Introduction

Hepatitis C is among the main causes of chronic liver disease and can progress to conditions such as cirrhosis and hepatocellular carcinoma. Among the main risk factors for virus transmission are injecting drug use, occupational exposure, intravenous therapy and blood transfusion [1, 2]. Hepatitis C is of great importance for public health and health support services, since the infection may present asymptomatic for an extended period of time, besides the possibility of chronicification, increasing the chances of transmission [3].

Data released by the World Health Organization in the Global Hepatitis Report, 2017, show that chronic HCV infection affected around 71 million people worldwide in 2015 [4]. In Brazil, the number of cases of hepatitis C remains high, especially in the south, whose capital, Porto Alegre, was the highlight among other Brazilian capitals in 2016, with 94.1 cases of hepatitis C per 100,000 inhabitants [5].

Hepatitis C screening is obligatory in Blood Bank for each donation of blood, independent of previous donations; this procedure became obligatory in hemotherapy services from 1993, including serological screening and optional molecular screening [6]. Later, in 2014, virus nucleic acid amplification (NAT) testing by molecular biology technique was also mandatory [7]. Screening tests for hepatitis C with two different methodologies, serology and NAT, were required from the Ministerial Directive #2712/2013 by Health Ministry [8].

In view of the prevalence of hepatitis C, effective monitoring of HCV seroprevalence in blood donors is essential for transfusion safety; however, this process causes a significant economic impact to the blood bank, resulting in the disposal of blood components, cost with operational and human resources. Therefore, it is a great challenge for hemotherapy to meet the demand, to guarantee the quality of the production of blood components, to improve its processes and to establish its public health responsibility in the conduct with the donor [9].

Therefore, the objective of this study was to draw a profile of the candidates for blood donation in the hemotherapy service of the Santa Casa de Misericórdia Hospital in Porto Alegre in relation to the HCV infection, using the information from the service database, as well as to the risk factors for HCV, as well as comparing the methodologies used for laboratory screening, after the implementation of the nucleic acid test (NAT), thus providing a discussion on relevant questions concerning the screening of hepatitis C in the blood bank.

Method

This is a retrospective transversal study, using secondary data referring to pre screening, clinical screening and laboratory screening, archived in a hemotherapy database of the Santa Casa de Misericórdia Hospital in Porto Alegre, after the implementation of the NAT, started in April 2014.
The pre-screening consists of measuring the pulse; body mass, blood pressure, hematocrit or hemoglobin. The clinical screening is performed through an interview about important parameters, contained in the technical regulation of hemotherapy procedures (Ordinance No. 158), related to the risk of the candidate’s inability to donate.

With the purpose of delimiting the inclusion of information in this research, clinical screening was classified into risk factors related to HCV (HCV RF) and other factors (RO). HCV-related risk factors included the presence of a tattoo, use of piercing, large surgery in the last 6 months, contact with hepatitis, sexually transmitted diseases, dental procedures, risk behavior (sharing of syringes, sexual risk behavior; accidents related to occupational activities ...), drug use, hepatitis after 11 years and transfusional history. However, other factors included the frequency and range of donations, minimum and maximum age allowed, body mass, pulse measurement, blood pressure measurement, hematocrit, hemoglobin, medical and gestational history, pathological antecedents, medications in use, consumption of alcoholic beverages, allergic episodes, habitual occupation and volume to be collected.

Once considered eligible for clinical screening, these donor candidates proceed to the next phase, where blood donation and sample collection are performed, which are then referred for laboratory screening.

All donors reported between April 2014 and July 2016 were included.

Sorological and molecular data from the bank of the hemotherapy laboratory service were used, which had the procedures described below as the analysis protocol. Samples were collected in a K3-EDTA anticoagulant tube, the aliquots are acquired together with the donor’s whole blood. In order to perform serological tests, the electrochemiluminescence methodology was used with the Cobas e411 Roche device, in which antibodies against the hepatitis C virus, NS3 and NS4 proteins were detected. The tests with samples with anti-HCV serology positive results are repeated with a second sample, obtained from the concentrated platelets or whole blood, and only after repetition it is considered reactive, unreactive or undetermined.

The anti-HCV results were reported using S/CO ratios. According to reference Seo YS, et al. (2009), patients were divided into viremia and no-viremia groups according to HCV RNA results, therefore this study used as predictive anti-HCV cutoff = 10 [10].

The quality control was guaranteed for the tests, analyzing daily positive and negative samples of internal control and commercial control, besides the external control of Biomanguinhos (Fiocruz) and Controlab. NAT HCV was performed with amplification of HCV RNA nucleic acid by real-time multiplex polymerase chain reaction (PCR).

