Published in final edited form as:
*Cent Eur J Med*. 2011 October ; 6(5): 672–678. doi:10.2478/s11536-011-0073-6.

# HEPATITIS C VIRUS GENOTYPES IN INJECTING DRUG USERS FROM ROMANIA

**Camelia Sultana**[1,2], **Codruta Vagu**[2], **Aura Temereanca**[1,2], **Camelia Grancea**[2], **Josefina Slobozeanu**[3], and **Simona Ruta**[1,2]

[1]Chair of Virology, Carol Davila University of Medicine and Pharmacy, 8 Eroilor Sanitari Bvd, Bucharest, Romania

[2]Emergent Diseases Department, Stefan S. Nicolau Institute of Virology, 285 Mihai Bravu Bvd, Bucharest, Romania

[3]Center for Integrated Assistance for Addicts CAIA Pantelimon, 255 Pantelimon Road, Bucharest, Romania

## Abstract

Due to the increasing number of infections related to injecting drug use, both the pattern of hepatitis C virus (HCV) transmission, and the circulating genotypes in Europe have changed. As there are little available data in this respect for Romania, the aim of our study was a preliminary analysis of the distribution of HCV genotypes circulating among injecting drug users (IDUs). Of the 45 IDUs evaluated (86.7% men, mean age - 27.6±3.7 years, mean age at first drug use - 17.5±3.9 years), 88.9% presented anti-HCV antibodies, with higher rates in those with an injecting history of more than 10 years; 57.8% of the subjects had detectable HCV viral load. Only 6.7% had markers of chronic hepatitis B infection, and none had anti-HIV antibodies. While HCV subtype 1b is still prevalent (in 50% of the viraemic subjects), other subtypes begin to emerge, especially in younger patients (1a - in 23.1%, 4 - in 11.5%, 3a – in 7.7% of the cases). These data indicate the possibility of major shifts in the distribution of the dominant subtype, underlining the need for close surveillance of HCV infections in IDUs, who can act as a bridging group toward the general population.

### Keywords

HCV prevalence; Romania; injecting drug users; HCV genotypes

## 1. Introduction

In Europe, according to national estimates, 8.8 million people (1.3% of the total population) are HCV infected [1]. In 2008, 29 927 confirmed cases of hepatitis C virus (HCV) infection were reported by the 27 EU and EEA/EFTA Member States, giving an overall notification rate of 8.97 per 100 000 population [2]. The most commonly affected age group is that of 25–44 years-old, with twice as many males infected compared to females. Injecting drug use accounts for 30% to 60% of all reported HCV cases in Europe, being the main risk factor in Northern European countries, and emerging as dominant in Southern European ones, where it replaces the iatrogenic transmission recorded 30 years ago [3].

Corresponding author: Simona Ruta, simona@simonaruta.ro.

**Conflicts of interest**
None to declare.

According to a recent report from the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), HCV antibody levels among national samples of injecting drug users (IDUs) vary from around 18% to 95%, with half of the countries reporting levels in excess of 40%, and only three countries - Bulgaria, Czech Republic, Slovenia - reporting a HCV prevalence of under 25% [4].

In Romania, results of a recent nationwide cross-sectional survey documented a 3.23% prevalence of anti-HCV in the general population [5]. Although during the last years drug use has increased significantly in Romania, only a limited amount of data about the prevalence of blood borne infectious diseases in IDUs is available at national level. In 2008, according to the National Anti-drug Agency (NAA), 16 800 injecting drug users were reported in Bucharest (0.9% of the city population). About one third of all IDUs were less than 25 years old, opioids were the most utilized drugs, and frequent use of non sterile injecting equipment was common practice [6].

In 2009 an omnibus-type survey at national level, reported by NAA and EMDCCA, regarding the attitudes and lifestyle of highschool students indicate a lifetime prevalence of illicit drug use of 10.7% among 12 $^{th}$ graders, with a regional extension outside the capital city [6]. A routine monitoring for the prevalence of drug related infectious diseases, reported by United Nations Office on Drugs and Crime (UNODC), showed HCV infection values among drug users from Romania over the European average – 83% in 2009 [7].

