HEPATITIS C – THREE DECADES OF THE PATH FROM DISCOVERY TO THE NOBEL PRIZE

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INTRODUCTION

This year, the Nobel Prize in Medicine was awarded to the three scientists who, 31 years ago, identified a new, blood-borne, primarily hepatotropic virus (BBV). This was the hepatitis C virus (HCV) that causes the infection with the same name, which is a global health problem linked to serious complications, such as liver cirrhosis and hepatocellular carcinoma (HCC). Because of this major discovery achieved by this year’s laureates of the Nobel Prize – Harvey Alter, Michael Houghton and Charles M. Rice, a discovery which the whole world has these scientists to thank, it is quite natural that, in the year when they are awarded this most prestigious prize, we should pay tribute to their work [1].

The hepatitis C virus is a new paradigm for the identification and control of viral infections, and its discovery has become a milestone in the fight against viral diseases in the 20th century. From the moment the HCV, as a new BB hepatotropic virus, had been identified, an era of rapid development of very sensitive laboratory tests for its detection started, which in turn, resulted in posttransfusion hepatitis C being eradicated in a large part of the developed world, with a tendency of decreasing the prevalence of posttransfusion HCV infection in the rest of the world to negligible percentages [2]. Also, this discovery has brought about advancements in the treatment of HCV infection, through the design of very efficient antiviral drugs, which completely put the HCV...
se šansa koja bi mogla da dovede do izlečenja i po prvi put postoji nada da će doći do iskorenjavanja HCV iz svetske populacije. Ipak, ovaj cilj je globalan i zahteva međunarodne napori kako bi se testiranje na HCV kao i savremena anti-virusna terapija novim, direktno delujućim anti HCV lekovima učinili dostupnim širom sveta. Ovo je imperativ koji su Alter, Hoton i Rajs postavili kao cilj koji treba da reše novi timovi naučnika [3].

NOVI, NEPOZNATI VIRUS UZROČNIK HEPATITISA – KAKO JE SVE POČELO

Već u prvoj polovini 20. veka bilo je jasno da postoje dve vrste hepatitisa virusne etiologije. Prvi otkriveni uzročnik virusnog hepatitisa bio je hepatitis A virus (HAV), koji se prenosi putem kontaminirane hrane, vode ili prljavim rukama i uspostavlja samo akutnu infekciju bez težih i dugoročnih sekvela po zdravlje ljudi. 60-tih godina istog veka je Baruh Blumberg identifikovao novi hepatitis virus koji se prenosi krvlju i drugim telesnim tečnostima. Bio je to hepatitis B virus (HBV), koji, pored akutne, može da uspostavi i hroničnu infekciju [4]. Utvrđeno je da HBV obično uspostavlja hroničnu infekciju tiho i neprimetno, te se infekcija često dijagnostikovala kada su nastajale teške kliničke forme ili kompleksije hronično uspostavljene infekcije, kao što su ciroza jetre i hepatocelularni karcinom. Blumberg je, 1976. godine, za identifikaciju novog virusnog patogena, dobio Nobelovu nagradu za medicinu. Otkriće HBV je vrlo brzo pokrenulo niz daljnjih istraživanja, koja su uslovljala rad na osetljivim dijagnostičkim testovima za rano otkrivanje HBV infekcije, kao i istraživanja na polju prevencije i terapije. Ove istraživanja su rezultovala otkrivanjem veoma uspešnih antivirnih lekova sa visokom genetičkom barjerom za nastanak virusne rezistencije, što ih čini izuzetno delotvornim terapijom kod pacijenata sa hroničnim B hepatitisom. Takođe, dizajnirana je efektna anti-HBV vakcina od koje se u skoroj budućnosti očekuje da će dovesti do iskorenjavanja HBV iz svetske populacije [5].

KAKO SE SKLAPALA SLAGALICA O NOVOM BB VIRUSU, UZROČNIKU HEPATITISA C

U periodu između 60-tih i 70-tih godina 20. veka, Alter je radio zajedno sa Blumbergom na usavršavanju metodologije za dokazivanje HBsAg, glikoproteina u omotaču HBV, koji kasnije postaje glavni kandidat za razvoj uspešne anti-HBV vaccine. Ranih 70-tih godina, Alter sa svojim naučnim timom, primećuje učestalu pojavu hepatitisa kod pacijenata koji su primili transfuzije krv, iako se već u to vreme postojali serološki testovi za dokazivanje HBV, koji su mogli da eliminišu HBV kao potencijalnog uzročnika posttransfuzijskog hepatitisa. infection under control, providing a chance that can lead to a cure and, for the first time, offering hope that the HCV will be eradicated from the world population. However, this goal is global and requires international efforts in order to make HCV testing as well as up-to-date antiviral therapy with new, direct-acting anti-HCV drugs, accessible all over the world. This is an imperative which has been set as a goal before new teams of researchers by Alter, Houghton and Rice [3].

