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Detection of drug resistance mutations of hepatitis C virus in patients with failure of the treatment with direct acting antivirals

Diana E. Valutite[1]✉, Aleksandr V. Semenov[1–3], Yulia V. Ostankova[1], Konstantin V. Kozlov[4], Aleksandr G. Borisov[5], Vladimir D. Nazarov[2], Areg A. Totolian[1,2]

[1]Saint Petersburg Pasteur Institute, Saint Petersburg, Russia
[2]Pavlov Saint Petersburg State Medical University, Saint Petersburg, Russia
[3]North-Western State Medical University named after I.I. Mechnikov, Saint Petersburg, Russia
[4]S.M. Kirov Military Medical Academy, Saint Petersburg, Russia
[5]Krasnoyarsk Science Center of the Siberian Branch of the Russian Academy of Sciences, Krasnoyarsk, Russia

Abstract
Background. The development of direct acting antivirals (DAAs) has spurred a revolution in treatment of patients with chronic hepatitis C. However, there are cases showing no response to treatment. In 5% of cases, the viral breakthrough is most likely caused by DAA resistance mutations in the hepatitis C virus genome.
The purpose of the study is to detect drug resistance mutations of hepatitis C virus in patients with DAA treatment failure.
Materials and methods. The study was performed on plasma samples from 3 patients diagnosed with chronic hepatitis C virus infection and demonstrating DAA virological treatment failure. All isolates had genotype 1b. Drug resistance mutations were detected by using direct sequencing of *NS3*, *NS5A*, and *NS5B* genome regions. The detection technique was developed at the Pasteur Research Institute of Epidemiology and Microbiology.
Results. Drug resistance mutations were detected in all cases. By using the Geno2pheno [hcv] 0.92 tool, nucleotide substitutions were detected in different viral genome regions and presumably caused resistance or decreased sensitivity to antivirals both present and absent in the sofosbuvir + daclatasvir combination therapy. Antiviral treatment failure in patients with chronic hepatitis C is caused by drug resistance mutations.
Conclusions. The developed technique is efficient for detection of drug resistance mutations in *NS3*, *NS5A*, and *NS5B* regions in cases of virological failure of DAA treatment.

Keywords: *hepatitis C virus*, *drug resistance mutations*, *direct acting antivirals*

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Научная статья
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Выявление мутаций лекарственной устойчивости вируса гепатита С у пациентов с неэффективной терапией препаратами прямого противовирусного действия

Валутите Д.Э.[1]✉, Семенов А.В.[1–3], Останкова Ю.В.[1], Козлов К.В.[4], Борисов А.Г.[5], Назаров В.Д.[2], Тотолян А.А.[1,2]

[1]Санкт-Петербургский НИИ эпидемиологии и микробиологии имени Пастера, Санкт-Петербург, Россия
[2]Первый Санкт-Петербургский государственный медицинский университет имени академика И.П. Павлова, Санкт-Петербург, Россия

[3]Северо-Западный государственный медицинский университет имени И.И. Мечникова, Санкт-Петербург, Россия
[4]Военно-медицинская академия имени С.М. Кирова, Санкт-Петербург, Россия
[5]Красноярский научный центр Сибирского отделения Российской академии наук, Красноярск, Россия

Аннотация

Актуальность. Появление препаратов прямого противовирусного действия (ППВД) стало большим достижением в лечении пациентов с хроническим гепатитом С. Однако выявляются случаи отсутствия ответа на лечение. В 5% случаев наиболее вероятной причиной вирусологического прорыва являются мутации устойчивости к ППВД в геноме вируса гепатита С.

Цель работы — выявить мутации лекарственной устойчивости вируса гепатита С у пациентов с неэффективной терапией ППВД.

Материалы и методы. Материалом исследования служили образцы плазмы крови 3 пациентов с подтвержденным диагнозом хронического гепатита С с вирусологической неэффективностью терапии ППВД. Генотип всех изолятов — 1b. Применяли метод определения мутаций лекарственной устойчивости на основе прямого секвенирования генов *NS3*, *NS5A*, *NS5B*, разработанный в НИИЭМ им. Пастера.

Результаты. Мутации лекарственной устойчивости были выявлены во всех случаях. Согласно базе данных Geno2pheno [hcv] 0.92, нуклеотидные замены были определены в разных генах вируса и, предположительно, обусловливали устойчивость или снижение чувствительности в отношении препаратов, как входящих в состав комбинированной терапии софосбувир + даклатасвир, так и отсутствующих в ней. Неэффективность терапии противовирусными препаратами у пациентов с хроническим гепатитом С обусловлена мутациями лекарственной устойчивости.

Выводы. Разработанный метод позволяет выявлять мутации лекарственной устойчивости в генах *NS3*, *NS5A*, *NS5B* при вирусологической неэффективности терапии ППВД.

Ключевые слова: *вирус гепатита С, мутации лекарственной устойчивости, препараты прямого противовирусного действия*

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Introduction

The hepatitis C virus (HCV) is a leading cause of chronic liver disease[1]. Acute hepatitis develops approximately in 20% of cases after initial infection with HCV; around 15–45% of patients spontaneously clear the virus within 6 months of infection without treatment [1].

Absence of treatment and progression of the chronic process lead to replacement of the healthy liver tissue with fibrous tissue, to distortion of the parenchyma architecture, and, eventually, to liver fibrosis and cirrhosis in 25–30 years. Once the disease has progressed to the state of cirrhosis, the risk of developing hepatocellular carcinoma over a year is 1–5% [2].

Historically, combination therapy with pegylated interferon (weekly subcutaneous injections) and ribavirin (daily orally) during 24-48 weeks was the standard treatment of chronic hepatitis C (CHC). This treatment produced a sustained virological response (SVR) — a PCR undetectable viral load during the treatment, 12 weeks (SVR12) and 24 weeks (SVR24) after the treatment — in more than 50% of patients with HCV genotype 1 after the 12-month treatment and in more than 80% of patients with HCV genotypes 2 and 3 after the 6-month treatment [3].

The successful treatment strategy resulting in complete eradication of the virus can be limited both due to the presence of the therapeutically challenging HCV genotype and due to other factors. Such common side effects as flu-like, asthenic vegetative, and neurological syndromes, weight loss, leukopenia, and thrombocytopenia can lead to dosage reduction, specific medication therapy, and even to premature treatment termination. Higher treatment rates have been demonstrated by young people, female patients, Caucasians, and patients with a low HCV viral load in blood plasma and low levels of preexisting fibrosis [4]. Patients with clinically apparent liver cirrhosis demonstrated a successful response to interferon and ribavirin treatment in 21.5% of all cases and in 11.3% of cases with HCV genotype 1, thus proving low efficiency of treatment for the above cohort of patients [5]. Non-responding patients were recommended to switch to long-term interferon monotherapy, which decreased serum aminotransferase levels, though it did not eradicate the virus [6].