Worldwide, HCV genotypes 1b and 2, associated with blood transfusions or nosocomial transmission are being replaced by 1a, 3/3a and 4, that are commonly found in injecting drug use related infections [8]. Romanian patients with chronic hepatitis C enrolled in several clinical studies were found to be almost exclusively infected with genotype 1 (subtype 1b in 93.46% of the patients from the ACHIEVE study, and genotype 1 - without subtype identification - in 99.13% of patients in ENMS study [9]. As there are little or no available data in this respect for IDUs, we developed a pilot study on the circulating HCV genotypes distribution, together with the seroprevalence of other blood borne viral infections in this particular risk group.

## 2. Materials, methods, and statistical analysis

### 2.1. Patients

Plasma samples were collected from 45 subjects with a history of intravenous drug use attending a methadone substitution program in one of the Centers for Integrated Assistance for Addicts in Bucharest, during September 2008 – March 2009. At enrollment, all subjects were administered a standardized questionnaire, that contained socio-demographic characteristics (age, gender, education), data about the drug consumption (drug exposure time, age at first drug use), and other potential risk factors for HCV / HBV / HIV infections. Informed consent was obtained from all participants. The study was approved by the Bioethics Committee of the Stefan S. Nicolau Institute of Virology.

### 2.2. Markers of viral infections

All samples were initially tested for total antibodies against hepatitis B core antigen (anti-HBc Ab), hepatitis B surface antigen (HBs Ag), antibodies against hepatitis C virus (anti-HCV Ab), against human immunodeficiency virus (anti-HIV Ab), and against human T cell leukemia virus I and II (anti-HTLV I & II Ab). Sera negative for HBsAg were tested for anti-HBs antibodies. Sera positive for HBsAg and/or anti-HBc antibodies were tested for markers of active HBV replication - hepatitis Be antigen (HBeAg) and HBV DNA. All serological markers were tested by third generation commercial EIA (DIA PRO Diagnostic, Bioprobes, Italy).

Detection of hepatitis B viral DNA was done using a commercial kit for quantitative in vitro nucleic acid amplification (Cobas AMPLICOR HBV Monitor test, Roche, Germany) with a linear range between 300 and $2\times10^5$ copies/mL, and a lower limit of detection of 300 HBV DNA copies/mL. Sera positive for anti-HCV antibodies, with signal-to-cut-off ratios lower than 3 (S/CO<3.0) were confirmed by recombinant immunobloting assay (DECISCAN HCV PLUS, BIO-RAD, France) in accordance with WHO recommendation. HCV viral load was determined by quantitative RT-PCR (Cobas Amplicor HCV Monitor, vers 2.0, Roche, Germany) - with a linear range between 600 - 700 000 IU/mL, and a lower detection limit of 600 IU/mL.

HCV genotyping was performed using a reverse hybridization line probe assay (VERSANT™ HCV Genotype 2.0 Assay, Siemens, Germany), according to the manufacturer instructions. Briefly, RNA extraction using a Qiagen kit was followed by RT-PCR for 5'UTR and core HCV regions, using co-amplification with two pairs of biotinylated primers to produce two distinct biotinylated DNA fragments of 240 and 270 base pairs, representing the above mentioned HCV genomic regions. The amplicons were hybridized to oligonucleotide probes specific to different HCV genotypes and subtypes, immobilized to a nitrocellulose strip; detection was done using an alkaline phosphatase labeled streptavidin conjugate and a BCIP/NBT chromogen substrate.

### 2.3. Statistical analysis

For statistical determination we used Fisher Exact test (for analyzing contingency tables), Mann-Whitney test (to compare two mean values), and Kruskal – Wallis test (to compare mean values of viral load in different HCV genotypes infections), from GraphPad InStat 3 Program; $p$ value < 0.05 was considered statistically significant.

## 3. Results

### 3.1. Patients' characteristics

The male/female ratio in the studied group was 6.5, mean age was 27.6 ± 3.7 years (range, 20 – 38 ys.); 31.1% of the subjects were younger than 25 years. Half of the participants graduated high-school, but were presently unemployed; most of them (95.5%) came from an urban area. The mean estimated duration of drug use was 9.8 ± 3.5 years (range 1 – 18 ys.); only one subject began injecting drugs recently (<1 year), and 20% of the subjects used drugs before 15 years of age; 31.1% reported sharing injecting drug equipment frequently. No notable gender differences in respect to demographic characteristics and history of drug abuse were recorded.

