consequences, especially when treatment is delayed mainly due to a lack of suspicion in the doctor and misdiagnosis [1].

Lyme disease (LD) is an infectious, vector-borne disease that is transmitted by Ixodes ticks and manifests itself in damage to various organs and systems (most often – ring-shaped erythema, damage to the nervous system, musculoskeletal system, heart, liver, and eyes).

According to scientists, in the territories of western Ukraine, the following species of Borrelia are pathogenic to humans: *B. burgdorferi s. s., B. garinii, B. afzelii, B. lusitaniae* and *B. valaisiana*, or *B. burgdorferi s. s., B. garinii, B. afzelii, B. valaisiana* and *B. spielmanii* – according to other authors. The infection of ticks with spirochetes of the B. burgdorferi s. l. complex in the environment is 7.0-25.0% [1, 2, 3, 4].

According to various authors, cardiac involvement in Lyme disease (LD) is a rare manifestation (from 4 to 10% in the United States and from 0.3% to 4% in Europe) with potentially fatal complications and is an understudied pathology [2, 3]. It is a transmissible widespread disease in Europe and a particularly dangerous infection. Lyme carditis (LC) usually develops in the stage of disseminated infection (after 1-3 months after infection) and is manifested by cardiac pain, palpitations, shortness of breath, dizziness, transient fainting, reduced tolerance to physical exertion [4, 5, 6]. According to the Centers for Disease Control and Prevention (CDC), the incidence of LD in European and American endemic areas can reach 500 cases per 100,000 population. The CDC reports an annual 30,000 cases of LD, 3-4% of which are diagnosed with myocarditis. There is a male predominance with a ratio of 3:1. Clinically, it proceeds in the following stages: early localized, early disseminated, and late persistent. The diagnosis of LD is based on anamnesis data on tick bites, clinical manifestations of the disease, serological studies (enzyme-linked immunosorbent assay (ELISA) and Western blot), which indicate the presence of specific anti-B. Burgdorferi s.l. IgM/IgG antibodies and data from instrumental examination methods (electrocardiogram (ECG), echocardiography (ECHO), and magnetic resonance imaging (MRI)). AV block is the most common manifestation of LC (90%), with high-grade AV block accounting for approximately two-thirds of cases [7, 8, 9]. In addition to AV block, other manifestations of LC may be observed, such as intraatrial block, atrial fibrillation, supraventricular tachycardia, bundle branch block, and ventricular tachycardia and fibrillation [3, 6, 9]. General rhythm and conduction disturbances are detected by ECG and Holter monitoring. The structural-functional state of the heart is assessed by echocardiography and MRI.

Aim: to study the possibilities of diagnostic tools in the verification of Lyme carditis, to investigate the genotype of the pathogen in a patient with Lyme carditis and to analyze the course of the disease in the process of etiotropic treatment.

We present a description of a clinical case of myocarditis associated with LD.

The study was conducted after the patient signed an informed consent and in compliance with the basic provisions of the "Rules of ethical principles of scientific medical research involving humans", approved by the Declaration of Helsinki (1964-2013), ICH GCP (1996), EU Directive No. 609 (of 24.11.1986), orders of the Ministry of Health of Ukraine of 23.09.2009 No. 690, of 14.12.2009 No. 944, of 03.08.2012 No. 616, approved by the Bioethics Commission of the Ternopil National Medical University named after I.Ya. Gorbachevskyi of the Ministry of Health of Ukraine (protocol No. 71 of 25 October 2022).

Clinical case

Patient F., 49 years old, was admitted to the cardiology department of Ternopil University Hospital on January 1, 2021, with complaints of chest pain when lying down, shortness of breath, dizziness upon change of body position, headache, and significant general weakness. The first symptoms appeared in the last decade of December 2021 when the patient experienced chest pain at rest and pronounced general weakness, which led to hospitalization in the cardiology department of Ternopil University Hospital. The patient resided in a rural area of Ternopil region, engaged in household activities, and reported multiple tick bites.