The advent of direct acting antivirals (DAAs) has revolutionized treatment of CHC patients. Interferon-free therapy has significantly increased SVR rates (over 90%), reduced the treatment duration (to 12 weeks), reduced the number of side effects (headaches and fatigue were most common among them), and permitted more convenient dosage regimens (once daily

1 WHO. Hepatitis C. Key facts. Available at: http://www.who.int/news-room/fact-sheets/detail/hepatitis-c

orally)[2]. Novel antivirals have demonstrated their efficiency in treatment of patients with liver cirrhosis and other complications, resulting in high SVR rates, which were difficult to achieve with the previous regimens [7].

The HCV genome encodes 4 structural proteins (core, E1, E2, P7) and 6 non-structural proteins (NS2, NS3, NS4A, NS4B, NS5A, NS5B) [8, 9]. Unlike interferon agents indirectly affecting replication and development of viral particles, DAAs directly target the virus and disrupt its life cycle.

Currently, there are 4 groups of antivirals targeting 3 viral non-structural proteins: NS3 protease inhibitors, NS5A replication complex inhibitors, NS5B RNA-polymerase nucleotide and non-nucleotide inhibitors [10]. In 2011, telaprevir and boceprevir, NS3 protease inhibitors, became the first approved DAAs [11]. Triple therapy with pegylated interferon, ribavirin and NS3 protease inhibitor improved treatment efficiency by 25% when administered to treatment-naïve patients with HCV genotype 1. At the same time, patients with liver cirrhosis and non-responders showed rather low SVR rates [12].

In Russia, therapeutic agents from all four groups of inhibitors are used in clinical practice. A combination of antivirals is the main strategy in treatment of CHC patients [13].

Pangenotypic sofosbuvir-based regimens are currently representing the main treatment options [14]. These regimens are most efficient for patients with HCV genotypes 1, 2 and 3, which are most typical of Russia[3]. In the meantime, some of the patients (around 5%) do not respond antiviral therapy. Failures can be associated with the phenomenon of virus resistance to antivirals [15]. According to some sources, 83% of treatment failure cases were characterized by a single or multiple resistance mutations found in genomic regions encoding DAA targeted proteins [16, 17].

The virus resistance is associated with nucleotide substitutions occurring randomly in HCV *NS3*, *NS5A*, *NS5B* regions during each replication cycle. Some substitutions prevent antiviral molecules from binding to their protein targets, thus making the virus resistant to the antiviral agent. Such substitutions are known as resistance-associated substitutions. Most of such mutations in the genome interfere with the ability of the wild-type virus to survive and reproduce under normal conditions; however, in DAA therapy, mutant strains have an advantage over the wild-type virus [18]. Detection of such mutations in patients who failed the treatment and in treatment-naïve patients is of critical importance.

At the moment, Russia and European countries do not have any routine test to detect DAA resistance mutations in HCV. Nevertheless, it is being developed in many countries, including Russia [19]. According to the EASL Recommendations for Treatment of Hepatitis C (2018), physicians who have easy access to reliable resistance tests can use these results to guide their decisions and select the further treatment strategies both for initial treatment and after relapse [15].

The purpose of the study is to detect drug resistance mutations in HCV patients who failed DAA treatment.

Materials and methods

The study was performed on plasma samples from 3 CHC patients who failed to respond the combination treatment with sofosbuvir + daclatasvir. The samples were provided by the Clinic of Infectious Diseases at the Kirov Military Medical Academy (St. Petersburg, Russia) and the hospital of the Krasnoyarsk Scientific Center of the Siberian Branch of the Russian Academy of Sciences in 2018. All the patients demonstrated virological treatment failure.

This study was performed at the HIV Infection Immunology and Virology Laboratory of the Pasteur Research Institute of Epidemiology and Microbiology in 2018–2019. The study was approved by the local ethics committee. All the patients gave written informed consent to participate in the study.

Biochemical values, METAVIR-scored fibrosis stage, viral loads and virus genotyping were assessed for all the patients. The data are shown in the **Table**.

Following the EASL Recommendations, all the patients were administered combination treatment with sofosbuvir, 400 mg + daclatasvir, 60 mg, 1 tablet taken once a day after a meal [15].

The treatment efficiency as well as SVR12 and SVR24 rates were assessed by measuring the viral load 12 and 24 weeks after the beginning of treatment. Highly sensitive tests did not detect any viral load in patient 1 during the screening performed 12 weeks after the start of antiviral therapy. The screening after 24 weeks showed a viral load of 1.1×10^6 copies/mL. During the screening performed 12 weeks after the beginning of treatment of patients 2 and 3, the viral load was 3.2×10^5 and 4.2×10^5 copies/mL, respectively.

The first analytical stage included assessment of the viral load and identification of the virus genotype

[2] State Register of Medicines. Full prescribing information for simeprevir. Available at: http://grls.rosminzdrav.ru/Grls_View_v2.aspx?routingGuid=17e9ef63-ea93-4c5a-8cc3-0e83bead0b61&t; State Register of Medicines. Full prescribing information for narlaprevir. Available at: http://grls.rosminzdrav.ru/Grls_View_v2.aspx?routingGuid=f60ec227-9db2-4c73-9b6a-fd1694cf11d6&t; State Register of Medicines. Full prescribing information for daclatasvir. Available at: http://grls.rosminzdrav.ru/Grls_View_v2.aspx?routingGuid=de1dd06c-e526-4e30-b8d8-794c2ade02f9&t; State Register of Medicines. Full prescribing information for sofosbuvir. Available at: http://grls.rosminzdrav.ru/Grls_View_v2.aspx?routingGuid=354729ed-4d65-4c32-9af1-4799a7104d2c&t

[3] WHO. Guidelines for the care and treatment of persons diagnosed with chronic hepatitis C virus infection 2018. Key facts. Available at: https://www.who.int/hepatitis/publications/hepatitis-c-guidelines-2018/

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Patients data

Data	Patient 1	Patient 2	Patient 3
Age, years	43	55	55
Sex	Male	Male	Male
ALT, U/liter	190	66	131,1
Total bilirubin, μmol/liter	29,5	5,9	7,7
Fibrosis stage (METAVIR)	F1	F1	F2
Viral load before treatment, copies/ml	$2,1 \times 10^6$	$1,6 \times 10^6$	$2,6 \times 10^6$
Genotype	1b	1b	1b
Treatment regimen	SOF+DAC	SOF+DAC	SOF+DAC

by using AmpliSens HCV-monitor-FL and AmpliSens HCV-genotype-FL reagent kits, respectively. The RNA was isolated from blood plasma by using the RIBO-prep kit for extraction of RNA/DNA from clinical materials (Central Research Institute of Epidemiology, Moscow).