11.2% of the subjects were married; only 8.9% reported being involved in a steady sexual relationship with just one partner in the last year. 48.9% reported having sexual intercourses with more then 10 occasional partners during their lifetime; the mean number of sexual partners was 10.52 ± 8.2 (range 1–50), and 13.3% were diagnosed with a sexual transmitted disease in the past. None of the study subjects had received blood transfusion or organ transplant. Other risk factors independently associated with parenteral transmitted infections were reported: tattoos (in 57.8% of cases), multiple invasive dental interventions (in 55.6% of cases), body-piercing (in 31.1% of cases), and surgery (in 26.7% of cases).

### 3.2. Serological markers of blood borne viral diseases

55.6% of the studied subjects had total anti-HBc antibodies as a marker of past or present hepatitis B infection, with similar prevalence rates recorded among IDUs with different durations of injecting history (Table 1), and a slightly lower prevalence of infection in younger patients (Table 2). 6.7% of the subjects were chronic HBsAg carriers and HBeAg

positive; 8.9% were HBV DNA positive - one patient had occult hepatitis B (defined as presence of HBV DNA and the absence of HBsAg antigen in plasma). 68% of the previously HBV infected subjects had serologic profiles suggestive for viral clearance (anti-HBc + anti-HBs antibodies; mean value of anti-HBs titer= 8.3 ± 59.5 mUI/mL).

None of the investigated IDUs tested positive for the presence of anti-HIV antibodies, and only one of them tested positive for the presence of anti-HTLV 1&2 antibodies. No notable gender differences in respect to distribution of the serological markers were recorded.

88.9% of the studied subjects had anti-HCV antibodies. Although not reaching statistical significance, higher HCV prevalence rates were recorded among subjects with a drug use history of more than 10 years (92.3% compared with 84.2% for those with shorter periods of drug use – Table 1), as well as in younger subjects (all subjects younger than 25 years were HCV positive *vs.* 80.6% of older ones - Table 2). No differences were recorded between HCV/HBV co-infected and HCV monoinfected IDUs in respect to demographic characteristics (mean age 26.9±3.7 ys. *vs.* 26.6 ±3.7 ys., mean estimated duration of drug use 9.3 ± 3.5 ys. *vs.* 10.9 ± 3.3 ys.), nor regarding the association with risk factors.

### 3.3. HCV genotypes

Three HCV genotypes (1, 3 and 4) were identified in the 26 IDUs with detectable HCV viral load. In descending order of prevalence, HCV subtype 1b was identified in 50% of cases (13/26), 1a in 23.1% (6/26), genotype 4 in 11.5% (3/26), and subtype 3a in 7.7% (2/26); in 2 cases it was not possible to differentiate between subtypes 1a and 1b.

None of the factors that we examined for an association with higher rates of HCV infection reached statistical significance for correlation with a particular genotype (age at initiation of drug injection, being male, longer duration of drug injecting, HBV co-infection rate), most probably due to the small size of the study sample. However, patients infected with HCV subtype 3a tend to be younger than the rest (mean age 24.5 ± 0.5 ys. *vs.* 27.5 ± 2.8 ys. for subtype 1b, 27.6 ± 3.7 ys. for subtype 1a, 27.8 ± 3.6 ys. for genotype 4); also, we observed that the mean viral load was higher in patients infected with genotypes 1a and 1b ($4.9\times10^6$ and $2.1\times10^6$UI/ml), compared with those infected with genotypes 3a and 4 ($1.3\times10^5$ and $1.03\times10^5$UI/ml; $p = 0.48$).

57.8% of the subjects had detectable HCV viral load; significantly higher viral loads were present in HCV monoinfected *vs.* HCV/HBV co-infected subjects ($4.8\times10^6 \pm 8.4\times10^5$ UI/mL *vs.* $2.3\times10^6 \pm 4.7 \times 10^5$ UI/mL, $p = 0.05$). No significant association was found between HCV viral load and duration of drug abuse (9.6 ± 2.8 ys. *vs.* 10 ± 3.4 ys., $p$=0.74 in those with low and high viral load, respectively).

