Anti-HCV prevalence in the general population of Lithuania

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Source of support: This work was funded by Lithuanian Science and Studies Foundation and by UAB Roche Lietuva

Summary

Background: The aim of this study was to assess risk factors for HCV acquisition and prevalence of anti-HCV in the general population of Lithuania.

Material/Methods: The study enrolled 1528 randomly selected adults from the 5 biggest cities of Lithuania and its rural regions. Screening for anti-HCV was performed by analysis of peripheral capillary blood with lateral flow immunochromatography and confirmation of positive cases by peripheral venous blood testing with 2-step chemiluminescent microparticle immunoassay.

Results: Anti-HCV prevalence in Lithuania is 2.78% and according to the standard European population the adjusted anti-HCV rate is 2.85%. It is more prevalent among men (crude rates: 4.02% males vs. 1.49% females, p=.0030) and this does not depend on age. Vilnius and Kaunas regions have higher infection rates than smaller rural regions (2.92% and 3.01% vs. 2.24%, 0.74% and 1.35%). Nowadays among our population HCV infection spreads mainly via intravenous drug use (OR=42.5, p<.0001). HCV transmission occurs through blood transfusions (OR=6.4, p=.0002), tooth removal (OR=4.1, p=.0048), childbirth (OR=5.0, p=.0224), multiple and a long-term hospitalization (OR=3.0, p=.0064), tattooing (OR=4.4, p=.0013), open traumas (OR=3.7, p=.0009) and intrafamilially (OR=11.3, p=.0002).

Conclusions: 2.78% of the population is anti-HCV-positive. The anti-HCV rate is higher in Vilnius and Kaunas in comparison with other regions. HCV spreads mainly through intravenous drug use, but intrafamilial and some nosocomial routes are also important. The anti-HCV prevalence did not depend on age. Despite active prevention of nosocomial HCV transmission, the incidence of HCV infection does not decrease due to virus spread mostly in “trusted networks” of intravenous drug users.

key words: hepatitis C virus • anti-HCV antibodies • infection • spread

Full-text PDF: http://www.medscimonit.com/fulltxt.php?ICID=882511

Word count: 3067

Tables: 4

Figures: 1

References: 66

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**Background**

Numerous publications present anti-HCV prevalence in particular groups: blood or plasma donors, hemodialyzed and post-transfusion drug users, subjects with high-risk behaviour, health care workers, and patients with liver diseases. Among these groups only data from studies of blood donor volunteers can more or less be treated as a reflection of anti-HCV prevalence in the general population. Such studies were conducted in many European countries shortly after HCV discovery [1–17]. Taking into account risk groups, blood donors’ studies gave a picture of anti-HCV prevalence and routes of hepatitis C virus (HCV) spread in various parts of Europe. A systematic review by Esteban et al summarized those data up to 2007 [18]. According to this comprehensive review, in general European population the lowest anti-HCV prevalence occurs in Northern (0.1–1.0%) and Central Europe (0.2–1.2%). In Southern Europe and Spain anti-HCV prevalence is 2.5–3.5%. The authors stressed that up to now there were no sufficient data about anti-HCV prevalence in Eastern Europe. Naoumov (1999) reviewed and summarized data from Eastern European studies of blood donors, medical personnel and hemodialysed patients [19]. It was ascertainment with reference to blood donor studies that the Eastern European population has a high prevalence of HCV infection – from 0.68% in Czech Republic to 4.9% in Romania. Later publications from this European region have confirmed this conclusion, but there are still no population-based studies published from Eastern European countries [20–22].

Prevalence of HCV infection in Lithuania has been studied in small groups of first-time blood donors, acute viral hepatitis patients and in risk groups (2.2% in first-time blood donors, 7.9% in commercial donors, 13.9% in commercial plasma donors, 48.3% in hemodialysed patients, 29.4% in prisoners, 9.4% in elderly nursing home residents, and 7.9% in hemodialysis staff) [23,24]. These data did not provide a clear picture of anti-HCV prevalence in the general population, but it allowed estimation of prevalence at from 2% to 3%.

We present a descriptive epidemiological study, prepared according to CONSORT [25] and STROBE [26,27] recommendations, aimed at assessing age-specific risk factors for HCV acquisition and prevalence of anti-HCV in the general population of Lithuania.

**Material and Methods**

**Study design**

An observational analytical cross-sectional study design was conducted to clarify the prevalence of anti-HCV and HCV transmission routes in the general population. Cross-sectional survey of random anonymous volunteers and testing them for anti-HCV was conducted May 17-22, 2010 in 5 cities of Lithuania (Vilnius, Kaunas, Klaipėda, Šiauliai, and Panevėžys) and corresponding rural regions where 75% of the population live (2 513 313 out of 3 329 039, according to the Lithuanian Statistical Department; http://www.stat.gov.lt/lt/).