The next stage included a reverse transcription reaction with the REVERTA-L reagent kit for cDNA synthesis from an RNA template (Central Research Institute of Epidemiology, Moscow).

Genotype-specific primers were used to obtain sequences of three viral regions with resistance-associated mutations: *NS3*, *NS5A*, and *NS5B*. The amplification products were purified and analyzed for the fragment size and concentration. The ABI Prism 3500 genetic analyzer (Applied Biosystems) was used to identify nucleotide sequences. The primary analysis of the obtained consensus nucleotide sequences was completed by using the NCBI BLAST program against the nucleotide sequences available in the international GenBank database. The fragments of *NS3*, *NS5A*, *NS5B* regions were assessed for presence of nucleotide substitutions causing drug resistance mutations; the assessment was performed with the Geno2pheno [resistance] 3.4 interpretation system[4].

Results

By using direct sequencing, we obtained satisfactory 800, 750 and 600 bp nucleotide sequences of target *NS3*, *NS5A*, *NS5B* genes for all 3 samples.

Phylogenetic relationships between the studied samples collected from CHC patients in Russia and the reference sequences from the international GenBank database are shown in the **Figure**. For the purpose of comparison, we used HCV nucleotide sequences of genotypes 1a, 1b, and 3a.

The dendogram analysis suggests that sequences of HCV *NS5B* gene of three genotypes cluster separately from each other. All three samples with virological failure are distributed on the neighboring tree branches belonging to HCV nucleotide sequences of genotype 1b, thus correlating with the genotypes previously identified with the help of the commercial test system.

The analysis of drug resistance mutations in all the patients showed the following drug resistance mutations:

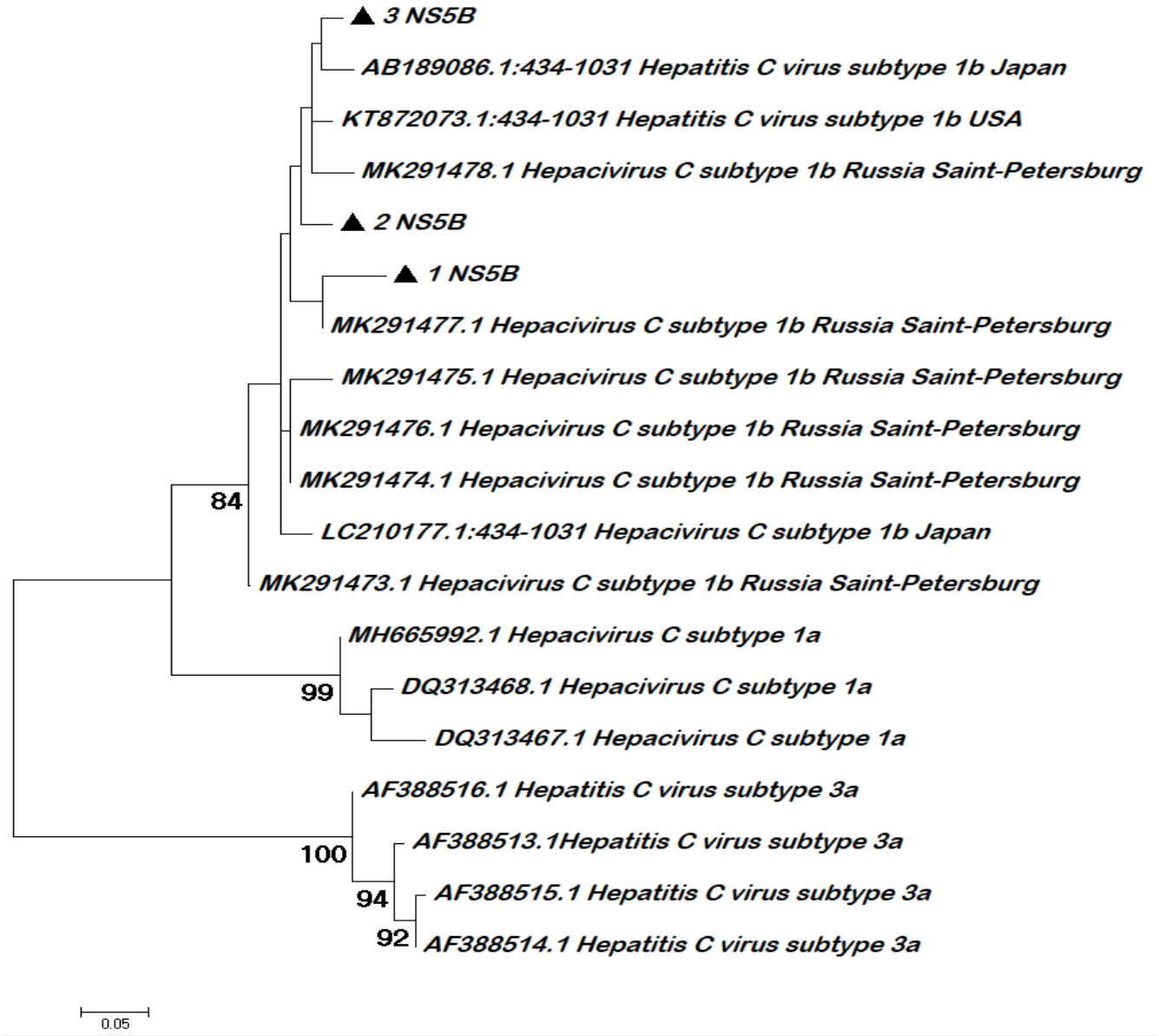
- patient 1: the *Y93H* mutation in the *NS5A* region; the mutation caused virus resistance to daclatasvir included in the regimen and to elbasvir, ledipasvir, ombitasvir, velpatasvir not included in the regimen;
- patient 2: the *L31V* mutation in the *NS5A* region resulted in decreased sensitivity to daclatasvir and ombitasvir;
- patient 3: similar to patient 2, the *L31V* nucleotide substitution was detected in the *NS5A* region. In addition, *L159F* and *Y56F* mutations were detected in the *NS5B* and *NS3* regions, respectively. The *L159F* nucleotide substitution causes a decrease in sensitivity to sofosbuvir. The *Y56F* nucleotide substitution in the *NS3* region causes a decrease in sensitivity to grazoprevir.