## 4. Discussion

In our study we found a very high prevalence of HCV infection in IDUs from Romania (88.9%), with the uppermost rates recorded among users with an injection history of more than 10 years. This finding is in accordance with recent reports from the Romanian National Antidrug Agency, showing a direct correlation between HCV prevalence and the duration of drug use [6]. As this pattern is different from that reported in other regions from Europe, where IDUs develop anti-HCV antibodies rapidly after drugs' initiation [10], it can offer a window of opportunity for public health strategies directed to control HCV spreading.

The percentage of subjects sharing needles or syringes was relatively low (31.1%), a fact that may reflect the efficiency of the needle exchange services. However, these data cannot be extrapolated to the whole IDU population in Romania, as long as this type of harm

reduction intervention is provided only by NGOs and remain located mainly in the capital city Bucharest; in addition sales of injecting equipment through pharmacies is not included in any national monitoring system [11].

Our results show the emergence of new HCV genotypes (1a, 3a and 4) in intravenous drug users from Romania, unreported in previous studies from general population. Although in Europe there is a wide range of circulating HCV genotypes, including 1b (the most prevalent worldwide), 2 and 4a throughout the Mediterranean area, during the last years genotypes 1a and 3a are detected with increasing frequency.

In Western European countries the prevalence of HCV genotype 1b decreased significantly a decade ago. In France, the prevalence of genotype 1a and 3a increased to 28.8% and 26.3%, respectively, in 2002 [12], in Germany, there was an increase in the rate of genotype 3 infection - from 33.6% in 2003 to 35.7% in 2005 [13], while in Italy in 2003 genotype 3 was found in about 12% of chronic hepatitis C patients [14]. More recently, studies from Eastern Europe reported high rates of HCV genotype 3, mainly associated to injecting drug use: 19.7% in Czech Republic [15], 31.3% in Poland [16], 23.2% in Serbia & Montenegro [17], and 33.3% in Bosnia & Herzegovina [18]. In Russia, genotype 1a was prevalent in the general population; however, due to emergent epidemics of HCV among drug injectors, genotype 3a (56.9%) appears to have surpassed 1a (11.9%) in younger people [19]. Two separate introductions of subtype 3a were reported in intravenous drug users in Estonia, where both subtype 1a and 3a are presently co-circulating in this community [20].

In our study, patients infected with subtype 3a are younger than the rest, indicating the new introduction of this subtype in Romania. Together with the high prevalence of subtype 1a in IDUs, this points out to the possibility of a major shift in the distribution of circulating HCV genotypes, with the expansion of viral strains with anterior low prevalence, but with more efficient transmission in selected risk groups. Successive HCV outbreaks among IDUs can further facilitate the secondary spread of these new genotypes in the general population. As long as several different subtypes are circulating, the emergence of HCV recombinants cannot be ruled out. This have been already described for HCV subtypes 1b/1a, as a crossover event which had taken place in the NS5B protein [21], and for HCV subtypes 2k/1b by homologous recombination during minus-strand synthesis [22].

An interesting point is that almost half of the IDUs in our study reported having more than 10 sexual partners, and more than 10% were diagnosed with a sexual transmitted disease in the past. Recently, the importance of sexual transmission of HCV infection has been reconsidered, mainly in men who have sex with men [23, 24]. High-risk sexual behaviors, traumatic sexual practices and ulcerative sexual transmitted diseases are facilitating HCV transmission; consequently, introduction of multiple HCV strains from injecting drug users in the general population, via unsafe sex, is possible.

Our data show that the rate of HCV/HBV and HCV/HIV co-infections in drug users are extremely low. Only 6.7% of the study subjects were HBsAg chronic carriers, a finding sustained by the data released in 2010 by NAA and EMDCCA, which show a stabilizing, low HBV prevalence in IDUs during the last years - 10.3% in 2009 [6]. This is likely to be maintained, as HBV incidence in children under 15 ys. decreased significantly (from 81 to 11 per 100,000 population and year) after the introduction in 1995 of HBV routine immunization for newborns [25].

