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Prevalence of hepatitis C in the general population in the Netherlands

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ABSTRACT

Background: Chronic hepatitis C virus (HCV) is transmitted by blood-blood contact and this leads to high HCV prevalence in risk populations such as haemophilia patients and intravenous drug users. The prevalence in the general Dutch population is unknown, although it appears to be very low in screened blood donors (0.0169%).

Aim: The objective of this study is to estimate the prevalence of HCV in a general population sample living in an urbanised region in the Netherlands.

Methods: We randomly selected 2200 EDTA blood samples that had been submitted for analysis of biochemical parameters to a regional servicing laboratory for general practitioners (SHO, Arnhem/Nijmegen, the Netherlands). HCV antibody testing was performed using a three-step approach. For initial screening, an enzyme immunoassay (Bioelisa HCV 4.0, Biokit, Spain) was used. Positive samples were subjected to a second, microparticle enzyme-linked immunoassay (AxSYM HCV version 3.0, Abbott laboratories, IL, USA). Genotypes were determined by Line Probe Assay.

Results: A total of four persons (two females, two males) (0.2%) tested positive for HCV antibodies. The average OD/cut-off ratio of the screening assay was 2.9 (range 1.0 to 7.3) and serological findings were confirmed using a specific second immunoassay. HCV RNA (genotype 1b) was found in the sera of two persons.

Conclusion: The HCV prevalence in our sample of the Dutch population was 0.2% which accords with earlier estimates from prevalence studies in the Netherlands.

KEYWORDS

Hepatitis C, prevalence, the Netherlands

BACKGROUND

Hepatitis C virus (HCV) is mainly transmitted through contact with blood and blood products. The majority of HCV-seropositive individuals will have persistent viraemia. More than half of all patients will develop chronic hepatitis, and in 20% infection will lead to cirrhosis with all the subsequent complications, such as ascites, encephalopathy, variceal bleeding and hepatocellular carcinoma.¹ Chronic HCV infection often runs an asymptomatic course and only 25 to 30% of infected persons seek medical attention for symptoms attributable to HCV infection.² Early detection is of key importance in order to prevent complications of HCV-related liver disease. The WHO estimates that 3% of the world's population is HCV-infected and in the USA it is a leading cause of liver transplantations.^{3,4} It is thus a significant clinical problem. However, there is wide variation in HCV prevalence in different parts of the world. For example, the prevalence in Scandinavia is less than 0.5%, whereas the prevalence in Egypt is over 20%.⁵

In the UK, the number of new HCV diagnoses rose from 2116 in 1996 to 7580 in 2005. Hospital admissions, transplants, and deaths related to HCV increased, and deaths from end-stage liver disease rose from 76 in 1997-8 to 216 in 2004-5. These results suggest that the number of people at risk for HCV-associated morbidity or mortality will double over the next decade in the UK.^{6,7}

In the Netherlands there have been only a few studies that focus on the prevalence of HCV. These studies were generally limited to high-risk populations, such as intravenous drug users and haemophilia patients. Some 54% of Dutch haemophilia patients are HCV carriers, and up to 74% of iv drug users are infected.⁸ In 2000, two nationwide prospective surveys among 2281 and 2286 dialysis patients resulted in an HCV prevalence of 2.9 and 3.4%, respectively.⁹

The prevalence in the general population is estimated to be much lower (0.1 to 0.4%). However, this is a crude estimation based on extrapolation of the prevalence among selected high- and low-risk groups.^{10,11} Thus, the actual prevalence in the population at large is unknown, hence the need for population-based serological studies. These data are desirable because they allow medical professionals and policymakers to develop and evaluate efforts with respect to treatment and prevention.

Therefore, the aim of this study is to determine the prevalence of HCV infection and the distribution of genotypes in the general Dutch population in urbanised regions of the East-Netherlands.

MATERIALS AND METHODS

Patients and setting

Data for the present study were collected prospectively from 2200 persons visiting general practices in the urbanised regions of Arnhem/Nijmegen in June 2006.