THE NEW UNKNOWN VIRUS CAUSING HEPATITIS – HOW IT ALL BEGAN

As early as the first half of the 20th century it became clear that there were two types of hepatitis of viral etiology. The first viral hepatitis pathogen to be discovered was the hepatitis A virus (HAV), which is transmitted by contaminated food, water and unclean hands and it causes only an acute infection without serious long-term consequences to people’s health. In the 1960s, Baruch Blumberg identified a new hepatitis virus transmitted by blood and other bodily fluids. This was the hepatitis B virus (HBV), which, in addition to acute, can also cause chronic infection [4]. It was established that HBV usually develops chronic infection quietly and imperceptibly, which is what caused the infection to be often diagnosed only when serious clinical forms or complications occurred, such as liver cirrhosis and hepatocellular carcinoma. In 1976, Blumberg was awarded the Nobel Prize in Medicine, for identifying this new viral pathogen. The discovery of HBV very quickly initiated a series of further research, which necessitated the work on sensitive diagnostic tests for early HBV infection detection, and sparked research in the area of prevention and therapy. This research resulted in the discovery of very efficient antiviral drugs with a high genetic barrier for the development of viral resistance, which makes these drugs exceptionally effective therapy in patients with chronic hepatitis B. Also, an efficient anti-HBV vaccine was developed, and it is expected to bring about the eradication of HBV from the world population in the near future [5].

HOW THE PUZZLE OF THE NEW BB VIRUS, THE CAUSE OF HEPATITIS C, WAS PIECED TOGETHER

In the period between the 1960s and the 1970s, Alter worked together with Blumberg on perfecting the methodology for detecting the HBsAg, a glycoprotein in the HBV capsule, which was later to become the main candidate for the development of an effective anti-HBV vaccine. In the early 1970s, Alter and his research team noticed a frequent incidence of hepatitis in patients who had received blood transfusions, although, at the time, there were serological tests for detecting HBV, which were able to eliminate HBV as a potential cause
Takođe, ni drugi poznati hepatotropni A virus nije bio povezan sa pojavom novog posttransfuzijskog hepatitisa. Bilo je jasno da se radi o novom patogenu, povezanom sa krvljom, koji je dovodio do zapaljenja jetre [6]. U to vreme nepoznati BBV koji se povezivao sa hepatitismom, nazvan je non-A, non-B (NANB). Ovo svoje zapažanje Alter i saradnici su morali da dokažu, što je i bio njihov sledeći istraživački korak, odnosno cilj je bio da se pokaže povezanost NANB sa hepatitismom koji je nastajao nakon transfuzija krv [7]. Tokom 1975. godine, Alter i saradnici su, u odsustvu drugih in vitro laboratorijskih postupaka za dokazivanje novog NANB virusa, svoje istraživanje nastavili na životinjskom modelu. Oni su na eksperimentalnom modelu šimpanzi pokazali da, nakon inokulacije seruma osoba sa kliničkim slikama novog virusnog NANB hepatitisa, u količinama ekvivalentnog transfuziji krv kod ljudi, dolazi do razboljevanja životinja. Već nakon prve infekcije postigli su uspeh, jer je pet od pet šimpanzi razvilo povećanje alaninaminotransferaze (ALT).

Prvi uspeli su ohrabrili naučni tim u daljim istraživanjima, te su se eksperimenti nastavili sa titriranjem inokulum dobijenog iz krv, sada pacijenata sa teškim kliničkim slikama NANB hepatitisa, što je koreliralo sa drastičnim povećanjima nivoa ALT kod eksperimentalnih životinja. Iz obolelih životinja je izolovan novi virus, uzročnik hepatitisa koji je povezan sa krvlju, koji je bio njihov sledeći istraživački korak, odnosno cilj je bio da se pokaže povezanost NANB sa hepatitismom koji je nastajao nakon transfuzija krv [7]. Tokom 1975. godine, Alter i saradnici su, u odsustvu drugih in vitro laboratorijskih postupaka za dokazivanje novog NANB virusa, svoje istraživanje nastavili na životinjskom modelu. Oni su na eksperimentalnom modelu šimpanzi pokazali da, nakon inokulacije seruma osoba sa kliničkim slikama novog virusnog NANB hepatitisa, u količinama ekvivalentnog transfuziji krv kod ljudi, dolazi do razboljevanja životinja. Već nakon prve infekcije postigli su uspeh, jer je pet od pet šimpanzi razvilo povećanje alaninaminotransferaze (ALT).