**Study population**

According to data on anti-HCV prevalence in Eastern Europe and the study by Ambrozaitis et al. on Lithuanian blood donors and some risk groups [23,24], we expected the prevalence of anti-HCV in Lithuania to be approximately P=3%±1%. After calculation of an adequate sample size with precision 1% (d=0.01) and level of confidence of 95% (Z=1.96), a cohort of n=1118 subjects was estimated by the formula $n=(Z^2P(1-P))/d^2$ [28].

People over age 18 who were willing to be tested for anti-HCV were voluntarily enrolled into the study by a convenience sampling of shoppers at one of the biggest supermarkets in Vilnius, Kaunas, Klaipėda, Šiauliai and Panevėžys. The only eligibility criteria were age ≥18 and residence in the selected cities and/or corresponding rural regions.

During this study, 1528 randomly selected adults of various ages were enrolled. Anti-HCV testing was wrongly performed or questionable for 14 (0.9%) participants or a tested person refused or could not repeat it. Anti-HCV tests was suitable for evaluation in a total of 1514 (99.1%) subjects (572 men and 942 women) – 651 (241 men and 410 women in Vilnius), 266 (128 men and 138 women in Kaunas), 313 (115 men and 198 women in Klaipėda), 148 (55 men and 93 women) in Panevėžys, and 136 (53 men and 103 women) in Šiauliai (Figure 1).

**Data collection**

The study was performed according to the World Medical Association Declaration of Helsinki, and all procedures were approved by the Lithuanian Bioethics Committee. All volunteers who wanted to know their anti-HCV status signed informal consent and answered an anonymous questionnaire with demographic data (age, sex, education, occupation, residence) and exposure to HCV risk factors (intravenous drug use, blood transfusions, blood donations, hemodialysis, tattooing, piercing, open trauma, surgery, multiple and the long-term hospitalizations, HCV infection among family members, dentistry, tooth removal, childbirth, abortions, and risky sexual behaviour).

Screening for anti-HCV was performed on the peripheral capillary blood using rapid lateral flow immunochromatography test (Core HCV-WB, Core Diagnostics, Birmingham B2 5HG, UK) with 99.99% diagnostic specificity and 100% sensitivity. All anti-HCV-positive subjects were admitted for further testing at the Centre of Hepatology, Gastroenterology and Dietetics of Vilnius University Hospital "Sanatoriškių klinikos", where anti-HCV were confirmed with 2-step chemiluminescent microparticle immunoassay (Architect System anti-HCV; Abbott, 65205 Wiesbaden, Germany).

The following limitations of such data collection are: a. Free anonymous testing for anti-HCV might be more attractive for having exposure to HCV risk (for example: intravenous drug users, subjects having family member infected with HCV) rather than for persons without suspected exposure. That is why detected anti-HCV prevalence can occur been higher than it really is. Such subjects could also be not fully overt in indication of possible risk factors, although the questionnaire used was fully anonymous. This can possibly distort data on HCV acquisition routes. b. It was not possible to control age and sex proportions of the participants. That is why in a study cohort we had 37.8% men and 62.2% women; although in the general population we have 45% men and 55% women. In the
study cohort the age distribution differed from the general population. All those limitations were taken into consideration during statistical analysis of our data.

**Statistical evaluation**

The anti-HCV rate was calculated separately for men and women on age-dependent strata. Crude rate in age-dependent strata and in the study population overall were calculated as a mean of rates among men and women.

Age- and sex-dependent standardized anti-HCV rate in the general population was calculated using the direct standardization method and separate calculation of standard vectors for men and women in age-dependent strata (\(w_i = n_i / N\) where \(n_i\) – number of males or females on age-dependent stratum, \(N\) – population overall) [29]. The standardized rate was calculated as a sum of proportional rates. This rate was further standardized to the European population.

Comparison of different routes of exposure was evaluated with Fisher's exact test. For every statistically significant (\(p \leq 0.05\) HCV route, an odds ratio was estimated using univariate logistic regression analysis with 95% confidence interval.

Differences in anti-HCV positivity between men and women on age-dependent strata were evaluated with Fisher's exact test.

