Journal of Virological Methods 171 (2011) 303–305

Contents lists available at ScienceDirect

Journal of Virological Methods

journal homepage: www.elsevier.com/locate/jviromet

Short communication

Twenty-four mini-pool HCV RNA screening in a routine clinical virology laboratory setting: A six-year prospective study

Katja Seme, Tina Močilnik, Mario Poljak*

Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana, Zaloška 4, 1000 Ljubljana, Slovenia

Article history:
Received 28 September 2010
Received in revised form 2 November 2010
Accepted 8 November 2010
Available online 16 November 2010

Keywords:
Hepatitis C
HCV
PCR
Mini-pool
Screening
Intravenous drug users

ABSTRACT

The usefulness of combined anti-HCV and 24 mini-pool HCV RNA screening strategy was re-evaluated after a six-year continuous routine use in a clinical virology laboratory, at which more than half of newly diagnosed hepatitis C patients are intravenous drug users. Pools of 24 samples were prepared from 20,448 anti-HCV negative serum samples and tested using an automated commercial PCR assay with a lower limit of detection of 50 IU/ml. After detection of anti-HCV negative/HCV RNA positive patients, responsible physicians provided follow-up samples. Thirty-eight (0.19%) anti-HCV negative/HCV RNA positive samples from 30 patients (28 intravenous drug users) were detected. Follow-up samples were available for 27/30 patients. Twenty, six and one patient seroconverted in the second, third and fourth available samples, respectively. The interval between the first HCV RNA positive and the first available anti-HCV positive sample was 17–517 days. The costs of detecting a single anti-HCV negative/HCV RNA positive patient were 1227 Euros. Combined anti-HCV and 24 mini-pool HCV RNA screening is a useful and cost effective strategy, not only in blood-transfusion settings but also in a routine clinical virology laboratory, at which a significant proportion of the tested population belongs to a high-risk population.
© 2010 Elsevier B.V. All rights reserved.

Detection of anti-HCV antibodies in a serum sample is a standard first step in the diagnosis of hepatitis C virus (HCV) infection. Only seropositive patients are then tested for the presence of HCV RNA by nucleic acid testing (NAT) (Chevaliez and Pawlotsky, 2009; Ghany et al., 2009). Since anti-HCV can appear within a window that can last up to almost three months, serological assays are not reliable for diagnosis of the early stages of HCV infection (Chevaliez and Pawlotsky, 2009; Scott and Gretch, 2007). Since HCV RNA appears in blood as early as two weeks after infection, the use of NAT can shorten substantially the diagnostic window (Ghany et al., 2009; Simmonds et al., 2002). Alternatively, HCV core antigen testing can be used for the same purpose (Seme et al., 2005).

In the last decade, the detection of HCV RNA by NAT in donated blood has been introduced in many countries (including Slovenia) in order to increase further the safety of blood and blood products (Coste et al., 2005; Mine et al., 2003; Roth et al., 2002; Stramer et al., 2004). Blood donations are mostly screened for HCV RNA in 6–96 mini-pools, although some blood transfusion centers favor individual-donation NAT screening (Busch et al., 2005; Coste et al., 2005; Mine et al., 2003; Roth et al., 2002; Stramer et al., 2004; Velati et al., 2008; Vermeulen et al., 2009). In addition, the detection of early phase of HCV infection enables timely initiation of treatment of acute hepatitis C, with a sustained virologic response rate of 82.5% (Corey et al., 2010). Identification of HCV infection in its early phase is particularly important in high risk populations, such as intravenous drug users, hemodialysis patients, organ donors and HIV infected individuals (Alter et al., 2004; Cox et al., 2005; Khan et al., 2004; Scott and Gretch, 2007). However, screening for hepatitis C based on HCV RNA detection, outside a blood transfusion setting, is still controversial and not recommended by current consensus guidelines (Ghany et al., 2009; Dhumeaux et al., 2003; National Institutes of Health, 2002).

