

## NAT TECHNOLOGY – A STEP FORWARD IN HEPATITIS C SCREENING IN BULGARIA

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### ABSTRACT

**INTRODUCTION:** Globally, blood transfusions are the cause of transmissible infections in between 10% and 15% of all patients. Currently, the testing of donor blood for transmissible infections is utilizing enzyme-linked immunosorbent assay ELISA and chemiluminescence method CLIA for screening and detection of hepatitis C (HCV Ag/Ab). Since February, 2020 all donor samples of blood taken in the 5 centers for transfusion hematology in Bulgaria have been tested for markers of transmissible infections, including HCV, by nucleic acid testing (NAT).

**AIM:** We have set the goal to analyze data produced by the implementation of NAT testing of donor blood for the period of 4 months (from 10th of February to 16th of June, 2020) regarding HCV infected samples.

**MATERIALS AND METHODS:** We present our experience of using NAT and data produced by its implementation for testing of donor blood for a period of 4 months, from the time NAT technology was implemented in Bulgaria.

**RESULTS AND DISCUSSION:** The performance of the multiplex Procleix Ultrio Elite assay as individual donor nucleic acid test for the detection of HCV is evaluated in a retrospective study. An advantage of the method is that it not only allows for timely identification of infected donor blood and saves lives of the recipients but also saves the lives of donors, as treatment of chronic hepatitis C continues to progress and is now effective in clearing the virus in more than half of the patients. In terms of safety and security of diagnosis and blood products obtained, the method has no alternative.

**CONCLUSION:** A recommendation is drafted: to adopt a strategy to increase the quality of healthcare by introducing a one-time HCV screening for adults aged 18 and above, for individuals at risk and for pregnant women to reduce further the risk of incidental transmission of the hepatitis C virus.

**Keywords:** *nucleic acid testing (NAT), hepatitis C, donor blood*

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### INTRODUCTION

Hepatitis C is a serious viral infection caused by the hepatitis C virus. It is one of the most serious diseases of the liver, with about 170 million people chronically infected worldwide and more than 350 000 dying each year from hepatitis C disease. Like hepatitis B, hepatitis C is also often referred to as the “silent killer” because people can live with the dis-

ease for years without knowing they are infected and contagious. In Europe, about 15 million people live with chronic HCV. A higher percentage of morbidity has been reported in Southern and Eastern Europe (1). The morbidity rates of acute HCV infections for the period 2000-2007 in Bulgaria were 1.52 cases per 100000 population, and for the period 2008-2016 - 1.14 cases per 100000 population (2). In Bulgaria, 110 000 people are infected with the hepatitis C virus, with only 0.3-0.5% being treated.

Initially, hepatitis C is acute, but often becomes chronic. Acute hepatitis C either resolves quickly without leaving permanent liver damage or becomes chronic. Most patients do not notice symptoms during the acute phase or the symptoms are often confused and people can live with the virus for many years before realizing they are infected. Between 15 and 45% of those infected with the virus are completely cleared of it during the acute phase. Chronic hepatitis C is a long-term liver infection that develops in 55-85% of patients with acute hepatitis C, and can lead to serious liver disease, including cirrhosis and liver cancer, thus being among the leading causes of liver transplants. A total of 20% of those infected develop cirrhosis of the liver. Unlike hepatitis B, there is currently no hepatitis C vaccine (1).

The latest global hepatitis report shows that only 20% of people living with HCV are aware of their status and that only 8% of those diagnosed with HBV and HCV infections received antiviral therapy (3).

A survey published recently showed that existing testing policies and practices may result in missed opportunities to diagnose both HBV and HCV due to lack of screening in most countries (4). In Bulgaria, those infected are on average twice less likely to be diagnosed as compared to the rest of Europe. The problem groups are people who inject drugs, patients on hemodialysis, HIV-positive, recipients of contaminated blood products, donated blood, or donated organs in the period before 1992, or infected by non-sterile medical appliances. Approximately 2.3 million of those in Europe infected with hepatitis C, are also infected with HIV.

Globally, blood transfusions are the cause of transmissible infections in between 10% and 15% of all patients. In Bulgaria, according to the annu-

al analyses of acute infectious diseases of NCIPD for 2015, 2016, and 2017 (<https://ncipd.org>), patients with hepatitis B and hepatitis C due to blood transfusion are on average 12% of all cases for which there are established data on the cause of the infection (for 2015 they are 13%, for 2016 - 14%, for 2017 - 9%). These data confirm the global incidence trend and prove that, although in many cases life-saving, blood transfusion carries risks that must be adequately addressed.