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Sledeći korak u dokazivanju novog virusa hepatitisa bio je da se dešuši genom virusa, odnosno da se izdvoji genomika sekvencsa koja je odgovorna za sintezu virusnih produkata – antigena virusa, koji senzibilizuju sintezu specifičnih anti-HCV antitela. Ovakvu zamisao, da se pomoću seruma pacijenata sa kliničkim slikama novog virusnog hepatitisa dokaže određena genom-
Kako su tradicionalne tehnike za izolovanje virusa bile limitirane, a eksperiment na majmunskom modelu je doneo jasnu povezanost virusa sa novim NANB hepatitисom, sledeći nedostajući deo slagalice, koja se zove hepatitис C, bila je molekularna identifikacija novog virusa. Hoton i njegovi saradnici su, 1982. godine, stvorili kolekciju DNK fragmenta od nukleinskih kiselina ekstrahovanih iz uzoraka krvi zaraženih simpanzi. Većina ovih fragmenta pripadala je genomu same simpanze, ali su Hoton i sradnici pretpostavili da će barem neka sekvencu biti poreklom od genetičkog materijala novog NANB virusa. Pod pretpostavkom da će anti-virusna antitela biti prisutna u krvi uzetoj od pacijenata sa hepatitисom, naučnici su koristili serume pacijenta za identifikaciju kloniranih virusnih fragmenta komplementarne DNK (cDNK) koji kodiraju virusne proteine. Nakon mnogo izvedenih testiranja, pronađen je jedan pozitivan kron. Dalji rad pokazao je da je ovaj kron izveden iz novog RNK virusa koji pripada porodici Flaviviridae. Molekularna identifikacija HCV bila je vrhunac timskog napora, koji je trajao 7 godina, te je, 1989. godine, NANB virus promenio naziv u današnje ime virusa, odnosno virus hepatitisa C [10].

Tako je Hoton, zajedno sa dvojicom kolega Ki Lim Čuom i Džordžom Kuom, postao prvi naučnik koji je identifikovao virus i formalno mu dodelio ime virus hepatitisa C. Takođe, njihov naučni rad rezultirao je dizajniranjem dijagnostičkog testa za identifikaciju virusa u krvi, koji je omogućio lekarima da, po prvi put, uvedu rutinski skrining na hepatitis C, testiranjem i davaoca krvi, koji je omogućio lekarima da, po prvi put, uvedu rutinski skrining na hepatitis C, testiranjem i davaoca krvi, koji je omogućio lekarima da, po prvi put, uvedu rutinski skrining na hepatitis C, testiranjem i davaoca krvi. Hoton i njegovi saradnici stvorili su kolekciju DNA fragmenta koja se zove slagalice Flaviviridae. Molekularna identifikacija HCV bila je vrhunac timskog napora, koji je trajao 7 godina, te je, 1989. godine, NANB virus promenio naziv u današnje ime virusa, odnosno virus hepatitisa C [10].

Istraživanja Altera i Hotona bila su od presudnog značaja u otkriću hepatitisa C. Ipak, nedostajao je još jedan suštinski deo „HCV slagalice“, koji je trebao da pruži odgovor na pitanje: Da li HCV može da izazove hepatitis? Čarls Rajs, istraživač na Univerzitetu Vašington u Sent Luisu, zajedno sa drugim naučnim timovima koji su radili sa različitim RNK virusima, pretpostavio je da bi region na 5’ kraju HCV genoma mogao da bude važan za otpočinjanje virusne genome sequence, more precisely its products, was the scientific idea conceived by Michael Houghton, who, during the 1980s, worked as a researcher at the pharmaceutical company Chiron Corporation in California, USA.