The patient's condition is of moderate severity, characterized by pain and shortness of breath. The skin appears pale and dry. Arrhythmic cardiac activity (bradyarrhythmia), weakening of the first heart sound, and a systolic murmur over the apex of the heart were observed. Auscultation revealed vesicular coarse breath sounds over the lower lung fields. The heart rate was 52 beats per minute, and the blood pressure was 135/85 mmHg. The liver was palpable at the level of the right costal arch with a rounded edge. Edema of the feet was noted.

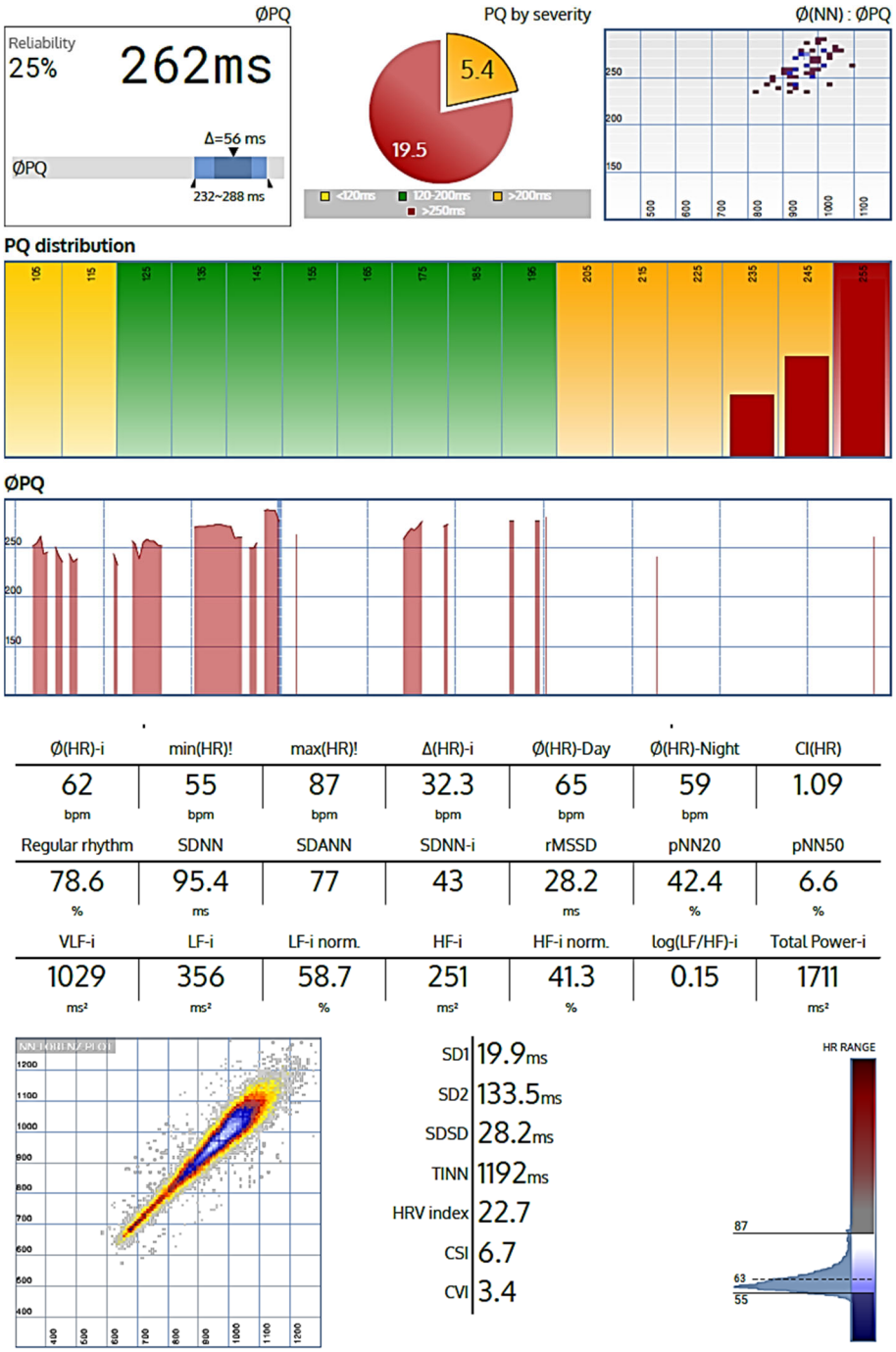
Laboratory data (January 2, 2021): leukocytosis – 9.5×10^9/L (eosinophils – 1%, basophils – 5%, segmented neutrophils – 51%, lymphocytes – 30%, monocytes – 13%), elevated erythrocyte sedimentation rate (ESR) of 32 mm/hour, moderately increased creatine phosphokinase-MB (CK-MB) of 31.0 U/L (reference range 0-24 U/L), significantly elevated C-reactive protein (CRP) of 28.06 mg/L (reference range 0.0-5.0 mg/L), increased anti-streptolysin-O