None of the investigated IDUs had antibodies against HIV. This dichotomy in the prevalence of blood borne viruses in IDU population can be attributed to several reasons. The estimated probability of HCV transmission per exposure to a contaminated syringe is 5 to 20 fold higher than that of HIV, probably due to higher viral titer and different range of

susceptible cells, as have recently been shown using a microculture assay [26]. Furthermore, there are host genetic factors that can influence susceptibility to viral infections. A strong association was reported between a high *CCL3L1* copy number and a reduced likelihood of HIV seropositivity in IDUs, while no such risk was demonstrated for HCV acquisition [27].

In addition, the HIV epidemic in Romania has unique features, related to the high number of parenterally acquired pediatric cases. Previous studies from our group have indicated that HCV co-infection is highly infrequent in these subjects - 0.8% [28]. This may account for the current absence of an epidemiological link between HCV and HIV risk groups. In fact, only 21 cases of HIV infection related to injecting drug use were identified in Romania during 1992–2010 in the 3959 tested IDUs [29], an insignificant number compared to the total of 31 521 cases in the EU [30]. Nevertheless, HIV prevalence in IDUs must be surveyed, as in countries bordering Romania (Russia, Ukraine and Moldavia), as well as in Estonia, this vulnerable population has been at the origin of recent concentrated outbreaks of HIV, HCV and HBV co-infections [31, 32, 33].

Concluding, we report the emergence of HCV infections with subtype 1a and other non 1b genotypes, especially in younger injecting drug users from Romania who, acting as a bridging group toward the general population, can trigger major changes in the HCV subtypes' distribution.

## Acknowledgments

SULTANA CAMELIA was supported by the Sectoral Operational Programme *Human Resources Development* (SOP HRD), financed from the *European Social Fund* and by the Romanian Government under the contract number POSDRU/89/1.5/S/64109

This paper was partially supported by Grant No. 5 P30 AI036211-15 from NIH, through Baylor International Pediatric AIDS Initiative, subcontract PO 5600167489

## References

1. European Centre for Disease Prevention and Control. Surveillance and Prevention of Hepatitis B and C in Europe. Stockholm: ECDC; 2010.
2. European Centre for Disease Prevention and Control. Annual Epidemiological Report on Communicable Diseases in Europe 2010. Stockholm: ECDC; 2010.
3. Esteban JI, Sauleda S, Quer J. The changing epidemiology of hepatitis C virus infection in Europe. J. Hepatol. 2008; 48:148–162. [PubMed: 18022726]
4. European Monitoring Centre for Drugs and Drug Addiction. Annual report on the state of the drugs problem in Europe. Lisbon: EMCDDA; 2010.
5. Gheorghe L, Csiki IE, Iacob S, Gheorghe C, Smira R, Regep L. The prevalence and risk factors of hepatitis C virus infection in adult population in Romania: a nationwide survey 2006 – 2008. J. Gastrointestin. Liver Dis. 2010; 19(4):373–379. [PubMed: 21188327]
6. European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) and National Antidrug Agency from Romania (NAA). National report on drugs situation for Romania, New developments, trends and in-depth information on selected issues. Bucharest: REITOX; 2010.
7. United Nation on Drugs and Crime. HIV, HBV and HCV Behavioral surveillance survey among injecting drug users in Bucharest, Romania. Bucharest: Speed Promotion; 2010.
8. Rantala, M.; Van de Laar, M. Surveillance and epidemiology of hepatitis B and C in Europe – a review. Eurosurveillance. 2008. http://www.eurosurveillance.org/2008/13/21/reviews/18880
9. Grigorescu M. HCV genotype 1 is almost exclusively present in Romanian patients with chronic hepatitis C. J. Gastrointestin. Liver Dis. 2009; 18(1):45–50. [PubMed: 19337633]
10. Sutton AJ, Hope VD, Mathei C, Mravcik V, Sebakova H, Vallejo F, et al. A comparison between the force of infection estimates for blood-borne viruses in injecting drug user populations across

the European Union: a modelling study. J. Viral Hepat. 2008; 15(11):809–816. [PubMed: 18761605]