As the traditional techniques for isolating viruses were limited, and the experiment on the monkey model had clearly indicated the link between the virus and the new NANB hepatitis, the next missing piece of the hepatitis C puzzle was the molecular identification of the new virus. In 1982, Houghton and his associates created a collection of DNA fragments from nucleic acids extracted from blood samples of infected chimpanzees. Most of these fragments belonged to the genome of the chimpanzees themselves, however Houghton and his team believed that at least some sequence would originate from the genetic material of the new NANB virus. Under the assumption that anti-viral antibodies would be present in the blood of patients with hepatitis, the scientists used the patients’ serum for identifying cloned viral fragments of complementary DNA (CDNA) encoding viral proteins. After many tests, one positive clone was found. Further work showed that this clone was derived from a new RNA virus belonging to the Flaviviridae family. The molecular identification of HCV was the climax of the team’s effort which lasted 7 years, and in 1989, as a result, the name of the NANB virus was changed to what it is called today, i.e. the hepatitis C virus [10].

In this way, Houghton, together with two of his colleagues, Qui-Lim Choo and George Ku, became the first scientist to identify the virus and to formally name it the hepatitis C virus. Additionally, their research resulted in the designing of a diagnostic test for the identification of the virus in the blood, which enabled doctors to introduce, for the first time, routine hepatitis C screening, by testing both the donor and the recipient of blood transfusion. Dr. Angela Rasmussen, a virologist who carried out her postdoctoral studies in Dr. Houghton’s team, working on discovering the hepatitis C virus, described the HCV as an intriguing and cunning pathogen which was inspirational to work on. The scientific discovery achieved by Dr. Houghton’s, who isolated the genetic sequence of the HCV, very clearly indicated that a new virus, which was the cause of a new type of hepatitis, had been discovered, thus joining the group of already known hepatotropic viruses, A and B [11].

Research carried out by Alter and Horton was of crucial importance in discovering the hepatitis C virus. However, another, key component of the HCV puzzle was missing, which needed to provide an answer to the question: Can HCV cause hepatitis on its own? Charles Rice, a researcher at the Washington University in St. Louis, together with other research teams working on different
replikacije [12]. Rajs je takođe primetio da određeni drugi regioni HCV genoma, svojim genskim produktima mogu da suprimiraju virusnu replikaciju, jer vrše inhibiciju genomske sekvence koja je započinje [13]. Metodama genetskog inžinjeringa, Rajs je dizajnirao HCV RNK, koja je obuhvatila region odgovoran za inicijaciju replikacije, uklonivši regione koje su se suprimirali. Kada je ovako modelirana HCV RNK ubrizgana direktno u jetru šimpanze, replikacijom su nastajali virioni koji su dokazani u krvi životinje, a takođe su primećene i patološke promene slične onima koje su viđene kod pacijenata sa hroničnim hepatitismom C [14].

Ovo je bio konačni dokaz da virus hepatitisa C može sam da prouzrokuje BB hepatitis.

TRIDESET GODINA KASNIJE — ŠTA SE DANAS ZNA O HCV

Tokom tri decenije od otkrića do danas, o HCV govorimo kao visoko varijabilnom, pozitivno jedno- lančanom RNK virusu sa visokom stopom mutacija. Ova biološka karakteristika je posledica njegovog životnog ciklusa i uloge virusne RNK-zavisne RNK polimeraze, enzima uključenog u virusnu replikaciju, koji nema mehanizme za ispravljanje slučajno nastalih grešaka. To obezbeđuje stvaranje različitih genomskih varijanti unutar inficiranog organizma — kvazipsičjesa, ali i različitih antigenskih varijanti [15]. Upravo antigenska varijabilnost HCV-a obezbeđuje da on vešto izbegava imunski odgovor domaćina, te ponaša uspostavljanje pERSISTENTE infekcije, a istovremeno predstavlja problem za razvoj uspešne vakcine. Razlika od 30% na dužini cegel genoma uslovljila je podelu na danas poznatih 8 HCV genotipova, dok još manja genomska razlika dalje klasifikuje HCV na 90 subtipova i 9 rekombinantnih formi [16]. Genotipovi HCV se međusobno značajno razlikuju u odgovoru na terapiju, te je njihovo dokaživanje deo rutinskog laboratorijskog protokola kod pacijenata koji su kandidati za otpočinjanje antivirusne terapije [17].