Patients had been referred to a servicing laboratory (SHO) by the general practitioner for analysis of biochemical parameters. After determination of the desired parameters, the remaining blood samples were stored at 4°C until transport to the laboratory of Radboud University Medical Centre in Nijmegen. All aspects of the protocol were reviewed and approved by the local medical ethical committee (CMO) Arnhem/Nijmegen, the Netherlands.

Laboratory methods

Immediately after arrival to the laboratory, samples were centrifuged. Sera were then stored in aliquots at -80°C.

Antibody testing

HCV antibody testing was performed using a three-step approach. For initial screening, an enzyme immunoassay (bioelisa HCV 4.0, Biokit, Barcelona, Spain) was used. Microtitre plates are coated with recombinant HCV antigens including core, NS3, NS4 and NS5. The cut-off value was determined by multiplying the mean optical density (OD) value of the low positive control by 0.9. Ratios of sample OD value and cut-off value of >1 were considered positive. Positive samples were further tested by a second, commonly used microparticle enzyme-linked immunoassay (AxSYM HCV version 3.0, Abbott Laboratories, IL, USA), because the Biokit assay is considered as sensitive as the AxSYM but somewhat less specific according to the information supplied by the manufacturer. Positive results in the AxSYM assay were then further tested using a recombinant immunoblot assay (INNO-LIA HCV™ Score, Innogenetics NV, Gent, Belgium).

Hepatitis C RNA detection and genotyping

Antibody positive samples were tested for the presence of viral RNA and the HCV genotype. For isolation of HCV RNA the COBAS® AmpliPrep (Roche Molecular Systems, Branchburg, USA) was used according to the manufacturer's instructions. Isolated HCV RNA was detected using the COBAS® TaqMan® HCV Analyzer real time PCR. Results are given in IU/ml. The detection range lies between 15 and 7×10^7 IU/ml. Hepatitis C genotype was determined using a Line Probe Assay LiPA, based on sequence variations found in the 5' untranslated region of the different HCV genotypes (VERSANT HCV Genotype Assay, Bayer HealthCare LLC, USA) as described before.¹² All assays were performed according to the manufacturer's instructions.

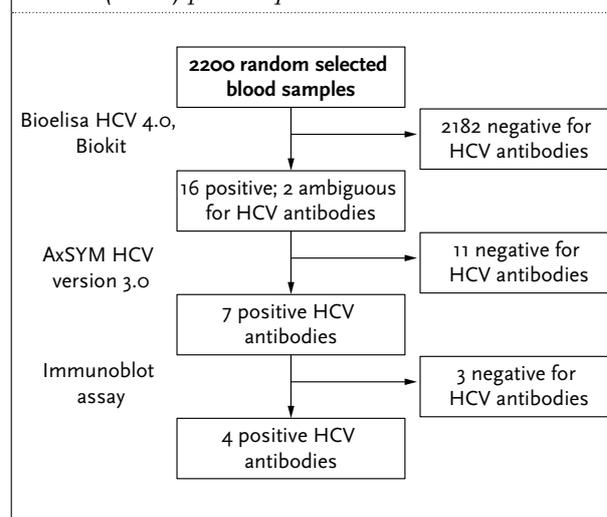
Statistical analysis

Quantitative results are reported as the mean ± standard deviation, and qualitative results are given as percentages. For comparison of proportions between groups, χ^2 , Fisher tests and independent T-tests were used. If a p value was below 0.05, the difference between proportions was considered statistically significant.

RESULTS

In total 2200 subjects were tested on HCV antibodies. This group consisted of 1254 (57%) females and 928 (42.2%) males with a mean age of 60.4 years (SD = 16.6). Demographical data on 18 patients were lacking, but they tested HCV negative using our testing strategy. *Figure 1* shows the strategy we followed to determine the HCV positivity.

Figure 1. Flowchart of strategy to determine hepatitis C virus (HCV) positivity



In the initial screening using the Biokit assay, 16 out of 2200 (0.7%) subjects had a positive test result with OD values varying from 1.016 to 8.466. Results from two additional samples were ambiguous with values of 0.919 and 0.949. There were no significant differences with respect to age and gender of subjects between those with a positive and those with a negative ELISA ($p=ns$) (table 1).