**Results**

According to a research carried out in the database for pre-screening and clinical screening, of 69,404 candidates to the donation, between April 2014 and June 2016, 56.73% of the candidates were male, while 43.27% of the female. Among of the male candidates to the donation, 79.35% were considered eligible donors, while 20.65% were rejected, of these 21.99% candidates were rejected regarding risk factors for HCV and 78.01% rejected for others factors. However, among of the female candidates to the donation, 63.23% were considered eligible donors, while 36.77% were rejected, of these 11.70% candidates were rejected regarding risk factors for HCV and 88.30% rejected for others factors (Table 1).

The percentage of refusal remains high over the analyzed period. Among the risk factors related to HCV, there were four factors, namely: recent large surgical procedure, presence of tattooing, use of piercing and risk behavior. The risk factor that stands out and growing during the years of this research was the risk behavior (45.19%), which is significantly more prevalent in the male gender, corresponding to 57.49% of men and 28.17% of women (Figure 1).

**Figure 1: Risk Factors related to HCV infection**

The chi-square test of adjusted residuals showed a significant association (p = 0.0001) between gender and risk factors for HCV. The use of piercing, large surgery and the presence of a tattoo were associated with the female gender; while the male gender showed association with drug use and risk behavior. There were no significant gender associations for the other risk factors.
Profile of blood donors in relation to hepatitis C

After clinical screening and interview, 50,230 candidates were considered suitable, 8,995 of whom were between 18 and 29 years old and 22,245 were over 29 years old. Among the women, 6,155 were between 18 and 29 years old and 12,675 were over 29 years old. From these candidates, samples were collected in EDTA for serology and NAT HCV, corresponding to each blood bag.

Once these 50,230 samples of suitable candidates were submitted for clinical screening and to serological qualification interview, 50,146 non-reactive and concordant results between serology and NAT and 84 anti-HCV reactive results were determined by the electrochemiluminescence methodology, which were selected and compared with the results of the methodologies used. Considering the serological cut-off point for anti-HCV 1.00, those who presented concordance between serology and NAT positive, who were 31 candidates, all had titers above 10, in counterpart; those with negative NAT, 23 had titers between 1 and 10 and 30 candidates had titers above 10 (Figure 2).

When considering the 84 donors, anti-HCV reactive, there was a concordance between the methodologies electrochemiluminescence and NAT in 31 cases (37.0%) and disagreement in 53 cases (63.0%) (Figure 3). These results showed a Kappa coefficient of 0.539, that is, the concordance between these analyzes was considered as moderate.

Discussion

Hepatitis C is characterized by a silent evolution, and its signs and symptoms are often non-specific and very similar to other diseases, such as parenchymal diseases of the liver, making clinical-laboratory diagnosis difficult [11]. This scenario is difficult for Public Health, since a large part of the infected population is unaware of its diagnosis. However, the South and Southeast regions still lead the number of reports of hepatitis C, according to data from the Ministry of Health, 86% of the cases, highlighting that the detection rate for HCV in 2016 for the South region was the highest in relation to other regions [12]. Among 1999 and 2015, 182,389 cases of HCV infection were detected in the national territory, corresponding to 32.5% of cases of viral hepatitis, in addition, 46,314 hepatitis C-related deaths were reported in the country [13].

The transfusion of blood and its products is an important form of transmission, although in recent years, after standardization of the clinical and serological screening processes, there was a significant inherent risk reduction. Despite this significant evolution in hemotherapy services, studies show that the
Profile of blood donors in relation to hepatitis C

The clinical and laboratory diagnosis for hepatitis C currently follows protocols recommended by the legislation, such as the Ministerial Directive #158/2016 by Health Ministry, which regulates technically hemotherapy procedures and the Clinical Protocol and Therapeutic Guidelines for Hepatitis C and co-infections. In the hemotherapy service, clinical screening is the initial phase, which precedes the blood donation, being an important tool for early monitoring of certain HCV-related risk factors in candidates, categorizing them as suitable, unsuitable permanently, unsuitable temporarily or unsuitable for an indeterminate time [16].

This classification from clinical screening, as demonstrated in this study, has a direct impact on the hemotherapy service, since 3,080 candidates were considered unsuitable for 12 months or, permanently, by risk behavior, large surgery, piercing and tattooing. In cases of risky sexual behavior and accidents related to occupational activities, they were considered temporarily unsuitable and in situations that evidenced the use of injectable illicit drugs, monitoring the increased risk of transmissible diseases, oral and genital cavity piercing and having been the only donor that seroconverted were considered as definitive unsuitable [16].