Diagnosis of blood and blood components for markers of transmissible infections is performed in the centers for transfusion hematology, according to the rules of good laboratory practice and in compliance with the requirements of the existing legislation. Only reagents authorized for use in the Republic of Bulgaria are used to test the blood taken. Until the end of 2019 each unit of blood taken is tested by mandatory serological tests for HIV-1, HIV-2, hepatitis B and hepatitis C. Currently, the diagnosis of donor blood for transmissible infections is utilizing enzyme-linked immunosorbent assay ELISA and chemiluminescence method CLIA for screening and detection of hepatitis C (HCV Ag/Ab). Since February 2020 all donor samples of blood taken in the 5 centers for transfusion hematology in Bulgaria, namely National Center for Transfusion Hematology (NCRH), Regional Center for Transfusion Hematology Plovdiv (RCTH Plovdiv), Regional Center for Transfusion Hematology Stara Zagora (RCTH Stara Zagora), Regional Center for Transfusion Hematology Pleven (RCTH Pleven) and Regional Center for Transfusion Hematology Varna (RCTH Varna), have been tested for the above markers of transmissible infections, including HCV, by nucleic acid testing (NAT). The main advantage of the new technology is the shortening of the so-called "window period" (the time from infection of the donor to the time of detection of this infection in the donor blood). NAT testing of donor blood for transmissible infections is without alternative in the increase of the safety of blood and blood components for transfusion and plasma for drug production. At the same time, the method leads to significant saving of financial resources and ensures that the highest standards for donor blood products available currently worldwide are met.

## AIM

With this respect we have set the goal to analyze data produced by the implementation of NAT testing of donor blood for the period of 4 months (from 10<sup>th</sup> of February to 16<sup>th</sup> of June, 2020) regarding HCV infected samples, since NAT technology was implemented in five big centers for transfusion hematology in Bulgaria. The performance of the multiplex Procleix Ultrio Elite assay as individual donor nucleic acid test for the detection of HCV was evaluated in a retrospective study.

## MATERIAL AND METHODS

### *Blood Donations*

A total of 49 390 specimens from blood donors were examined in the period from February 10<sup>th</sup>, 2020 to 16<sup>th</sup> of June, 2020. The results were provided by five regional blood transfusion centers in Bulgaria: National Center for Transfusion Hematology (NCRH), Regional Center for Transfusion Hematology Plovdiv (RCTH Plovdiv), Regional Center for Transfusion Hematology Stara Zagora (RCTH Stara Zagora), Regional Center for Transfusion Hematology Pleven (RCTH Pleven) and Regional Center for Transfusion Hematology Varna (RCTH Varna). Blood donors in Bulgaria meet certain eligibility criteria specified by national guidelines.

### *Screening Methods*

Testing of blood samples for HCV uses serologic assays that detect human antibodies generated as a response to HCV (anti-HCV) or/and HCV antigen. Donor blood samples were tested with enzyme-linked immunosorbent assay (ELISA) or immunoenzymatic assay (EIA) using third and fourth generation diagnostic kits implemented in the national blood transfusion centers and following the guidelines of the manufacturers: qualitative enzyme immunoassay Monolisa HCV Ag-Ab ULTRA V2 (Bio-Rad, France) based on the detection of anti-HCV antibodies and capsid antigen in serum or human plasma; Architect Anti-HCV (Abbot, Germany) chemiluminescent microparticle immunoassay (CMIA) for the qualitative detection of anti-HCV antibodies, and CMIA-based Architect HCV Ag assay (Abbot, Germany) for the quantitative determination of HCV core antigen in human serum and plasma. The following automated instruments were used for the analyses: Architect Anti-HCV Ag core (ABBOTT,

Wiesbaden, Germany), ETI-Max 3000 (DiaSorin, Italy), Evolis System (Bio-Rad Laboratories, France), and Elisys Quattro (HUMAN Diagnostics, Germany). All reactive samples were tested at least twice and anti-HCV or HCV Ag positive were tested by alternative NAT using Procleix Panther System (Grifols, Spain), and the qualitative transcription-mediated amplification multiplex Procleix Ultrio Elite assay as an individual donor RNA test for the detection of combined signals for HCV, HBV, and HIV in individual donor blood samples was applied. Positively reactive samples resulting from the combinatory NAT analysis are tested by discriminatory Procleix Ultrio Elite test for HCV RNA. Sera were diagnosed positive with viral hepatitis C by at least one of the applied detection methods: antibodies against the hepatitis C antigen, hepatitis C antigens or hepatitis C RNA, and after a confirmatory testing in the national reference laboratory. Donor blood testing positive for HCV is discarded and the donor identification number is entered into the national information system, which automatically excludes these donors from the blood donors' database. If such individual is identified during the screening for eligibility at any of the regional centers for transfusion hematology, he would be automatically eliminated as a potential blood donor.