titer (ASO) of 220.2 IU/mL (normal range up to 200 IU/mL), elevated serum mucoid to 10.0 IU (reference range 0-6 IU), troponin T of 26.61 (reference range 12.7-24.9 ng/mL), and NT-proBNP of 896 pg/mL (reference range 0-125 pg/mL).

Data on cardiac rhythm and conduction were obtained through ECG and Holter monitoring. ECG recordings were obtained using the YUKARD-200 electrocardiograph by UTAS (Ukraine) in the supine position with 12 leads. The paper speed was set at 50 mm/s, and 1 mV corresponded to 10 mm. Each ECG analysis focused on signs of myocardial ischemia, rhythm disturbances (extrasystoles, atrial fibrillation), conduction abnormalities (atrioventricular conduction disturbances, His bundle branch block, AV block), and the presence of cardiac hypertrophy. Holter monitoring is an electrocardiographic diagnostic method that involves recording ECG continuously for 24 hours during a patient's daily activities, followed by analysis of the recorded data. This method allows for the detection of cardiac abnormalities, even if they occur infrequently, as the ECG is recorded continuously. The recording is performed on 2, 3, or more channels (up to 12 channels), with 2-channel and 3-channel recorders being the most common. In some cases, it is possible to obtain mathematically reconstructed 12-lead ECG from the 3-channel recording, which can be useful for the specific diagnosis of extrasystoles. However, such "reconstructed" ECG and the recording from a 12-channel recorder may not match the surface ECG recorded in the standard 12 leads, so Holter recording data (including true 12-lead recordings) cannot replace a standard ECG. The ECG was recorded using the Bittium Faros 360 recorder, which provides three-channel ECG with adjustable sampling rate up to 1000 Hz, 3D acceleration with a sampling rate of up to 100 Hz, and an available API with a range of up to 100 meters. The following data were obtained (January 3, 2021): sinus rhythm alternating with ectopic beats at an average rate of 67 beats per minute, minimum heart rate of 48 beats per minute, maximum heart rate of 102 beats per minute. Complex rhythm and conduction disturbances were also detected, including first-degree and transient second-degree atrioventricular block, polymorphic ventricular extrasystoles, and episodes of paired beats and pauses longer than 2.4 seconds with compensatory contractions (Figure).