Table 1. Anti-hepatitis C virus (HCV) prevalence tests according to gender and age

| | HCV test 1 | | HCV test 2 | |
|-------------------|----------------------|--------------------|----------------------|-------------------|
| | Negative (n=2166) | Positive (n=16) | Negative (n=2178) | Positive (n=4) |
| Age* (mean/SD) | 60.4 (16.6) | 61.4 (17) | 60.4 (16.6) | 60.0 (16.1) |
| n% females** | 1244 (57.1) | 10 (0.8) | 1252 (57.5) | 2 (50) |
| n% males** | 922 (42.3) | 6 (0.6) | 926 (42.5) | 2 (50) |

*No age information about seven subjects, **no gender information about 11 subjects.

These 16 positive and two ambiguous samples were subjected to further testing using an AxSYM assay. This established the presence of HCV antibodies in seven out of 16 positive samples (OD values of 37.36 to 82.82). Next we performed an immunoblot assay to confirm the HCV infection and were able to confirm the presence of specific anti-HCV antibodies in these four samples (two males, two females). Thus, the HCV prevalence in this sample can be estimated at 0.2%. Lastly, we searched for HCV RNA in these samples and detected viral RNA in two out of four samples. Both samples contained genotype 1b.

In order to test the robustness of our findings, we designed a novel study using our cohort and the dataset of an earlier study as a replication cohort.^{15,16} We detected a positivity rate of 4/2200 while the other study found 6/7373 positive.^{15,16} Next we calculated whether both datasets were statistically different. Using Fisher's exact test we found that they were not ($p=0.2$). Subsequently we combined the datasets which yield a much larger cohort of 9573, containing ten HCV-positive patients. The new prevalence was 0.10%, with a calculated 95% confidence interval of 0.039 to 0.17%.

DISCUSSION

We performed a cross-sectional study to assess the epidemiology of HCV infection and we were able to estimate the prevalence at 0.2% among the general population residing in the eastern part of the Netherlands. Most of the previous research in this field had been

conducted in high-risk groups or in blood donors, which may over- or under-estimate the actual burden of HCV infection in the general population. How do these data compare with other studies in this field?

The prevalence (0.2%) from this study is appreciably higher compared with healthy blood donors from the Netherlands. In 2001 approximately 0.0169% of new blood donors tested positive for HCV antibodies.¹³ In comparison, the HCV prevalence rates among healthy blood donors range from 0.01 to 0.02% in the North-Western Europe, to 1 to 5% in Southern Europe.¹⁴

While interpreting these results, it is important to realise that blood donors are a self-selected group of individuals who have lower rates of blood-borne infections compared with the general population. Many of the people with risk factors for infection due to HCV (parenteral drug addiction, previous history of hepatitis) are rejected as blood donors. Therefore, these prevalence rates probably underestimate the actual HCV carrier rate.

To this end, in another Dutch study a nationwide call was sent out for volunteers to give sera. The general public was invited by postal request to donate blood samples and the study went to great lengths to obtain a well-balanced age mix among the different geographic regions. This study measured HCV antibodies among a sample of 7373 volunteers and found that only six tested anti-HCV positive (0.08%).^{15,16} When we combined this dataset with our own data, the cohort now encompasses a sample of 9573 patients and yields an HCV prevalence of 0.10% (95% CI 0.039 to 0.17%).

In our study, we analysed sera obtained from subjects who underwent blood analysis for various reasons requested by their general practitioners in the Arnhem/Nijmegen region. This is a potential source of bias as we selected subjects who had a reason to go to their general practitioner and have blood taken. However, blood analysis in primary care is a common procedure, often used for the exclusion of severe disorders or for the reassurance of the physician or the patient that there is no severe pathology of underlying symptoms.

As the incidence of serious diseases is low in the general practice population, it can be assumed that the observed prevalence of HCV is representative for the general population.¹⁷⁻¹⁹

This study was performed in the eastern part of the Netherlands, which is an urban region with a smaller immigration population of 13.7% in Gelderland compared with 25.1% in the Utrecht/North-Holland/South-Holland regions, which may affect the findings.²⁰

Anti-HCV testing is performed in different settings, including hospitals, other healthcare facilities and also for screening purposes. Therefore the most desirable HCV screening is the test that has a very high specificity.