All the patients were administered combination treatment with glecaprevir (the *NS3* inhibitor) + pibrentasvir (the *NS5A* inhibitor), as no resistance mutations to them were detected. Patients 2 and 3 reached SVR12 and SVR24 by following the regimen. Patient 1 decided not to follow the recommendations and discontinue the treatment.

Discussion

Treatment of CHC with pegylated interferon and ribavirin is not a totally efficient method for eradication of the virus and it may cause a number of problems. The solution to some of them was found with the ad-

[4] Geno2pheno [hcv] 0.92.
Available at: https://hcv.geno2pheno.org/index.php



The phylogenetic analysis of nucleotide sequences of the HCV *NS5B* gene fragment from CHC patients in Russia as compared to the reference sequences available in the international GenBank database.
The samples collected from patients failing DAA treatment and examined in this study are marked with triangles.
The bootstrap value is ≥ 60%.

vent of direct-acting antivirals. Sofosbuvir is an inhibitor of NS5B protein RNA-dependent RNA polymerase. In sofosbuvir-containing regimens, therapeutic efficacy is achieved in more than 95% of cases [20]. Sofosbuvir is characterized by pangenotypic, high antiviral activity, thus being the best-choice medication for most patients. Daclatasvir is a direct-acting inhibitor of the HCV NS5A protein. The combination with sofosbuvir suppresses the viral replication by targeting two regions at a time, thus providing therapeutic efficacy in more than 90% of the patients [16].

DAA combination regimens including NS5A inhibitors are most relevant. According to the EASL Recommendations (2016), HCV resistance testing prior to treatment makes sense if the regimen includes NS5A inhibitors [21].

Despite the high barrier to resistance due to the combination of two antiviral agents, in all three cases, the treatment failure evidenced as detected viral RNA in the plasma during the treatment can indicate the developing dominance of the resistant strain over the wild-type virus during antiviral treatment and, consequently, the occurring resistance to the administered regimen. It is quite possible that resistance mutations of the circulating HCV strain existed in these patients before the beginning of treatment, thus being a cause

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of their non-response to the antiviral treatment. For patients 2 and 3, it is reasonable to assume that viral strains with DAA resistance mutations circulated before the treatment, thus explaining the non-response to the antiviral therapy and failure to achieve SVR12 rates. On the contrary, as SVR12 was achieved in patient 1, it can be assumed that the resistant HCV strain dominated over the wild type during the treatment. However, there is a risk of reinfection of this patient with another viral strain. Thus, when performed before the treatment, the sequencing of HCV genes, in which nucleotide substitutions are associated with DAA resistance, makes it possible to detect mutations to therapeutic agents planned for administration. The analysis of nucleotide sequences helps avoid administration of inadequate regimens for patients, thus being of importance for a health care facility or individual patients with no need to change the regimen. Furthermore, the HCV sequencing prior to treatment helps assess the phylogenetic relatedness of viral strains in case of a viral breakthrough, thus making it possible to differentiate between on-treatment reinfection and disease relapse.

HCV strains harboring *Y93H* polymorphism detected in patient 1 are associated with a high resistance level and are found in NS5A-inhibitor treatment-naïve patients [22]. Strains with this mutation can persist for a long time even after the DAA treatment is discontinued. Moreover, the above nucleotide substitution causes increased production of DAA resistant viruses, though it is associated with higher sensitivity to protease inhibitors. Thus, the detailed profile of HCV variants with the *Y93H* polymorphism is required for making an optimum choice of treatment strategy for CHC patients.

The treatment failure with first-line antivirals should be followed by the second-line pangenotypic DAA regimen. Russia has registered elbasvir,[5] ledipasvir,[6] ombitasvir,[7] and velpatasvir,[8] which are included in combination pangenotypic therapy [22]. The *Y93H* nucleotide substitution leads to cross-resistance to the above antivirals. Thus, some mutations leading to drug cross-resistance can cause failure of treatment of CHC patients when both the first-line therapy and alternative DAA treatment options are administered. In this case, the pressing problems are those of pharmacoeconomic nature, as there can be need to change between multiple regimens and there can be complications resulting from absence of positive effect of the antivirals.

In patients 2 and 3, the *L31V* single nucleotide substitution in the *NS5A* region decreases sensitivity to some inhibitors. However, the combination of *L31V* and *Q54L* mutations can enhance sensitivity to other DAAs [23].

In clinical study P7977-2025, 16 (26%) out of 61 patients showed no response to the sofosbuvir + ribavirin treatment after the liver transplantation. Six (37.5%) patients out of 16 did not achieve the SVR12 rate and, importantly, had *C316N* amino acid substitution detected in the virus. The other 45 (74%) patients achieved SVR12 and had no *C316N* substitution [24]. In clinical study NEUTRINO, 4 (6%) patients out of 66 had HCV isolates with *C316N* mutation before the treatment. Among them, 50% of the patient did not respond the sofosbuvir + ribavirin treatment. In the above study, 85% of patients with HCV strain without *C316N* mutation achieved SVR12 [20, 25]. Therefore, post-transplantation patients who need DAA treatment should be tested for HCV resistance to DAAs to eliminate both the risk of a viral breakthrough during the treatment and the risk of non-response to the treatment.

In addition to drug resistance-associated mutations, one polymorphic variant was detected in the *NS3* gene in patient 1, while patients 2 and 3 had 6 and 7, respectively. It should be noted that the *F147S* mutation was detected in all 3 cases, while *S7A*, *A66G*, *K87A*, and *F147S* mutations were detected in 2 out of 3 cases.

6 polymorphic variants were detected in the *NS5B* gene in patient 1, while patients 2 and 3 had 6 and 3 polymorphic variants, respectively. *S231N* and *R254K* mutations were detected in 2 out of 3 cases.

7 polymorphic variants were detected in the *NS5A* gene in patient 1, while patients 2 and 3 had 15 and 12 polymorphic variants, respectively. Notably, *K6R*, *S17T*, *L34V*, and *K44R* mutations were detected in all 3 cases, while *T64S*, *V164A*, *V174T*, and *L183V* mutations were detected in 2 out of 3 patients.