Ultrasound (US) examination was performed using the Philips HD 11 XE ultrasound system (USA) with a 2-4 MHz frequency transducer. The SonoCT composite imaging technology provides spatial alignment of the transmitted and received beams, resulting in simultaneous acquisition of multiple lines of sight without the need for additional manipulations of the transducer and real-time alignment of these lines, thereby producing exceptionally clear images. The contrast imaging option allows the use of the HD11 XE system for detecting harmonic signals of contrast agents using S3-1 and C5-2 transducers. A uniform field is created, providing a more uniform contrast signal throughout the sector. Adaptive image processing with XRES eliminates graininess artifacts and enhances boundary sharpness, improving the performance of diagnostic analysis. The combination of SonoCT and XRES modes allows for obtaining high-quality and precise images, enabling early and accurate diagnosis. Standard echocardiographic imaging planes were used to assess the size, volume, and contractile function of the heart: the long-axis, short-axis, and four-chamber views. The 2D mode, M-mode, and Doppler ultrasound (color Doppler, pulse-wave Doppler, and continuous-wave Doppler) were employed using standard approaches. Measurements included anterior-posterior dimensions of the aorta and pulmonary artery (PA), dimensions of the left ventricle (LV) and LV cavity, thickness of the interventricular septum and posterior wall of the LV in diastole, and end-diastolic dimension of the LV [10,11]. Cardiac ultrasound data (January 4, 2021): left atrium – 4.1 cm (volume -50 cm^3). The thickness of the LV wall is 1.1-1.2 cm, with normal contractility. A small amount of fluid (2 mm) was detected in the pericardium above the right atrium during diastole. The end-diastolic dimension (EDD) is 5.4 cm. The size of the right ventricle is 2.3 cm. The ejection fraction is 60%. In the dynamic echocardiogram on February 5, 2021, the left atrium volume (LA) was 48 cm^3, thickening of the mitral annulus, and small areas in the lower septum and lower segments with dyskinetic motion. The ejection fraction slightly decreased over time, to 52%.

Cardiac magnetic resonance imaging (MRI) is the most important non-invasive diagnostic method for the assessment, monitoring, and risk stratification of patients with non-ischemic myocarditis. According to the 2021 recommendations for the evaluation and diagnosis of chest pain by the American College of Cardiology/American Heart Association/American Society of Echocardiography/American College of Chest Physicians/Society of Academic Emergency Medicine/Society of Cardiovascular Computed Tomography/Society for Cardiovascular Magnetic Resonance, cardiac MRI plays a crucial role in differentiating myocarditis from other causes of acute chest pain in patients with myocardial injury and non-obstructive coronary arteries on anatomical evaluation. Cardiac MRI is also used to differentiate myocarditis from myopericarditis and to determine the presence and extent of myocardial or pericardial inflammation and fibrosis [12].



Holter monitoring data

MRI was performed on the "Vantage Titan, 1.5 T" system (Japan). Data processing for MRI was conducted on a cardiac workstation, analyzing MRI slices of the heart in standard projections and corresponding scanning modes. Cardiovascular magnetic resonance (CMR) is a set of magnetic resonance imaging methods designed to assess the morphology of the cardiovascular system, ventricular function, myocardial perfusion, tissue characterization, quantitative measurement of blood flow, and non-invasive evaluation of ischemic heart disease [13, 14, 15].

On cardiac MRI (January 12, 2021), signs of myocarditis were observed, indicating an active inflammatory process with infiltration of perifocal fibrotic areas. Minimal exudative effusion in the pericardial cavity was noted. The overall contractile function of the left ventricle was preserved, and the chamber volumes were within normal range.

In the first stage of serological diagnosis, total specific antibodies to antigens of the *B. burgdorferi sensu lato* complex were determined in the blood serum using the enzyme immunoassay method with test systems from Euroimmun AG (Germany): IgM antibodies – *Anti-Borrelia burgdorferi* ELISA (IgM), IgG antibodies – *Anti-Borrelia plus* VIsE ELISA (IgG). Antibodies were detected using an optimized mixture of lysates from pathogenic strains of *Borrelia* affecting humans. The test system *Anti-Borrelia plus* VIsE ELISA (IgG) additionally includes recombinant VIsE, a variable major protein sequence similar to *B. burgdorferi* (variable major protein-like sequence, expressed). In the second stage, blood samples with intermediate and positive results were confirmed using the immunoblot method with the EUROLINE *Borrelia* RN-AT test system from Euroimmun AG (Germany). Immunoblotting is important for identifying a range of specific Borrelia antigens with varying specificity.