**RESULTS**

**Anti-HCV positivity**

Among 1514 tests, 37 (2.44%) anti-HCV-positive subjects were detected (25 men, 14 women), of these there were 3
(8.1%) people (2 from Vilnius region and 1 from Klaipėda) who knew their anti-HCV status because HCV infection had been diagnosed previously and/or they were treated for chronic hepatitis C, and 34 (91.9%) had never been tested for anti-HCV (Table 1).

In our cohort, anti-HCV positivity did not depend on age (p=.7216), but more men than women had been exposed to HCV (crude rates: 4.02% vs 1.49%, p=.0030). The mean of anti-HCV rates was 2.76% and was similar to the rate in the general population (2.78%).

After direct standardization of sex-adjusting rates by age distribution of standard European population prevalence of anti-HCV, a rate of 2.85% was obtained.

We did not standardize the anti-HCV rate of our cohort to the world population because the population of Lithuania is older and anti-HCV prevalence dependence on age was not ascertained.

In the study cohort anti-HCV prevalence depended on region of the country. Rural Lithuanian regions are less affected by HCV infection than the capital and bigger cities (Table 2).

**HCV infection routes**

After analyzing the questionnaire data, we found that intravenous drug use is the most important risk factor for HCV spread in our population (Table 3).
Blood transfusions, tooth removal, childbirth, and long-term or multiple hospitalizations can be assigned to HCV acquisition risks (Table 3). Statistically, other healthcare procedures (blood donation, hemodialysis, surgery, dentistry, abortions) are not associated with HCV acquisition. People who had a single surgery had generally similar rates of infection to the general population, but people having more than 2 surgeries were more often anti-HCV positive (Table 4).

Nine people had HCV-infected relatives. Among the 5 (19.2%) anti-HCV-positive people, 2 had HCV-infected fathers, 1 had an HCV-infected sister, 1 had an HCV-infected wife, and 1 had an HCV-infected husband. Among the 4 (1.4%) anti-HCV-negative people, 2 had HCV-infected fathers, 1 had an HCV-infected brother, and 1 had an HCV-infected sister. Risky sexual behaviour was not confirmed as a risk factor (Table 3).

Being rather often declared, piercing was ascertained as a safe procedure concerning HCV spread, but the tattoo is not safe. Open traumas were also confirmed as an HCV acquisition risk.

**Discussion**

Based on the results of this study we estimate that the anti-HCV prevalence in Lithuania is 2.78%, and adjusted to the standard European population the anti-HCV rate is 2.85%.

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**Table 3. Risk factors of HCV acquisition.**

| Risk factor                             | Anti-HCV positive, n/% | Anti-HCV negative, n/% | Fisher exact test, p | Univariate logistic regression OR | p      |
|-----------------------------------------|------------------------|------------------------|---------------------|-----------------------------------|--------|
| Risky sexual behaviour                   | 0/0                    | 8/2.35                 | NS                  |                                   |        |
| Intravenous drug use                     | 8/27.6                 | 3/0.9                  | 7.98×10⁻¹⁰          | 42.54                             | <.0001 |
| Blood transfusions                       | 7/24.1                 | 16/4.3                 | .0009               | 6.424                             | .0002  |
| Blood donations                          | 10/33.3                | 80/21.7                | NS                  |                                   |        |
| Hemodialysis                             | 1/3.7                  | 1/0.3                  | NS                  |                                   |        |
| Tattoo                                   | 8/27.6                 | 27/7.3                 | .0029               | 4.416                             | .0013  |
| Piercing                                 | 8/27.6                 | 184/49.9               | .0066               | 0.323                             | .0085  |
| Open trauma                              | 13/44.8                | 59/17.7                | .0013               | 3.733                             | .0009  |
| Surgery                                  | 18/62.1                | 179/52.8               | NS                  |                                   |        |
| More than one surgery                     | 4/30.8                 | 37/24.3                | .0196               |                                   |        |
| Multiple and long-lasting hospitalizations| 11/37.9                | 56/16.7                | .0099               | 3.056                             | .0064  |
| Dentistry                                | 22/75.9                | 243/71.5               | NS                  |                                   |        |
| Tooth removal                            | 24/82.8                | 182/53.7               | .0029               | 4.141                             | .0048  |
| Childbirth                               | 7/70.0                 | 54/31.6                | .0324               | 5.056                             | .0224  |
| Abortions                                | 1/10.0                 | 23/13.5                | NS                  |                                   |        |
| HCV infection in family member            | 5/19.2                 | 4/1.4                  | .0008               | 11.270                            | .0002  |