High genetic polymorphism is typical of all RNA-viruses, including HCV. Yet, the phenomenon of frequently occurring polymorphic variants needs further study, as such variants can cause both decreased sensitivity and decreased resistance to antivirals. The analysis of natural polymorphic patterns is highly important, as these patterns can potentially function as drug resistance mutations of the virus. With development of novel antivirals and novel regimens, attention should be given to frequently occurring nucleotide substitutions, which previously were not associated with resistance as drug resistance mutations. Statistical data on DAA treatment are being continuously updated and

[5] State Register of Medicines. Full prescribing information for elbasvir. Available at: https://grls.rosminzdrav.ru/Grls_View_v2.aspx?routingGuid=4d2cce52-e4e9-4349-acc8-73f0c84d52c3&t

[6] State Register of Medicines. Full prescribing information for ledipasvir. Available at: https://grls.rosminzdrav.ru/Grls_View_v2.aspx?routingGuid=74291100-1f06-4e05-9c49-a8ff0356170e&t

[7] State Register of Medicines. Full prescribing information for ombitasvir. Available at: https://grls.rosminzdrav.ru/Grls_View_v2.aspx?routingGuid=ad5159a1-91c1-42ec-aec6-e65d4e5313dd&t

[8] State Register of Medicines. Full prescribing information for velpatasvir. Available at: https://grls.rosminzdrav.ru/Grls_View_v2.aspx?routingGuid=c9275dea-5954-42ea-97c1-cd9ae05ccfbe&t

it is quite possible that a weaker response or non-response to existing antivirals can be caused by the mutations that are still not associated with drug resistance or by new mutations.

Although sofosbuvir-containing regimens are characterized by a high barrier to genetic resistance, treatment failures are still reported as caused by drug resistance mutations in one or several regions of the virus. The lack of routine HCV tests for resistance to antiviral agents not only aggravates the problem of optimum regimen selection, but also involves pharmacoeconomic aspects when other regimens have be administered after failed treatment with first-line antivirals. Such clinical cases emphasize the importance of the test for resistance to administered antivirals in case of a viral breakthrough and virological failure as well as before the administration of treatment.

The level of resistance to antivirals depends on several factors: The genetic barrier to drug resistance; the type of nucleotide substitution in the viral genome; the occurrence rate of the resistance-associated substitution in the virus population; existing combinations of nucleotide substitutions, which are present in the virus population. These factors can significantly increase the resistance to some treatment methods [26]. The high mutation rate and the speed of replication of RNA-containing viruses underlie the genetic diversity of the virus pool persisting in an individual. Therefore, mutations, including drug resistance-associated mutations, can be spontaneously generated in any patient. Thus, the pool of closely related virus variants known as quasispecies can contain a small population of drug-resistant variants before the beginning of antiviral treatment [27].

The Sanger sequencing method is efficient in detecting a major virus variant (a quasispecies), which prevails in the virus population of the studied clinical sample, though it does not detect minor genomes of drug-resistant quasispecies present in the sample and accounting for less than 15% of the total virus pool, while the next-generation sequencing provides highly reliable identification of minor mutations accounting for 1–2% of the population, helps assess the occurrence rate for each mutation in the virus population, and evaluates the possibility of several mutations combined in one viral genome. This sequencing method can be important for treatment efficiency and for treatment strategies [28]. Nevertheless, direct sequencing is more affordable and economically feasible, especially when working with individual patients in the population where the level of drug resistance of the virus to antiviral agents is low.

Sequencing of viral genome regions will not only provide data on exiting resistance mutations, but also will be important for analyzing epidemiological situations in and outside the country through identification of virus genotypes and subgenotypes as well as through the phylogenetic analysis [29].

Conclusion

The development of a universal test for detecting DAA resistance in HCV is of high importance both for administration of treatment and for any changes in the treatment, if required.