The obtained immunoblot analysis (January 11, 2021) for *Borrelia burgdorferi* revealed specific IgG antibodies, VIsE (*Borrelia afzelii*) – detected, VIsE (*Borrelia burgdorferi*) – detected, p41 – detected, OspC *(Borrelia afzelii*) – detected.

Based on clinical and laboratory-instrumental data, the infectious disease specialist diagnosed the patient F. with early disseminated Lyme disease, carditis, and third-degree AV block.

On the basis of clinical and laboratory-instrumental data, acute myocarditis associated with Lyme disease was diagnosed in patient F. Complex rhythm and conduction disturbances were observed, including sinus bradycardia, first-degree AV block, transient second-degree AV block (Mobitz type 2), third-degree AV block, ventricular polymorphic extrasystole, and blocked supraventricular extrasystoly. High cardiovascular risk. The patient has functional class II heart failure according to the NYHA (New York Heart Association) classification.

The patient received metabolic and etiological antibacterial therapy with doxycycline at a dose of 200 mg/day in two doses for 21 days.

Patient F. was discharged from the hospital with an improvement in general condition. During the outpatient stage, the patient was recommended to continue treatment for heart failure.

On April 19, 2021, a 24-hour Holter monitoring of ECG was performed to monitor the disease progression. During the observation, an irregular sinus rhythm was recorded. The following arrhythmias were detected: tachycardia with a total duration of 4 hours 59 minutes 48 seconds; supraventricular extrasystole- 30 times: single – 27, paired – 2; ventricular arrhythmia: 16 episodes of monomorphic. Conduction disturbances included stable first-degree AV block and transient second-degree block, Mobitz type 2. T-wave inversion was observed.

At the time of examination, the patient reported shortness of breath only during intense physical exertion. Objectively, the cardiac activity was rhythmic. The heart rate was 66 bpm. Blood pressure reached the target value of 130/80 mmHg.

On February 7, 2022, the Holter monitoring of ECG did not reveal clinically significant arrhythmias. The baseline rhythm was sinus with an average heart rate of 64 bpm. During the day, the average heart rate was 65 bpm, and during the night, it was 59 bpm. Analysis of the PQ segment showed AV conduction slowing throughout the monitoring period. Prolongation of QTc was observed during 25% of the monitoring period (mean QTc – 445 ms, range 414-490 ms). Negative T-wave was observed in a lead, reflecting the potential of the anterior wall of the left ventricle. Heart rate variability was slightly reduced: SDNN – 95.4 ms. LF/HF ratio – 1.42.

Lyme carditis is a natural focal zoonosis caused by Borrelia species of the *Borrelia sensu lato* complex, which are transmitted to humans through the bite of Ixodes ticks [16, 17, 18, 19].

The prevalence and incidence of Lyme carditis are continuously increasing. Climate changes play a significant role in the expansion of ticks [1, 4]. The increase in Lyme carditis cases can also be attributed to increased awareness among general practitioners regarding its epidemiology and clinical features, as well as greater public knowledge [20].

Lyme disease affects both males and females almost equally, although Lyme carditis occurs more frequently in males with a male-to-female ratio of 3:1 [15, 21]. The patient we examined, Mr. F., resided in a rural area within an endemic zone for Lyme carditis, which served as an additional risk factor for Lyme infection. According to the patient's medical history, he had experienced multiple tick bites. Calculation of the risk score using the SILC (Suspicious Index in Lyme Carditis) scale, aiming to determine the likelihood of Lyme carditis in the patient, classified him into the high-risk group (8 points). Specifically, he scored 1 point for being younger than 50 years old, 1 point for male gender, 1 point for outdoor activities/residence in an endemic zone, 2 points for constitutional symptoms (malaise, dyspnea, presyncopal states), and 3 points for tick bite. In the presence of a high risk of borrelial myocarditis according to the SILC scale, a two-stage serological examination of the patient's blood samples is recommended. In the differential diagnosis of patients with myocarditis, physicians should

maintain a high level of suspicion regarding the possible borrelial etiology of the disease to ensure timely diagnosis and treatment [22].