Consequently, this might lead to a lower sensitivity. Therefore in line with recommendations published elsewhere we decided to perform a second confirmatory ELISA in the positive samples.²¹ Indeed, this was beneficial as it led to exclusion of the suspicion of HCV infection in nine of the 16 samples (56%).

It is possible that a larger sample would have led to different results but in view of the abovementioned studies it is probable that the real HCV prevalence in the Netherlands is well below 0.5%, though we cannot exclude that there is a wide region-region variation.

Other possible limitations include the absence of detailed information on whether these patients were aware of their infection and whether they had had treatment. In addition, HCV RNA could not be detected in two of the four subjects with HCV-positive antibodies. This can be explained by a decline in viral load during storage of the tubes or because the two subjects had cleared the HCV. It is known that around 20% of patients spontaneously clear HCV.⁴

The genotypes detected in our sample (1b) is the most common genotype in the Netherlands. A recent study performed by de Vries *et al.* showed that genotype 1 is found in approximately 50% of HCV patients in the Netherlands.²²

How does this prevalence compare with other countries? In the USA, the prevalence of HCV infection is estimated at 1.6%.³ Various studies performed throughout Europe on the prevalence of HCV in general population indicate prevalences from 0.63% in Germany, 0.9% in Belgium to 1.2% in France.²³⁻²⁵ In Southern Europe the prevalence varies from 1.6% in Spain to 4.8 to 26.0% in Italy.^{26,27} The HCV prevalence is higher in Southern Italy compared with Northern Italy. The large variation is most probably due to differences in the quality of the healthcare system. In the recent past, healthcare facilities in Southern Italy made extensive use of glass syringes, and/or non-sterile syringe use facilitating nosocomial HCV transmission.²⁸

Our results show lower HCV infection rates in the Dutch population compared with those found in other countries in Europe. Further, this accords with a North-South gradient in HCV prevalence in Europe.

CONCLUSION

The current study provides information on HCV prevalence in the general population. We found an HCV prevalence of 0.2% in the general population. Combining our data with other observations provides a point estimate of 0.10%, with a 95% confidence interval of 0.039 to 0.17%, which is clearly lower than in other European countries.

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New developments in the treatment of systemic lupus erythematosus

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Systemic lupus erythematosus (SLE) is the prototype of a systemic autoimmune disease. Many organ systems can be affected and a multitude of autoantibodies can be present. Although the aetiopathogenesis of the disease has not been fully elucidated yet, there is increasing evidence that genetic factors, next to environmental influences, play a major role. Certain polymorphisms in genes that are involved in immune responsiveness appear to be skewed in lupus. In recent years particular attention has been given to the role of apoptosis in lupus. Defective clearance of apoptotic cells leads to accumulation of these cells. In addition, intracellular antigens are, whether or not in modified form, expressed on the surface of these apoptotic cells. Handling of these (antigenetically modified) apoptotic cells by macrophages/dendritic cells may result in (auto)immune responses to these intracellular antigens. Thus, together with other developments, new insights have been gained into the pathogenesis of SLE.

Besides these breakthroughs in pathogenesis, new treatment modalities have become available for SLE. Although corticosteroids and immunosuppressives are still the mainstay of treatment in SLE, biologicals are now being tested with great promise for the future. As mentioned, aberrant B-cell activity with production of numerous autoantibodies appears to underlie many clinical characteristics of lupus. B-cell targeting via monoclonal antibodies in various forms is now a realistic goal in SLE. Interference in co-stimulation by small molecules or blocking B-cell activating factors is another way of inhibiting (autoreactive) B-cells. So, the horizon is open for many new exciting clinical trials in SLE. All of these new developments in pathogenesis and treatment of SLE will be discussed in depth during the Seventh European Lupus Meeting that will be held in Amsterdam, 7-10 May 2008. The programme promises exciting news on autoimmunity in general and SLE in particular. For more information: www.lupus2008.nl.