**Table 4. Multiple surgeries and anti-HCV positivity.**

| Total                               | Number of surgeries | Number of surgeries |
|-------------------------------------|---------------------|---------------------|
| Anti-HCV positive, n/%              | 0                   | 1                   | 2                   | 3                   | 4                   | 5                   | 6                   | p       |
| 13/7.9                              | 0                   | 9/69.2              | 0                   | 2/15.4              | 1/7.7               | 0                   | 1/7.7               | .0196   |
| Anti-HCV negative, n/%              | 152/92.1            | 1                   | 114/75.0            | 26/17.1             | 7/4.6               | 2/1.3               | 2/1.3               | 0       |

Blood transfusions, tooth removal, childbirth, and long-term or multiple hospitalizations can be assigned to HCV acquisition risks (Table 3). Statistically, other healthcare procedures (blood donation, hemodialysis, surgery, dentistry, abortions) are not associated with HCV acquisition.

People who had a single surgery had generally similar rates of infection to the general population, but people having more than 2 surgeries were more often anti-HCV positive (Table 4).
This rate is a bit higher than was expected. Perhaps the free anonymous testing provided by our study was more attractive for people possibly having exposure to HCV (eg, intravenous drug use, having a family member with HCV infection) than for those without such risks.

Our estimated anti-HCV prevalence is similar to that in Russia, but it is higher than in Poland, Estonia and Latvia [18]. However, the conclusion related to anti-HCV prevalence in Eastern European countries was based on studies of blood donors, as in case of Poland [22,30], or on the basis of personally communicated data, as in case of the Baltic states [19].

We could not find a single observational study presenting anti-HCV prevalence in the general populations of Estonia, Latvia, Poland or in other neighbouring countries (Russia, Byelorussia or Ukraine). In Lithuania blood donors, some small risk groups and chronic hepatitis C patients were investigated [23,31–34]. Similar data are obtainable in Latvia and Estonia [35–39].

The data on anti-HCV prevalence from a large cohort of 4216 healthy people from various regions of Russia and Mongolia were presented in 1996 [40]. According to this study, anti-HCV prevalence in those regions varied from 0.7% in Central Russia to 10.7% in Mongolia. There is no current data about HCV infection spread in Russia including the Kaliningrad region where an explosion of intravenous drug abuse was observed during the last decade. Namely this Russian region has a common border with Lithuania.

A study of northern Ukrainian schoolchildren and blood donors found a 0.8% anti-HCV prevalence for schoolchildren and 2.3% for blood donors; anti-HCV prevalence directly correlated with the age – people ages 40–49 years had 4.0% positivity, while those older than 60 years had 6.25% [41]. Those data were published in 1993 and do not reflect the present situation of HCV infection in Ukraine. In addition, the correlation of anti-HCV prevalence with age was not reported anywhere in the literature, nor did we find such correlation in our cohort.

In Byelorussia, 1.26% anti-HCV prevalence was reported without reference to the source [21].

On the basis of the above-mentioned publications, Esteban and co-authors presented anti-HCV prevalence in Eastern Europe (Figure 1 in review) [18]. However, these data are not sufficient to provide a true picture of anti-HCV prevalence or the number of HCV carriers in this region. Those authors presumed that anti-HCV prevalence could be higher not only in Eastern Europe, but also in the general European population because there are not sufficient current data [18]. Epidemiological studies of the general population would be useful in order to clarify the situation of HCV infection in Europe in light of increasing intravenous drug abuse.

According to the obtained data, the main HCV infection route in Lithuania is currently intravenous drug use (OR=42.5). In our country about 80% of intravenous drug users are infected with HCV and men more often than women engage in this behavior [42]. This could explain the higher rate of anti-HCV positivity found in men of our cohort. Intravenous drugs are more accessible in cities (especially in the capital); accordingly, we found lower anti-HCV prevalence in rural regions.

Observations and data of other investigators taken together reinforce the fact that intravenous drug use is now the principal route of HCV spread in the European population [18,33].

Former nosocomial routes such as blood donations, surgeries, and hemodialysis seem to be well controlled in our country. Nevertheless, blood transfusion can still be a source of HCV (OR=6.4). In Lithuania every donor’s blood has been tested for anti-HCV since 1994 and since 2004 for HCV RNA. Sufficient HCV RNA quantity for detection appears only after 4–5 days of HCV infection. HCV RNA is tested separately in every donation, not in pools, and NAT sensitivity is 3.01 IU/ml. Still, there exists a possibility for HCV transmission with blood or its products. Safety of donor’s blood is still a worldwide health care problem which could be partially solved by introducing more sensitive NAT methods. However, it will not be solved completely, especially in the case of paid donations [31,32,43].