According to Radesich C. et al, Lyme carditis is diagnosed in untreated adults in 0.3-4% of cases in Europe and 4-10% of cases in the United States. The authors attribute this difference to variations in virulence between European and North American isolates, as well as their lesser cardiotropism [23]. Overall, based on differences in DNA nucleotide sequence, 21 genotypes of the *Borrelia burgdorferi sensu lato* complex have been identified [1]. In western regions of Ukraine, 5 species that are pathogenic to humans are prevalent (*B. burgdorferi s.s., B. garinii, B. afzelii, B. valaisiana, and B. spielmanii*) [24]. As known, patients with cardiac involvement in Lyme carditis are hospitalized with continuous ECG monitoring in the presence of significant PR interval prolongation (>300 ms), other arrhythmias, or pericardial effusion. The results of the preliminary examination served as the basis for hospitalization of patient F.

In order to determine the etiological factor of myocarditis in patient F., we conducted a two-stage serological study of the patient's blood samples. In the first stage, we used the enzyme immunoassay method, and in the second stage, blood samples with intermediate and positive results were confirmed by immunoblotting, establishing the involvement of *Borrelia burgdorferi s.s.* and *Borrelia afzelii* in the development of clinical manifestations of Lyme carditis.

Today, it is known that prolonged persistence of *B. burgdorferi* in the host's body and its binding to host tissue cells, extracellular matrix, and vascular network occurs through the suppression of its surface protein immunogenicity, inactivation of effector organisms, antigenic mimicry, and presence in the extracellular matrix. Several proteins present in B. burgdorferi, including P66 and decorin-binding protein, play an important role in enhancing tropism for myocardial tissue [25]. Thanks to these proteins, *B. burgdorferi* can infect all parts of the heart, including the conduction system around the atrioventricular node, the external or internal membrane of the heart, the cardiac muscle, and less commonly, the coronary vessels or heart valves [8, 9]. It is evident that in addition to the cardiotropism of the genotypes of *B. burgdorferi* and *Borrelia afzelii* identified in our patient, the occurrence of Lyme carditis in patient F. may have been facilitated by moderate left ventricular hypertrophy (1.1-1.2 cm LV wall thickness) detected during cardiac ultrasound examination. However, we did not identify any obvious comorbid pathology that could account for these changes in the patient's examination.

The development of cardiac diseases is influenced by many factors, including both modified and non-modified factors (such as age, gender, and the presence of cardiovascular diseases in close relatives). Additionally, attention should be paid to the history of previous infections, as there is a hypothesis that various bacterial and viral agents are also responsible for endothelial changes in blood vessels, which can potentially worsen the course of myocarditis. For example, literature reports suggest that cytomegalovirus, hepatitis C virus, *Mycoplasma pneumoniae*, and *Chlamydia pneumoniae* may contribute to the development of atherosclerotic vascular lesions [26, 27]. According to the patient, he does not have any harmful habits (does not smoke, consumes alcohol no more than once a week up to 30 g/day in terms of pure alcohol), and denies a history of infectious diseases.

A typical manifestation of Lyme carditis resulting from the toxic effects of Borrelia is various degrees of AV block, which can be diagnosed using ECG and Holter monitoring. In our examined patient, F., instrumental examinations revealed first-degree AV block, transient second-degree AV block (Mobitz type 2), third-degree AV block, and other rhythm and conduction disturbances.

According to Scheffold et al. and other authors, conduction and rhythm disorders in patients with LC can be resolved with timely administration of appropriate etiotropic antibiotic therapy [15].

For patients with symptomatic bradycardia due to Lyme carditis that is unresponsive to medical therapy, temporary cardiac pacing is recommended [8, 22]. The average heart rate in our patient was 67 bpm according to Holter monitoring, and it was managed with medication without the need for temporary cardiac pacing.