11. UNGASS. Country Progress Report for Romania, Reporting period: January 2008–December 2009. Romania: UNGASS; 2010.
12. Bourlière M, Barberin JM, Rotily M, Guagliardo V, Portal I, Lecomte L, et al. Epidemiological changes in hepatitis C virus genotypes in France: evidence in intravenous drug users. J. Viral Hepat. 2002; 9(1):62–70. [PubMed: 11851904]
13. Huppe D, Zehnter E, Mauss S, Boker K, Lutz T, Racky S, et al. Epidemiology of chronic hepatitis C in Germany-an analysis of 10,326 patients in hepatitis centres and outpatient units. Z. Gastroenterol. 2008; 46(1):34–44. [PubMed: 18188814]
14. Cenci M, De Soccio G, Recchia O. Prevalence of hepatitis C virus (HCV) genotypes in central Italy. Anticancer Res. 2003; 23(6D):5129–5132. [PubMed: 14981978]
15. Nemecek V, Strunecký O. Genotypic heterogeneity of hepatitis C virus (HCV) from blood donors in the Czech Republic. Epidemiol. Mikrobiol. Imunol. 2009; 58(2):63–72. [PubMed: 19526920]
16. Chlabicz S, Flisiak R, Kowalczuk O, Grzeszczuk A, Pytel-Krolczuk B, Prokopowicz D, et al. Changing HCV genotypes distribution in Poland - Relation to source and time of infection. J Clin Virol. 2008; 42(2):156–159. [PubMed: 18353714]
17. Svirtlih N, Delic D, Simonovic J, Jevtovic D, Dokic L, Gvozdenovic E, et al. Hepatitis C virus genotypes in Serbia and Montenegro: The prevalence and clinical significance. World J Gastroentero. 2007; 13(3):355–360.
18. Ahmetagić S, Salkić N, Čičkušić E, Zerem E, Mott-Divković S, Tihić N. Hepatitis C virus genotypes in chronic hepatitis C patients and in first time blood donors in Northeastern Bosnia and Herzegovina. Bosnian Journal of Basic Medical Sciences. 2009; 9(4):278–282. [PubMed: 20001992]
19. Paintsil E, Verevochkin SV, Dukhovlinova E, Niccolai L, Barbour R, White E, et al. Hepatitis C virus infection among drug injectors in St Petersburg, Russia: social and molecular epidemiology of an endemic infection. Addiction. 2009; 104(11):1881–1890. [PubMed: 19712125]
20. Tallo T, Norder H, Tefanova V, Krispin T, Schmidt J, Ilmoja M, et al. Genetic characterization of hepatitis C virus strains in Estonia: fluctuations in the predominating subtype with time. J. Med. Virol. 2007; 79:374–382. [PubMed: 17311333]
21. Colina R, Casane D, Vasquez S, García-Aguirr L, Chunga A, Romero H, et al. Evidence of intratypic recombination in natural populations of hepatitis C virus. J. Gen. Virol. 2004; 85:31–37. [PubMed: 14718617]
22. Kalinina O, Norder H, Magnius LO. Full-length open reading frame of a recombinant hepatitis C virus strain from St Petersburg: proposed mechanism for its formation. J. Gen. Virol. 2004; 85:1853–1857. [PubMed: 15218169]
23. Van de Laar TJ, Van der Bij AK, Prins M. Increase in HCV incidence among men who have sex with men in Amsterdam most likely caused by sexual transmission. J. Infect. Dis. 2007; 196:230–238. [PubMed: 17570110]
24. Danta M, Brown D, Bhagani S. Recent epidemic of acute hepatitis C virus in HIV-positive men who have sex with men linked to high-risk sexual behaviours. AIDS. 2007; 21:983–991. [PubMed: 17457092]
25. Pitigoi D, Rafila A, Pistol A, Arama V, Molagic V, Streinu-Cercel A. Trends in hepatitis B incidence in Romania, 1989–2005. Eurosurveillance. 2008; 13(1–3):70–74.
26. Paintsil E, He H, Peters C, Lindenbach BD, Heimer R. Survival of Hepatitis C Virus in Syringes: Implication for Transmission among Injection Drug Users. J. Infect. Dis. 2010; 202(7):984–990. [PubMed: 20726768]
27. Huik K, Sadam M, Karki T, Avi R, Krispin T, Paap P, et al. CCL3L1 Copy Number is a strong genetic determinant of HIV seropositivity in caucasian intravenous drug users. J. Infect. Dis. 2010; 201(5):730–739. [PubMed: 20095832]
28. Ruta SM, Matusa RF, Sultana C, Manolescu L, Kozinetz CA, Kline M, et al. High prevalence of hepatitis B virus markers in Romanian adolescents with HIV. Med. Gen. Med. 2005; 7:68–72.
29. National AIDS Committee. Department for AIDS monitoring from Romania, Statistic data on HIV/AIDS infection in Romania. Bucharest: CNLAS; 2010. (in Romanian)