REFERENCES

1. Zabala V., Tong M., Yu R., Ramirez T., Yalcin E.B., Balbo S., et al. Potential contributions of the tobacco nicotine-derived nitrosamine ketone (NNK) in the pathogenesis of steatohepatitis in a chronic plus binge rat model of alcoholic liver disease. *Alcohol Alcohol.* 2015; 50(2): 118–31. https://doi.org/10.1093/alcalc/agu083
2. Bertino G., Ardiri A., Proiti M., Rigano G., Frazzetto E., Demma S., et al. Chronic hepatitis C: This and the new era of treatment. *World J. Hepatol.* 2016; 8(2): 92–106. https://doi.org/10.4254/wjh.v8.i2.92
3. Vogel W. Treatment of chronic hepatitis C patients not responding to combination therapy with ribavirin and interferon alpha — hype or hope? *Wien. Klin. Wochenschr.* 2004; 116(15-16): 508–10. https://doi.org/10.1007/bf03217702
4. Strader D.B., Seeff L.B. A brief history of the treatment of viral hepatitis C. *Clin. Liver Dis. (Hoboken).* 2012; 1(1): 6–11. https://doi.org/10.1002/cld.1
5. Esmembetov K.I., Esmembetova N.I. Antiviral therapy of liver cirrhosis due to chronic hepatitis C with interferon. *Klinicheskaya meditsina Kazakhstana.* 2015; (4): 21–34. (in Russian)
6. Koike K. Antiviral treatment of hepatitis C: present status and future prospects. *J. Infect. Chemother.* 2006; 12(5): 227–32. https://doi.org/10.1007/s10156-006-0460-0
7. Ivashkin V.T., Maevskaya M.V., Abdurakhmanov D.T., Bakulin I.G., Geyvandova N.I., Zubkin M.L., et al. The modern options of chronic hepatitis C antiviral therapy with daclatasvir: results of named patient program. *Rossiyskiy zhurnal gastroenterologii, gepatologii, koloproktologii.* 2017; 27(6): 52–62. (in Russian)
8. Hijikata M., Kato N., Ootsuyama Y., Nakagawa M., Shimotohno K. Gene-mapping of the putative structural region of the hepatitis C virus genome by in vitro processing analysis. *Proc. Natl. Acad. Sci. USA.* 1991; 88(13): 5547–51. https://doi.org/10.1073/pnas.88.13.5547
9. Lin C., Lindenbach B.D., Pragai B.M., Mccourt D.W., Rice C.M. Processing in the hepatitis C virus E2-2 region: identification of p7 and 2 distinct e2-specific products with different C termini. *J. Virol.* 1994; 68(8): 5063–73. https://doi.org/10.1128/jvi.68.8.5063-5073.1994
10. Aghemo A., De Francesco R. New horizons in hepatitis C antiviral therapy with direct-acting antivirals. *Hepatology.* 2013; 58(1): 428–38. https://doi.org/10.1002/hep.26371
11. Sarrazin C., Kieffer T.L., Bartels D., Hanzelka B., Müh U., Welker M., et al. Dynamic hepatitis C virus genotypic and phenotypic changes in patients treated with the protease inhibitor telaprevir. *Gastroenterology.* 2007; 132(5): 1767–77. https://doi.org/10.1053/j.gastro.2007.02.037
12. Lin C., Kwong A.D., Perni R.B. Discovery and development of VX950, a novel, covalent, and reversible inhibitor of hepatitis C virus NS3.4A serine protease. *Infect. Disord. Drug Targets.* 2006; 6(1): 3–16. https://doi.org/10.2174/187152606776056706
13. Blokhina N.P., Malyshev N.A., Nurmukhametova E.A. Hepatitis C treatment: state-of-the-art and prospection. *Tuberkulez i znachimye infektsii.* 2013; (1): 73–8. (in Russian)
14. Burnevich E.Z., Nikulkina E.N., Filatova A.L. Sofosbuvir-based antiviral regimens for chronic hepatitis C in Russia. *Klinicheskaya farmakologiya i terapiya.* 2018; 27(2): 10–7. (in Russian)
15. European Association for the Study of the Liver. EASL recommendations on treatment of hepatitis C 2018. *J. Hepatol.* 2018; 69(2): 461–511. https://doi.org/10.1016/j.jhep.2018.03.026
16. Vermehren J., Susser S., Dietz J., Von Hahn T., Petersen J., et al. Retreatment of patients who failed DAA-combination therapies: real-world experience from a large hepatitis C resistance, database. *Hepatology.* 2016; 64(2): 188.
17. Kichatova V.S., Kyuregyan K.K. Modern view on resistance to direct antiviral drugs in the treatment of viral hepatitis C: analytical review. *Infektsionnye bolezni: novosti, mneniya, obuchenie.* 2019; 8(2): 64–71. https://doi.org/10.24411/2305-3496-2019-12009 (in Russian)
18. Feld J.J. Resistance testing: Interpretation and incorporation into HCV treatment algorithms. *Clin. Liver Dis. (Hoboken).* 2017; 9(5): 115–20. https://doi.org/10.1002/cld.631
19. Valutite D.E. Testing the molecular genetic method for detecting mutations in the drug resistance of hepatitis C virus. In: *Collection of Abstracts of the VIII International Youth Medical Congress «St. Petersburg Scientific Readings» [Sbornik tezisov VIII mezhdunarodnogo molodezhnogo meditsinskogo kongressa «Sankt-Peterburgskie nauchnye chteniya»].* St. Peretsburg; 2019: 180. (in Russian)
20. Lawitz E., Mangia A., Wyles D., Rodriguez-Torres M., Hassanein T., Gordon S.C., et al. Sofosbuvir for previously untreated chronic hepatitis C infection. *N. Engl. J. Med.* 2013; 368(20): 1878–87. https://doi.org/10.1056/nejmoa1214853
21. European Association for the Study of the Liver. EASL recommendations on treatment of hepatitis C 2016. *J. Hepatol.* 2017; 66(1): 153–94. https://doi.org/10.1016/j.jhep.2016.09.001
22. Nitta S., Asahina Y., Matsuda M., Yamada N., Sugiyama R., Masaki T., et al. Effects of resistance-associated NS5A mutations in hepatitis C virus on viral production and susceptibility to antiviral reagents. *Sci. Rep.* 2016; 6: 34652. https://doi.org/10.1038/srep34652
23. Lemm J.A., O'Boyle D., Liu M., Nower P.T., Colonno R., Deshpande M.S., et al. Identification of hepatitis C virus NS5A inhibitors. *J. Virol.* 2010; 84(1): 482–91. https://doi.org/10.1128/jvi.01360-09
24. Charlton M., Gane E., Manns M.P., Brown R.S., Curry M.P., Kwo P.Y., et al. Sofosbuvir and ribavirin for treatment of compensated recurrent hepatitis C virus infection after liver transplantation. *Gastroenterology.* 2015; 148(1): 108–17. https://doi.org/10.1053/j.gastro.2014.10.001
25. Wang Y., Rao H.Y., Xie X.W., Wei L. Direct-acting antiviral agents resistance-associated polymorphisms in chi-

nese treatment-naïve patients infected with genotype 1b hepatitis C virus. *Chin. Med. J. (Engl).* 2015; 128(19): 2625–31.
https://doi.org/10.4103/0366-6999.166038
26. Amer F., Yousif M.M., Hammad N.M., Garcia-Cehic D., Gregori J., Rando-Segura A., et al. Deep-sequencing study of HCV G4a resistance-associated substitutions in Egyptian patients failing DAA treatment. *Infect. Drug Resist*. 2019; 12: 2799–807.
https://doi.org/10.2147/idr.s214735
27. Bellocchi M.C., Aragri M., Carioti L., Fabeni L., Pipitone R.M., Brancaccio G., et al. NS5A gene analysis by next generation sequencing in HCV nosocomial transmission clusters of HCV genotype 1b infected patients. *Cells*. 2019; 8(7): 666.
https://doi.org/10.3390/cells8070666
28. Georg von Massow G., Garcia-Cehic D., Gregori J., Rodriguez-Frias F., Macià M.D., Escarda A., et al. Whole-genome characterization and resistance-associated substitutions in a new HCV genotype 1 subtype. *Infect. Drug Resist*. 2019; 12: 947–55.
https://doi.org/10.2147/idr.s195441
29. Semenov A.V., Ostankova Yu.V., Gerasimova V.V., Bichurina M.A., Kozlov A.V., Mukomolov S.L., et al. Molecular and epidemiological features of hepatitis C virus isolates from different regions of the Republic of Sakha (Yakutia). *Infektsiya i immunitet.* 2015; 5(4): 359–72. (in Russian)