Tooth removal was also confirmed as an HCV transmission route in our population. The possibility of HCV transmission through saliva is questionable, but it cannot be ruled out. Taking into consideration the HCV RNA presence in saliva of 35–55% of chronic hepatitis C patients, there is a possibility to transmit HCV to dentists and others [44–49].

Childbirth, being a risk factor for HCV acquisition, was an unexpected finding for us. This HCV infection route was not investigated. Anti-HCV prevalence among pregnant women and mother-to-child HCV transmission have been discussed in the literature [50–54]. The number of anti-HCV-positive women in our cohort was only 14 and only 10 answered the question about childbirth – 7 (70%) had children – and 171 anti-HCV-negative women, answered a question about childbirth – 54 (32%) had children. So, the small group of anti-HCV-positive women in our cohort may indicate why childbirth became statistically significant as an HCV risk factor. We failed to find a single study of anti-HCV prevalence in women before and after childbirth. Such a study would be useful to confirm or deny our controversial finding and clarify a possible source of HCV infection if this exists in the maternity clinic.

Confirming that multiple and long-term hospitalization is an HCV risk factor raises the question of the specific route of transmission in hospitals [55]. The possible infection source can be asymptomatic HCV carriers who are admitted for surgery or conservative treatment, for example, heart disease, and they are not tested for HCV by diagnostic algorithm. A health care worker infected with HCV but not diagnosed can also be a source of infection [56–60].

There were only 2 patients in the study cohort who underwent hemodialysis: 1 was anti-HCV positive and the other one was not. Such limited data does not allow us to draw conclusions, but studies before 1995 indicated to a high prevalence of anti-HCV in hemodialyzed patients [23].

It is necessary to pay attention to a new risk factor for HCV acquisition in our population – HCV infection among family
members (OR=11.3). Nine people (5 anti-HCV-positive and 4 anti-HCV-negative) indicated presence of HCV infection in family members (usually it is sister or brother, but also it can be a spouse or parent); it confirmed a possibility of household and/or sexual HCV acquisition. However, risky sexual behaviour was not proved to be an HCV acquisition route. Still, intrafamilial HCV spread is not well elucidated, although it has been recorded and studied since the early 1990s. It was only recognized that family members of HCV-infected patients had a 3- to 5-fold higher prevalence of anti-HCV than the general population and are often infected with the same HCV strain [61-64].

Open traumas with a big blinding area can be considered as a risk for HCV acquisition but its reasons are unclear (OR=3.7). Receiving a tattoo is a risk factor for HCV infection (OR=4.4), possibly because of violation of sanitary norms during the procedure.

Our previous study of chronic hepatitis C cohort [33] found a changing trend in HCV transmission, from nosocomial to less controlled intravenous drug use or HCV infection in family member. HCV infection source depended on age of patients. We found that older patients often get HCV during surgeries or long-term and multiple hospitalizations, while younger ones get it through intravenous drug use and tattooing.

Considering the asymptomatic course of infection, a high HCV rate in intravenous drug users and existence of their "trusted networks", a new peak of HCV infection can be expected in the near future [65,66]. This is important not only for Lithuania, but also for other European countries where intravenous drug use is becoming a core of the HCV epidemic [18].

HCV spread through intravenous drug use is not only a healthcare problem; it is a social problem as well. It cannot be resolved only with prevention or other healthcare tools, especially in the absence of effective immunization. Only a complex of coordinated means directed to intravenous drug use prevention, public education and preventive efforts can be effective in prevention of HCV spread.

**Conclusions**

In our population, anti-HCV prevalence is 2.78%; adjusted to the standard European population, prevalence is 2.85%. This rate is higher in Vilnius and Kaunas in comparison with other regions of Lithuania.

The anti-HCV prevalence does not depend on age.

The main HCV transmission route is intravenous drug use; intrafamilial transmission is significant as well.

Blood transfusions, tattooing, open trauma, tooth removal, uncertain nosocomial transmission during multiple and long-term hospitalizations and childbirth are also HCV infection routes.

Despite active prevention of nosocomial HCV transmission, the incidence of HCV infection does not decrease, due to virus spread in "trusted networks" of intravenous drug users.

Nowadays, HCV transmission control cannot be carried out without controlling intravenous drug use and active prevention educational tools.

**Acknowledgements**

The authors acknowledged Prof. Jolanta Dadoniene and Dr. Viktor Skorniakov for invaluable help in proper statistical analysis of the obtained data. The authors also express gratitude to the State Science and Study Foundation and UAB Roche Lietuva for financial support of this work.

**Conflict of interest**

The authors who have taken part in this study declare that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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