These situations lead to higher costs and time-consuming procedures for the hemotherapy service, and can be minimized through effective educational campaigns and consequently, the reduction of the disability indexes by clinical screening. National and regional blood donation campaigns are often isolated initiatives as a measure to contain an emergency deficiency of blood levels and little is discussed about the complexity of different donor profiles under the socio-economic, political, educational and cultural view, often individuals lacking information regarding the established criteria of unsuitability for hepatitis C [17].

In addition, the high prevalence of risk behavior in the male gender is worrying, linking a profile of inability to blood donation to activities that compromise the safety of the recipient, regarding the possibility of sharing syringes, sexual behavior of risk and accidents related to occupational activities. Faced with this problem, a vulnerable group stands out, which should be part of the focus of campaigns for the prevention of hepatitis C and qualification of blood donors.

For blood donation, laboratory screening provides for the serological quantification, i.e., the detection of antibodies anti-HCV by immunoassays and the detection of HCV-RNA by molecular biology technique, which demonstrates a great scientific technical advance in the search for this pathology and adds safety in the diagnosis. For this purpose, the NAT comes with the proposal to advance in the search for this pathology and adds safety in the biology technique, which demonstrates a great scientific technical qualification of blood donors.

From the serological point of view, some authors suggest that the presence of antibodies with high titres are indicative of infection, the biomarker of which becomes more accurate for investigation as titration increases, attributing more specificity to serological analysis and predictive value, while that lower titers can be attributed to other causes, including biological false-negative results [9, 20]. In the presence of NAT for non-reactive HCV, the presence of circulating antibodies may still represent the immunological scar, a situation in which the individual already had contact with hepatitis C, but laboratory assessment does not mean the presence of the infection [12].

However, during the investigation of anti-HCV antibodies, some groups require more attention, since they demonstrate late immunological production after the infection, they are immunocompromised patients, recipients of transplanted organs, patients on hemodialysis and HIV co-infected, and may represent a limitation for the quantification of anti-HCV [21, 22].

Regarding viral detection, during the preventive phase HCV establishes the infection in susceptible hepatocytes, which can effectively vary RNA levels, reaching an undetectable viral concentration, consequently with non-reactive NAT. On the other hand, many serological tests that are too sensitive become able to detect non-uniform peaks of antibodies [23]. In cases where HCV infection is established, in the acute phase the presence of HCV-RNA can be detected in serum after the second week of exposure, rapidly increasing the level of RNA, and after decay, no longer being detected 6 months after the onset of the infection [24]. Non-detection of viral RNA has been related to low viremia and/or persistence in extrahepatic tissues, nevertheless, may be related to infection hidden by HCV [25, 26, 27].
Profile of blood donors in relation to hepatitis C

Hidden hepatitis C is poorly recognized and often under diagnosed. This pathology was first described in 2004. In these cases, patients do not present viremia, but have reactive HCV in the peripheral blood mononuclear cells and liver cells; this clinic has been evaluated when considering patients with renal insufficiency, because they present a risk of reactivation of HCV infection due to immunosuppressive therapy after transplantation [28, 29].

HCV presents a great genetic heterogeneity, resulting from the individual’s humoral system, determining genetic subtypes that are associated with disease severity, pathogenesis and individual response. Trillions of viral particles are produced, associated with the high rate of mutations, generating a large number of variants in the individual [30]. To date, HCV has been classified into seven genotypes, and in Brazil there is a prevalence of genotype 1 (64-72 %) [31].

In addition, laboratory analyses become able to identify cases of clearance of the virus in which the virus is limited to hepatocytes. In this case viral RNA become undetectable in the blood, while immunological tests are able to detect antibodies [32]. Therefore, it is accepted the hypothesis that the variation of the cytokine profile of the individual is suitable to contribute to this process. Spontaneous clearance occurs when it is associated with some host factors such as age under 40 years, female gender, onset of jaundice, and genetic factors such as interleukin 28B polymorphism (IL28B) [33, 34]. In recent studies, it has been shown that patients who presented the polymorphism identified as IL28B showed spontaneous cure of the HCV infection during acute infection, and may present low titers of circulating antibodies, since there was contagion [35].

In this study, the serological inability index for hepatitis C was 0.2%, convergent to that found in other studies, such as the Uberaba Regional Hemocenter, which found a 0.3% unfitness for the same reason and reiterated by Hemoprod (2013) with 0.25% of inability, in this context [36, 8].