СПИСОК ИСТОЧНИКОВ

1. Zabala V., Tong M., Yu R., Ramirez T., Yalcin E.B., Balbo S., et al. Potential contributions of the tobacco nicotine-derived nitrosamine ketone (NNK) in the pathogenesis of steatohepatitis in a chronic plus binge rat model of alcoholic liver disease. *Alcohol Alcohol.* 2015; 50(2): 118–31.
https://doi.org/10.1093/alcalc/agu083
2. Bertino G., Ardiri A., Proiti M., Rigano G., Frazzetto E., Demma S., et al. Chronic hepatitis C: This and the new era of treatment. *World J. Hepatol.* 2016; 8(2): 92–106.
https://doi.org/10.4254/wjh.v8.i2.92
3. Vogel W. Treatment of chronic hepatitis C patients not responding to combination therapy with ribavirin and interferon alpha — hype or hope? *Wien. Klin. Wochenschr.* 2004; 116(15-16): 508–10.
https://doi.org/10.1007/bf03217702
4. Strader D.B., Seeff L.B. A brief history of the treatment of viral hepatitis C. *Clin. Liver Dis. (Hoboken).* 2012; 1(1): 6–11.
https://doi.org/10.1002/cld.1
5. Есмембетов К.И., Есмембетова Н.И. Противовирусная терапия цирроза печени в исходе хронического гепатита С препаратами интерферона. *Клиническая медицина Казахстана.* 2015; (4): 21–34.
6. Koike K. Antiviral treatment of hepatitis C: present status and future prospects. *J. Infect. Chemother.* 2006; 12(5): 227–32.
https://doi.org/10.1007/s10156-006-0460-0
7. Ивашкин В.Т., Маевская М.В., Абдурахманов Д.Т., Бакулин И.Г., Гейвандова Н.И., Зубкин М.Л. и др. Современные возможности противовирусной терапии с использованием даклатасвира при лечении больных хроническим вирусным гепатитом С: результаты программы индивидуального доступа. *Российский журнал гастроэнтерологии, гепатологии, колопроктологии.* 2017; 27(6): 52–62.
8. Hijikata M., Kato N., Ootsuyama Y., Nakagawa M., Shimotohno K. Gene-mapping of the putative structural region of the hepatitis C virus genome by invitro processing analysis. *Proc. Natl. Acad. Sci. USA.* 1991; 88(13): 5547–51.
https://doi.org/10.1073/pnas.88.13.5547
9. Lin C., Lindenbach B.D., Pragai B.M., Mccourt D.W., Rice C.M. Processing in the hepatitis C virus E2-2 region: identification of p7 and 2 distinct e2-specific products with different C termini. *J. Virol.* 1994; 68(8): 5063–73.
https://doi.org/10.1128/jvi.68.8.5063-5073.1994
10. Aghemo A., De Francesco R. New horizons in hepatitis C antiviral therapy with direct-acting antivirals. *Hepatology.* 2013; 58(1): 428–38.
https://doi.org/10.1002/hep.26371
11. Sarrazin C., Kieffer T.L., Bartels D., Hanzelka B., Müh U., Welker M., et al. Dynamic hepatitis C virus genotypic and phenotypic changes in patients treated with the protease inhibitor telaprevir. *Gastroenterology.* 2007; 132(5): 1767–77.
https://doi.org/10.1053/j.gastro.2007.02.037
12. Lin C., Kwong A.D., Perni R.B. Discovery and development of VX950, a novel, covalent, and reversible inhibitor of hepatitis C virus NS3.4A serine protease. *Infect. Disord. Drug Targets.* 2006; 6(1): 3–16.
https://doi.org/10.2174/187152606776056706
13. Блохина Н.П., Малышев Н.А., Нурмухаметова Е.А. Лечение гепатита С: настоящее и будущее. *Туберкулез и значимые инфекции.* 2013; (1): 73–8.
14. Бурневич Э.З., Никулкина Е.Н., Филатова А.Л. Софосбувир-содержащие схемы противовирусной терапии хронического гепатита С, актуальные в Российской Федерации в 2018 г. *Клиническая фармакология и терапия.* 2018; 27(2): 10–7.
15. European Association for the Study of the Liver. EASL recommendations on treatment of hepatitis C 2018. *J. Hepatol.* 2018; 69(2): 461–511. https://doi.org/10.1016/j.jhep.2018.03.026
16. Vermehren J., Susser S., Dietz J., Von Hahn T., Petersen J., et al. Retreatment of patients who failed DAA-combination therapies: real-world experience from a large hepatitis C resistance, database. *Hepatology.* 2016; 64(2): 188.
17. Кичатова В.С., Кюрегян К.К. Современный взгляд на резистентность к препаратам прямого противовирусного действия при лечении вирусного гепатита С. *Инфекционные болезни: новости, мнения, обучение.* 2019; 8(2): 64–71.
https://doi.org/10.24411/2305-3496-2019-12009
18. Feld J.J. Resistance testing: Interpretation and incorporation into HCV treatment algorithms. *Clin. Liver Dis. (Hoboken).* 2017; 9(5): 115–20.
https://doi.org/10.1002/cld.631
19. Валутите Д.Э. Апробация молекулярно-генетического метода выявления мутаций лекарственной устойчивости вируса гепатита С. В кн.: *Сборник тезисов VIII международного молодежного меди-*