### *Statistical Analysis*

The collected data were organized and analyzed using GraphPad Prism 6.0 software. Qualitative data were presented as frequency and percentage.

## RESULTS AND DISCUSSION

In the period from 10.02.2020 to 16.06.2020 a total of 52 of all tested samples were confirmed positive for anti-HCV by the national reference laboratory, among them one old sample from year 2015. Nine samples were positive as determined only by serological tests made in 2020 prior to the introduction of NAT testing in the national blood transfusion system, with a reference conformational test produced during the study period. All other 42 blood donor samples were analyzed by both serological and molecular NAT tests with an at least one positive result for hepatitis C by either of the test types. Two of the donor blood samples reported to the national information system were just below the cut-off value of the HCV assay and these were listed among the

HCV positive and were also excluded from the blood bank. One of the blood samples was co-infected with syphilis.

Analysis of the results showed that most blood donations originated from males – 36 890 (74.6%), which is as much as a recent study established for a sample of 17 502 739 donations collected for a period of six years in Poland (5) and corresponds to a roughly estimated male/female blood donor ratio 3:1 for Bulgaria in the last decade. The shares of infected blood from male donors vs. female ones, as found by this study, is 71% to 29%, respectively, which is proportional to the number of male:female blood donations.

In spite of the increasing analytical sensitivity of HCV screening tests over the years, the number of detected infections in donor blood samples is very low – 0.105%. This might be explained by the nationwide implementation of the united information system collecting data from all centers for transfusion hematology in Bulgaria since 2014, which allows to discard blood donors detected with transmissible disease infections earlier and thus to eliminate repeating infected blood donors.

Analysis of the structure of infectious pathology and morbidity of acute infectious diseases in Bulgaria in recent years shows that the incidence of viral hepatitis C per 100 000 is 1.18 (data are for 2017, excluding those for influenza and ARI, tuberculosis, AIDS and sexually transmitted infections), the total number of patients is 84, with a relative share to the incidence of acute infectious diseases in Bulgaria of 0.16% and mortality from HCV infection - 1.19%. Of the total number of 3132 cases of viral hepatitis registered in the same year, viral hepatitis C accounted for 2.68% and the level of morbidity remains unchanged (morbidity 1.18‰) for several consecutive years (6). For a period of a year prior to NAT implementation in Bulgaria (2016) in the National Reference Laboratory for Hepatitis Viruses at the National Centre of Infectious and Parasitic Diseases out of 1802 samples received for differentiation of viral hepatitis or for screening of hepatitis markers, 1019 (57%) were tested for anti-HCV and 127 (12%) were positive (7). It is important to note that these cases are identified mainly by the gastroenterological departments and general practitioners. The number of cases reported

for the country per year as compared to results produced only by NAT analysis of blood donations seem to be underestimating the real situation – despite the overlapping of a number of cases reported, there still exists a slight increase as produced by the results from donor blood NAT testing. Blood transfusion centers testing may identify either passed infections, chronic infections or early-stage infected individuals, who are in the window period in the acute infection, usually still asymptomatic. Transfusion practice considers carriers of anti-HCV only potentially infectious and blood donations are discarded. Furthermore, the analytical serological assays implemented in Bulgarian blood transfusion centers are qualitatively analyzing the presence of anti-HCV and capsid HCV Ag altogether. Only CMIA-based Abbot Architect Anti-HCV and Architect HCV Ag assays distinguish between the presence of Anti-HCV and core HCV antigen, respectively, in human serum and plasma and thus might distinguish between past and current infections in case of negative Ag results. This could be the case for 10 of the blood donors, who were reported negative by NAT testing and positive by the serological Monolisa HCV Ag-Ab ULTRA V2 testing. By the implementation of molecular diagnostic test for HCV RNA referred to as NAT, viremic donors might be distinguished from donors who have cleared the virus. The HCV NAT becomes positive approximately 1 to 2 weeks after the initial HCV infection (8). That is how the NAT test has become the gold standard supplemental test for patients who have a positive HCV EIA screening test (9,10). NAT can determine whether a patient with a positive HCV antibody test has a current (active) or a resolved HCV infection. That could be the case for three of the blood donations, which were tested NAT negative, Ag-Ab Ultra V2 test assay positive, and HCV core Ag negative. One could suspect a past infection in these donors. However, an additional testing by other methods is required to also exclude the presence of capsid Ag and the presence of HCV.