HCV može da uspostavi i akutnu i hroničnu infekciju. Oko 30% pacijenata (15% – 45%) spontano izluči virus unutar 6 meseci od infekcije i u odsustvu specifične terapije. Ipak, ~70% pacijenata (55% – 85%) razvije hroničnu bolest sa rizikom od nastanka ciroze jetre ili HCC, u visokom procentu od 15% do 30%, unutar dva desetogodišnjeg perioda [18].

Noviji podaci procjenjuju da danas u svetu ima oko 110 miliona ljudi sa dokazom prethodne HCV infekcije (prisustvo anti-HCV antitela), dok je 71 milion hronično inficiranih [19]. HCV infekcija je jedna od retkih infektivnih bolesti čiji mortalitet raste u RNA viruses, speculated that the region at the 5’terminus of the HCV genome could be important for the initiation of virus replication [12]. Rice also noticed that certain other regions of the HCV genome can suppress virus replication with their gene products, as they inhibit the genome sequence that initiates it [13]. Through methods of genetic engineering, Rice designed HCV RNA, which included the region responsible for replication initiation, eliminating the regions suppressing it. When thus designed HCV RNA was injected directly into the liver of a chimpanzee, virions were replicated, which were detected in the blood of the animal, and pathological changes were also noted, similar to those observed in patients with chronic hepatitis C [14]. This was final proof that the hepatitis C virus could cause BB hepatitis on its own.

THIRTY YEARS LATER — WHAT IS KNOWN ABOUT THE HCV TODAY

During the thirty years since its discovery until now, HCV has been known as a highly variable, single-stranded, positive-sense RNA virus, with a high mutation rate. This biological characteristic is the result of its life cycle and the role of the viral RNA-dependent RNA polymerase, an enzyme involved in virus replication, which lacks the repair mechanism for correcting accidental mistakes. This enables the creation of different genome variants within the infected organism — quasispecies, but also different antigen variants [15]. It is this antigen variability of the HCV that enables it to skillfully avoid the immune response of the host, which, in turn, facilitates the establishing of persistent infection, at the same time posing a problem to the development of a successful vaccine. Due to a 30% difference in the length of the entire genome, a categorization into 8 different HCV genotypes, known today, has been made, while an even smaller genome difference classifies the HCV into 90 subtypes and 9 recombinant forms [16]. HCV genotypes differ amongst themselves significantly in their response to therapy, which is why their detection is a part of the routine laboratory protocol in patients who are candidates for antiviral therapy [17].

HCV can cause both acute and chronic infection. Around 30% of patients (15% – 45%) spontaneously eliminate the virus within 6 months of infection even without specific therapy. However, ~70% of patients (55% – 85%) develop chronic illness with a high risk (15% – 30%) of developing liver cirrhosis or HCC within a 20-year period [18].

More recent data estimate that there are around 110 million people in the world with proven previous HCV infection (detected anti-HCV antibodies) and 71 million chronically infected patients [19]. HCV infection is one of the rare infectious diseases whose mortality...
rate has been rising in the past decades. According to available data, mortality has risen since the year 2000, by 22%, and it is estimated that around 700,000 patients die, every year, as the result of HCV infection complications. In order to put a stop to this trend, it is necessary to establish efficient therapy for chronic HCV infection, which, according to the latest recommendations of the World Health Organization, proposes treatment with pan-genotypic direct-acting antiviral drugs (DAA). This is a new, interferon free regimen, which efficiently suppresses viral replication and, within a relatively short time period of 8 to 12 weeks, establishes a stable virologic response (undetectable HCV-RNA) in as many as 95% of patients [20]. Depending on the subgenotype of the virus, the presence or absence of cirrhosis, HCV therapy protocols with the new DAA are different, i.e. the combinations of drugs vary [21].

With more testing, and with new anti-HCV therapy being made available, it is believed that, within the next decade, it will be possible to eradicate HCV infection, even without a vaccine. A large part of the HCV puzzle has been pieced together. The steps, without which there would be no other steps further, were made by Alter, Houghton and Rice. However, the HCV remains an intriguing pathogen, and, until it is eradicated, it will be the object of attention of expert and research teams.
professor postaje na istom fakultetu 1995. godine. Od 2001. godine, profesor je na Univerzitetu Rockefeller u Njujorku. U periodu od 2001 - 2018. godine bio je načelnik i izvršni direktor Centra za proučavanje hepatitisa C, na Univerzitetu Rockefeller, gde je i danas aktivni istraživač.

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**Conflict of interest:** None declared.

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