When evaluating serological inability, considering anti-HCV reactive, of these 84 candidates, 50 were male (59.5%) and 34 were female (40.5%). This male predominance is relevant and demonstrated in other studies, such as Barroso & Brito Junior (2012), which reported 66.67% male donors unsuitable for anti-HCV and 33.33% female donors. Similar data are ratified by the National Sanitary Surveillance Agency (ANVISA) in conjunction with the State University of Rio de Janeiro, which found 62.39% of unsuitable male donors and the Epidemiological Bulletin, in which 106,637 confirmed cases of hepatitis C were reported in the individual and the cost for the hemotherapy service. For this reason the discussion regarding the 53 discordant analyzes becomes relevant, since they are analytically evaluated in a different way, part with a high serological titer, considered inconclusive, and another part with reduced titer, often considered false-positive, both not reactive in molecular biology.

The recall, re-assessment and therapeutic orientation of the donor with altered results for the analysis performed are responsibilities of the Blood Bank, as recommended in the legislation. In cases where the donor is called for results confirmation, some important situations are highlighted, an exhausting process to the retest donor, related to the doubt in the diagnosis, the addition of cost in the laboratorial analyses for the service, which should be reanalyzed and, finally the possibility of under diagnosis, by not considering a laboratory discrepancy relevant, which may represent an early diagnosis, when well interpreted and based on the scientific evidence referenced in this study [16, 38].

According to data from the Health Ministry investments related to treatment for hepatitis C can reach the prediction of expenditure per patient between 2 to 20 thousand reais to the public health service, it is still worth emphasizing that exams, consultations, medical procedures and progression of the pathology are additional costs, which must be added together. It is estimated that the annual cost per patient presenting recurrent complications of hepatitis C progression, such as cirrhosis and liver transplantation, is around 22 and 87 thousand real [39,40].

Therefore, it is important to adopt preventive and early intervention initiatives, establishing a connection between public health and hemotherapy, once inconclusive results, with only a reactive methodology, can provide laboratory subsidies and use of resources for diagnosis. Considering the complexity of HCV and its relevance to health care, research for hepatitis C should be thorough and substantiated.

Although analytical science is up-to-date to provide assurances of safety in blood screening by promoting a diagnostic network coverage, confronting hepatitis C with a pharmacoeconomic approach requires, above all, a well-structured public health policy. In this context, updated epidemiological information, making it easy of the data collection systems, scientific information of the pathology in question, with this improvement of the professionals involved in the diagnosis, and conducts with informed decision making are fundamental and necessary for the process as a whole [13].
Conclusion

From this study it was possible to outline a profile for candidates for blood donation with hepatitis C, whose pathology was predominantly related to four risk factors, highlighting the high risk behavior index, in addition, laboratory discrepancies were detected, with the presence of reactivity in antibody detection and non-detection of viral RNA, these were discussed and related to methodological limitations, to the history of diseases and to the physiology of the individual. These informations about hepatitis C are fundamental for the implementation of preventive measures, through education and awareness campaigns, these being directed to minimize the transmission and effectiveness of clinical and laboratorial diagnosis, as well as guaranteeing the safety of the practice of transfusion medicine.

The hemotherapy service in the last years has been supplied with analytical tools that minimized the transfusion risk. The advent of the research for hepatitis C virus is of extreme relevance, since this pathology affects the Brazilian population effectively, being conducted as a problem of public health.

Serological screening has recently been complemented with the molecular technique, making it mandatory to perform the NAT, which provides the advantage of detecting the virus and, consequently, reducing the detection time of the current pathology. However, despite methodological progress, some discrepancies are evident and should be treated cautiously, since the final product will be referred to patients who need it safely.

Some situations related to the development of the pathology were referenced, justifying the occurrence of analytical divergences, reiterating the importance of knowing the information surrounding the blood donation procedure and the diagnostic investigation, adding new functions to the service that investigates the donor. In addition, knowing the profile of individuals who showed reactivity in laboratory research has a great epidemiological value, as well as those that were detected, with the presence of reactivity in antibody detection and non-detection of viral RNA, these were discussed and related to methodological limitations, to the history of diseases and to the physiology of the individual. These informations about hepatitis C are fundamental for the implementation of preventive measures, through education and awareness campaigns, these being directed to minimize the transmission and effectiveness of clinical and laboratorial diagnosis, as well as guaranteeing the safety of the practice of transfusion medicine.

Therefore, when considering pharmacoeconomics as a relevant science, HCV research should be complete and effective, bringing the hemotherapy activity closer to public health, and especially respecting the candidate for blood donation, who may be at risk of being under diagnosed and returning late to the health service. Finally, it reiterates the importance of these results as discussion tools to guide the Public Health campaigns linked to Hepatic C and to qualify potential blood donors.

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References