цинского конгресса «Санкт-Петербургские научные чтения». СПб.; 2019: 180.
20. Lawitz E., Mangia A., Wyles D., Rodriguez-Torres M., Hassanein T., Gordon S.C., et al. Sofosbuvir for previously untreated chronic hepatitis C infection. *N. Engl. J. Med.* 2013; 368(20): 1878–87. https://doi.org/10.1056/nejmoa1214853
21. European Association for the Study of the Liver. EASL recommendations on treatment of hepatitis C 2016. *J. Hepatol.* 2017; 66(1): 153–94. https://doi.org/10.1016/j.jhep.2016.09.001
22. Nitta S., Asahina Y., Matsuda M., Yamada N., Sugiyama R., Masaki T., et al. Effects of resistance-associated NS5A mutations in hepatitis C virus on viral production and susceptibility to antiviral reagents. *Sci. Rep.* 2016; 6: 34652. https://doi.org/10.1038/srep34652
23. Lemm J.A., O'Boyle D., Liu M., Nower P.T., Colonno R., Deshpande M.S., et al. Identification of hepatitis C virus NS5A inhibitors. *J. Virol.* 2010; 84(1): 482–91. https://doi.org/10.1128/jvi.01360-09
24. Charlton M., Gane E., Manns M.P., Brown R.S., Curry M.P., Kwo P.Y., et al. Sofosbuvir and ribavirin for treatment of compensated recurrent hepatitis C virus infection after liver transplantation. *Gastroenterology*. 2015; 148(1): 108–17. https://doi.org/10.1053/j.gastro.2014.10.001
25. Wang Y., Rao H.Y., Xie X.W., Wei L. Direct-acting antiviral agents resistance-associated polymorphisms in chinese treatment-naïve patients infected with genotype 1b hepatitis C virus. *Chin. Med. J. (Engl).* 2015; 128(19): 2625–31. https://doi.org/10.4103/0366-6999.166038
26. Amer F., Yousif M.M., Hammad N.M., Garcia-Cehic D., Gregori J., Rando-Segura A., et al. Deep-sequencing study of HCV G4a resistance-associated substitutions in Egyptian patients failing DAA treatment. *Infect. Drug Resist*. 2019; 12: 2799–807. https://doi.org/10.2147/idr.s214735
27. Bellocchi M.C., Aragri M., Carioti L., Fabeni L., Pipitone R.M., Brancaccio G., et al. NS5A gene analysis by next generation sequencing in HCV nosocomial transmission clusters of HCV genotype 1b infected patients. *Cells*. 2019; 8(7): 666. https://doi.org/10.3390/cells8070666
28. Georg von Massow G., Garcia-Cehic D., Gregori J., Rodriguez-Frias F., Macià M.D., Escarda A., et al. Whole-genome characterization and resistance-associated substitutions in a new HCV genotype 1 subtype. *Infect. Drug Resist*. 2019; 12: 947–55. https://doi.org/10.2147/idr.s195441
29. Семенов А.В., Останкова Ю.В., Герасимова В.В., Бичурина М.А., Козлов А.В., Мукомолов С.Л. и др. Молекулярно-эпидемиологические особенности изолятов вируса гепатита с из разных регионов Республики Саха (Якутия). *Инфекция и иммунитет*. 2015; 5(4): 359–72.

Information about the authors

Diana E. Valutite✉ — doctor of clinical laboratory diagnostics, Department for diagnosing HIV infection and AIDS-related diseases, St. Petersburg Pasteur Institute, St. Petersburg, Russia, dianavalutite008@gmail.com, https://orcid.org/0000-0002-0931-102X

Aleksandr V. Semenov — D. Sci. (Biol.), Head, Laboratory of virology and immunology of HIV, Deputy director for innovation, St. Petersburg Pasteur Institute; Assoc. Prof., Department of immunology, Pavlov First St. Petersburg State Medical University; Assoc. Prof., Department of clinical laboratory diagnostics, North-West State Medical University named after I.I. Mechnikov, St. Petersburg, Russia, https://orcid.org/0000-0003-3223-8219

Yulia V. Ostankova — PhD (Biol.), senior researcher, Laboratory of molecular immunology, St. Petersburg Pasteur Institute, St. Petersburg, Russia, https://orcid.org/0000-0003-2270-8897

Konstantin V. Kozlov — D. Sci. (Med.), Assoc. Prof., Department of infectious diseases (with a course of medical parasitology and tropical diseases), S.M. Kirov Military Medical Academy, St. Petersburg, Russia, https://orcid.org/0000-0002-4398-7525

Aleksandr G. Borisov — PhD (Med.), leading researcher, Laboratory of molecular cell physiology and pathology, Scientific Research Institute of Medical Problems of the North, Krasnoyarsk, Russia; Assoc. Prof., Department of infectious diseases, Krasnoyarsk State Medical University named after Prof. V.F. Voino-Yasenetsky, Krasnoyarsk, Russia, https://orcid.org/0000-0002-9026-2615

Vladimir D. Nazarov — PhD (Med.), research fellow, Laboratory of autoimmune diagnostics, Center for molecular medicine, Academician I.P. Pavlov First St. Petersburg State Medical University, St. Petersburg, Russia, https://orcid.org/0000-0002-9354-8790

Areg A. Totolian — D. Sci. (Med.), Prof., Academician of RAS, Head, Laboratory of molecular immunology, Director, St. Petersburg Pasteur Institute, St. Petersburg, Russia; Head, Department of immunology, Academician I.P. Pavlov First St. Petersburg State Medical University, St. Petersburg, Russia, https://orcid.org/0000-0003-4571-8799

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Информация об авторах

Валутите Диана Эдуардовна✉ — врач клинической лабораторной диагностики отделения ВИЧ-инфекции и СПИД-ассоциированных заболеваний НИИЭМ им. Пастера, Санкт-Петербург, Россия, dianavalutite008@gmail.com, https://orcid.org/0000-0002-0931-102X

Семенов Александр Владимирович — д.б.н., зав. лаб. иммунологии и вирусологии ВИЧ-инфекции, зам. директора по инновационной работе НИИЭМ им. Пастера; доцент каф. иммунологии ПСПбГМУ им. акад. И.П. Павлова; доцент каф. клинической лабораторной диагностики СЗГМУ им. И.И. Мечникова, Санкт-Петербург, Россия, https://orcid.org/0000-0003-3223-8219

Останкова Юлия Владимировна — к.б.н., с.н.с. лаб. молекулярной иммунологии НИИЭМ им. Пастера, Санкт-Петербург, Россия, https://orcid.org/0000-0003-2270-8897

Козлов Константин Вадимович — д.м.н., доц. каф. инфекционных болезней (с курсом медицинской паразитологии и тропических заболеваний) ВМА им. С.М. Кирова, Санкт-Петербург, Россия, https://orcid.org/0000-0002-4398-7525

Борисов Александр Геннадьевич — к.м.н., в.н.с. лаб. молекулярно-клеточной физиологии и патологии НИИ медицинских проблем Севера, Красноярск, Россия; доц. каф. инфекционных болезней КрасГМУ им. проф. В.Ф. Войно-Ясенецкого, Красноярск, Россия, https://orcid.org/0000-0002-9026-2615

Назаров Владимир Дмитриевич — к.м.н., н.с. лаб. диагностики аутоиммунных заболеваний НМЦ молекулярной медицины ПСПбГМУ им. акад. И.П. Павлова, Санкт-Петербург, Россия, https://orcid.org/0000-0002-9354-8790

Тотолян Арег Артёмович — д.м.н., проф., акад. РАН, зав. лаб. молекулярной иммунологии, директор НИИЭМ им. Пастера; зав. каф. иммунологии ПСПбГМУ им. акад. И.П. Павлова, Санкт-Петербург, Россия, https://orcid.org/0000-0003-4571-8799

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