Scientists from Varna Regional Blood Transfusion Center approached the NAT analyses from a slightly different point of view. A total of 36 NAT-positive blood samples were established for the studied period of four months in RCTH Varna, after the analysis of 6844 donor samples (0.53 %) and all 36 samples underwent discriminatory analysis for HB-

sAg, HCV, and HIV (Table 1). Five of the samples tested positive for HCV (13.9%), 1 sample was HIV positive (2.8%), and 19 – HBsAg positive (52.8%). Discriminatory tests failed to identify one simultaneously NAT positive and ELISA HBsAg seropositive blood sample. And serological assays failed to identify as positive a total of 17 (47.2%) out of all 36 NAT-positive samples, which makes almost half of them. Discriminatory tests that were carried out afterwards confirmed six of those to be HBsAg positive and the remaining 11 were unidentified by serological or discriminatory tests for the presence of any Ab-Ag indicative for any of the three types of transmissible infections – HCV, HBV or HIV, and donations were also discarded. One possibility is that some of

false-negative EIA include patients with acute HCV infection, persons with major immunosuppression (advanced HIV infection or organ transplantation recipients), and persons with chronic renal failure on long-term hemodialysis, while false-positive tests can occur with increased gamma globulin production, with autoimmune diseases, and following immunizations. The role of NAT in these cases would be the detection of the virus within 1-2 weeks of infection, or detection of actual virus rather than immune response. That is where NAT testing may contribute to the clarification of results. Excluding any of the donations from the blood storages could possibly save lives.

*Table 1. Results from NAT testing in the Regional Center for Transfusion Hematology - Varna in the period of 10<sup>th</sup> February to 16<sup>th</sup> June, 2020*

	NAT	Discriminatory NAT	ELISA	Number of Samples
HCV	+	+	+	5
HIV	+	+	+	1
HBsAg	+	+	+	12
	+	-	+	1
	+	+	-	6
Unspecified	+	-	-	11
Totally				36

those cases could possibly be in the window period, which is quite long, e.g. about two months for HCV. In case of a very low viral load at the early stage of the infection it may fail to be detected by the discriminatory tests. In 2017, when NAT technology was still not implemented in the country, 284 cases of unspecified virus hepatitis were established for Bulgaria (incidence 4.00‰), and the incidence in the previous seven years varied between 2.7 and 3.9 per 100 000 population. Patients were tested for serological markers of HAV, HBV, and HCV, but were not laboratory confirmed for any of the viral types of hepatitis. Screening of patients with unspecified acute hepatitis indicated 32% presence of anti-HBc in HBsAg-negative and anti-HBs-negative population (7). ELISA nonreactive samples theoretically may originate from patients who are either in the window phase prior to seroconversion or from immunocompromised patients (11). Circumstances associated with a

The value of NAT technology should be underlined by the fact that it might also be transferred beyond the scope of blood transfusions - organ utilization of HBV and/or HCV NAT-negative organs is increased over time despite positive antibody status (NAT-negative but seropositive), as reported based on evaluation of results and utilization information for organ donor referrals from 2004 to 2017, out of whom 1.8% were HCV antibody-positive and NAT-negative (12). The use of NAT for deceased donor screening correlated with increased utilization of donor organs. This approach could be of value with regard to donor blood and blood product used for treatment, especially in the developing pandemic situation of COVID-19. Convalescent plasma products for the treatment for COVID-19 patients should be subjected to NAT analysis to eliminate risk from transmissible infectious diseases, including HCV. In addition, with the emergence of direct antiviral agents,

there is an increase in data showing the short-term outcomes and success of hepatitis C treatment, which would reduce HCV spread in the population.

## CONCLUSION

We believe that, although the number of samples analyzed is limited to the blood donations from the period since NAT was implemented as a routine practice in the blood transfusion centers in Bulgaria, data presented in this study are of value to assess the first outcomes of NAT implementation and possibly reroute the analytical approaches applied in the blood transfusion system in Bulgaria and the National Reference Laboratory. An advantage of the method is that it not only allows for timely identification of infected donor blood and saves lives of the recipients but also saves lives of the donors, as treatment of chronic hepatitis C continues to progress and is now effective in clearing the virus in more than half of the patients. In terms of safety and security of diagnosis and blood products obtained, the method has no alternative. In conclusion, a recommendation could be drafted based on the above results: to adopt a strategy to increase the quality of healthcare by introducing a one-time HCV screening for adults aged 18 and above, for individuals at risk and for pregnant women to reduce further the risk of incidental transmission of hepatitis C virus.

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