Unfortunately, the diagnosis of Lyme carditis is complicated by the polymorphism of clinical manifestations, the absence of clear history of tick bites, the frequent absence of erythema migrans, and the possibility of asymptomatic disease [14, 21]. Direct detection of the infectious agent in LC is almost impossible, so serological testing is necessary to confirm the diagnosis. In endemic areas for LC, early serological testing and the availability of instrumental diagnostic methods ensure a favorable prognosis with timely diagnosis and appropriate treatment of LC, often allowing for the restoration of cardiac conduction and avoiding the need for pacemaker implantation [14].

The novelty and practical value of using the immunoblot method in our study lie in excluding false-positive results of enzyme immunoassay in the first stage and establishing the involvement of specific pathogenic species of Borrelia (*Borrelia*

burgdorferi s.s. and Borrelia afzelii) in the clinical manifestations of LC in the second stage.

Currently, there is no universally recognized standard etiotropic therapy for LC. Broad-spectrum antibiotics from pharmacological groups such as third-generation cephalosporins, tetracyclines, and macrolides are used in medical treatment. The choice of antibiotics is based on preclinical and clinical studies of the drug, taking into account its pharmacokinetic properties, degree of organ involvement, duration of the disease, clinical form, and general condition of the patient. In addition to the equal efficacy of doxycycline hydrochloride, amoxicillin, and cefuroxime axetil, literature reports also describe the use of doxycycline, which is most commonly used in the treatment of LC, and its therapeutic effect in diseases caused by *A. phagocytophilum, B. miyamotoi, and Babesia spp*. These findings were also taken into account, as well as the results of a study conducted at the I.Ya. Horbachevsky Ternopil National Medical University, Ministry of Health of Ukraine, regarding coinfection with multiple tick-borne pathogens simultaneously. The high effectiveness of doxycycline hydrochloride against mobile forms of Borrelia and the possibility of prescribing it for a period of 10 to 21 days were considered, while amoxicillin or cefuroxime axetil are prescribed for 14 to 21 days [2, 4, 6, 16, 17, 24].

In patient F, after prolonged antibiotic therapy, an improvement in AV conduction was achieved, and the third-degree AV block disappeared in the dynamic ECG monitoring. Ultrasound examination and MRI allowed for timely detection of functional and structural myocardial abnormalities of inflammatory origin. Serological testing in patient F confirmed the presence of specific Borrelia antigens, *Borrelia burgdorferi* s.s. and *Borrelia afzelii*, which presumably exhibited cardiotropic properties and caused myocardial involvement in Lyme carditis. Thanks to the timely diagnosis of LC using modern diagnostic methods and the administration of etiotropic antibiotic treatment, significant improvement in clinical dynamics and restoration of impaired conduction were achieved in patient F, in addition to the treatment of heart failure.

CONCLUSIONS

1. The use of the immunoblot method in the second stage of laboratory testing in a patient with Lyme carditis allowed for the exclusion of false-positive results from the initial serological test (first stage) and established the involvement of two Borrelia species, specifically Borrelia burgdorferi s.s. and Borrelia afzelii, in the clinical manifestations of Lyme carditis.
2. The combined infection of *B. burgdorferi s.s.* and *B. afzelii* presumably contributed to their cardiotropic effect and caused Lyme carditis.
3. The application of modern specific serological methods in the diagnosis of myocarditis and the identification of the etiological factor, followed by the administration of appropriate antibiotic therapy, resulted in positive clinical and ECG dynamics in the patient.

Contributors:

Myndziv K.V. – formal analysis, research, data curation, writing – initial draft, writing – review and editing;

Yarema N.I. – conceptualization, validation, data curation, writing – review and editing, conducting;

Vereshchahina N.Ya. – conceptualization, methodology, validation, formal analysis, research, data curation, writing – review and editing, conducting;

Bondarchuk V.I. – software, resources, visualization;

Hevko U.P. – methodology, resources, visualization;

Vayda O.V. – software, resources.

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Conflict of interests. The authors declare no conflict of interest.

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