cases, since one of them was assigned to the ANA Hep-2 negative. The immunoblot for systemic sclerosis was appropriate in all cases 6/6 andANA Hep-2 was also positive. All patients were included in group 4 (Fig. 2).

The anti-dsDNA test was performed in 12/144 cases ($_{4.3}$ 8.3 $_{14.1}$%) with ANA Hep-2 1:160 (6/12 cases). The feasibility of anti-dsDNA is 66.7%, because ANA Hep-2 was positive. Most of the patients were in groups 4 and 5 (83.3%). Antikeratin antibodies (AKA) — 1/144 ($_{0.02}$ 0.7 $_{3.8}$%), RF 5/144 ($_{1.1}$ 3.4 $_{7.9}$%) in 4 cases of ANA Hep-2 negative, anti-MCV

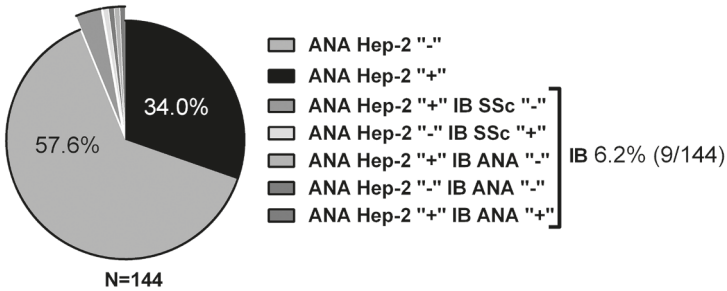

Fig. 2. The use of immunoblot SSc and ANA

9/144 ($_{2.9}$ 6.3 $_{11.5}$ %) in 5 cases of ANA Hep-2 negative, all were in group 4. Anti-neutrophil cytoplasmic autoantibodies (ANCA) were studied in 17/144 cases ($_{7.0}$ 11.8 $_{18.2}$ %). The purposeful determination was in 11/17 cases of negative ANA Hep-2 ($_{3.8}$ 64.7 $_{85.8}$ %), but in majority the ANCA was appointed simultaneously (group 4). In one case the ANCA panel determination was made.

Conclusion

The study revealed that every third patient was assigned diagnostic tests as part of confirming connective tissue disease by simultaneously determining almost all available tests. And only in five patients the algorithm was followed, and rational screening was performed in 1/3 of the cases. Also, it should be noted that the low frequency of application of ANA immunoblots significantly reduced the effectiveness of diagnosis. Thus, the most rational is the consistent purposeful screening and aimed screening tests than their simultaneous use.

It is also advisable to provide more information about the primary screening methods for connective tissue diseases to medical practitioners, namely internists and general practitioners, who serve as a primary diagnostic link for this category of patients. This approach will increase the effectiveness of screening for connective tissue diseases as well as for other autoimmune disorders.

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Authors' information:

Diana A. Batsatsa — MD; diabats@mail.ru
Tatiana V. Bulgakova — MD, PhD; bulgakovat@mail.ru
Sergei N. Bardakov — MD, PhD; episaxis@mail.ru