30. EuroHIV. HIV/AIDS Surveillance in Europe. Mid-year report 2007 No. 76. Saint-Maurice: French Institute for Public Health Surveillance; 2007.

31. Shakarishvili A, Dubovskaya LK, Zohrabyan LS, St Lawrence JS, Aral SO, Dugasheva LG, et al. Sex work, drug use, HIV infection, and spread of sexually transmitted infections in Moscow, Russian Federation. Lancet. 2005; 366:57–60. [PubMed: 15993234]

32. Jabłonowska E, Małolepsza E. Causes of death in HIV-infected patients in the region of Lodz, Poland from 1995 through 2005. Cent Eur J Med. 2009; 4(2):179–183.

33. Joint United Nations Programme on HIV/AIDS. The Changing HIV/AIDS Epidemic in Europe and Central Asia, UNAIDS. Geneva: WHO Library; 2004.

## Table 1

Serological markers of tested patients by the duration of drug use

| Parameter | Subjects N=45 | History of drug use 0 – 9 ys. N=19 (M-16;F-3) | History of drug use >10 ys. N=26(M-23;F-3) | *p* value |
|---|---|---|---|---|
| HCV infection rate | 40 (88.9%) | 16 (84.2%) | 24 (92.3%) | 0.64 |
| Detectable HCV RNA | 26 (57.8%) | 11 (57.9%) | 15 (57.7%) | 1.00 |
| Anti-HBc positive | 25 (55.6%) | 10 (52.6%) | 15 (57.7%) | 0.77 |
| HBs Ag positive | 3 (6.7%) | 2 (10.5%) | 1 (3.8%) | 0.56 |
| Detectable HBV DNA | 4 (8.9%) | 2 (10.5%) | 2 (7.7%) | 1.00 |
| Anti-HIV positive | 0 | 0 | 0 | |
| Anti HTLV positive | 1 (2.2%) | 1 (5.3%) | 0 | 0.42 |

**Table 2**

Characteristics of the patients by age group

| **Age group** | **20–25 ys. (N=14)** | **25–30 ys. (N=18)** | **30–40 ys. (N=13)** | ***p* value** |
|---|---|---|---|---|
| HCV infection rate (anti-HCV positive) | 14 (100%) | 13 (72.2%) | 12 (92.3%) | 0.49 |
| HBV coinfection rate anti-HBc positive | 6 (42.8%) | 11 (61.1%) | 8 (61.5%) | 0.21 |
| HBsAg positive | 1 (7.1%) | 2 (11.1%) | 0 | 1.00 |
| HBV DNA positive | 1 (7.1%) | 3 (16.7%) | 0 | 0.61 |
| Mean HCV viral load (IU/mL ±SD) | $2.1 \pm 4.2 \times 10^5$ | $1.6 \pm 5.5 \times 10^5$ | $6.21 \pm 8.2 \times 10^5$ | 0.13 |
| Mean estimated duration of drug use (ys. ± SD) | 8.4 ± 3.71 | 10.19 ±2.45 | 10.9 ± 3.5 | 0.14 |
| Sharing needles | 5 (35.7%) | 4 (22.2%) | 5 (38.5%) | 0.59 |