In a previous two-year pilot study usefulness of combined anti-HCV and 24 mini-pool HCV RNA screening was evaluated on a total of 6432 anti-HCV negative specimens and 18 (0.28%) anti-HCV negative/HCV RNA positive serum samples obtained from 12 patients were detected (Seme et al., 2007). In this report, the results of a six-year continuous use of a combined hepatitis C screening strategy are presented. The present study was performed in the national hepatitis C reference laboratory which serves the majority of hospitals and outpatient clinics in Slovenia where intravenous drug users predominate among newly diagnosed hepatitis C patients (Seme et al., 2009).

A six-year prospective study was carried out between June 1, 2004 and May 31, 2010. During this period, 22,548 consecutive serum specimens were tested routinely for the presence of anti-HCV antibodies using Ortho HCV Assay (Ortho Diagnostic Systems,

* Corresponding author. Tel.: +386 1 543 7453; fax: +386 1 543 7418.
E-mail addresses: katja.seme@mf.uni-lj.si (K. Seme), tina.mocilnik@mf.uni-lj.si (T. Močilnik), mario.poljak@mf.uni-lj.si (M. Poljak).

0166-0934/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.jviromet.2010.11.008

Table 1
Laboratory findings of 30 anti-HCV negative/HCV RNA positive patients identified by 24 mini-pool HCV RNA screening. Bolded are anti-HCV negative/HCV RNA positive samples.

Patient no.	Time interval between HCV RNA pos. and anti-HCV pos. sample	HCV RNA ($\log_{10}$ IU/ml) in initial and follow-up samples	HCV genotype
1	**0**/17	**NT**/NT	3
2	**0**/19	**7.12**/5.56	1a
3	**0**/21	**7.50**/6.86	3
4	**0**/22	**7.07**/NT	1a
5	**0**/23	**4.66**/3.59	3
6	**0**/24	**5.65**/4.03	1b
7	**0**/26	**4.37**/NT	3
8	**0**/28	**5.97**/3.57	1b
9	**0**/28	**7.41**/7.17	1b
10	**0**/31	**6.71**/6.76	3
11	**0**/32	**7.73**/6.89	1a
12	**0**/35	**5.81**/6.13	3
13	**0**/38	**3.39**/<1.48	1a
14	**0**/44	**5.32**/<1.48	3
15	**0**/49	**3.71**/2.93	1a
16	**0**/55	**6.71**/3.59	1b
17	**0**/78	**5.73**/<1.48	1
18	**0**/162	**5.93**/5.73	3
19	**0**/192	**3.40**/<1.48	NT
20	**0**/517	**5.67**/<1.48	1a
21	**0**/**13**/48	**6.52**/**6.86**/6.73	3
22	**0**/**26**/63	**5.22**/**5.53**/4.39	3
23	**0**/**12**/64	**4.43**/**4.96**/4.23	3
24	**0**/**50**/100	**5.01**/**4.79**/3.34	2b
25	**0**/**22**/279	**NT**/**NT**/6.95	1a
26	**0**/**23**/469	**7.52**/**7.14**/6.28	3
27	**0**/**11**/**99**/154	**4.90**/**3.39**/**5.10**/4.50	3
28	**0**	**4.75**	1a
29	**0**	**6.52**	NT
30	**0**	**4.20**	NT

NT, not tested because there was not enough sample for testing.

Neckargemünd, Germany) and in 2100 (9.3%) the results of initially anti-HCV reactive specimens were confirmed as anti-HCV positive by the Inno-Lia HCV Ab III Update Assay (Innogenetics, Zwijndrecht, Belgium). The remaining 20,448 anti-HCV negative specimens were tested by 24 mini-pool HCV RNA screening as described previously (Seme et al., 2007) and 38 (0.19%) anti-HCV negative/HCV RNA positive samples from 30 patients were detected (Table 1). Immediately after the recognition of an anti-HCV negative/HCV RNA positive patient, the responsible physician was contacted, informed of the result and asked for a follow-up sample. The patients were followed actively until anti-HCV seroconversion.

Twenty-eight out of 30 (93.3%) anti-HCV negative/HCV RNA positive patients were intravenous drug users. The probable mode of acquisition of HCV infection could not be established for two patients (patients 13 and 29, Table 1). Twenty-six patients responded to the invitation for follow-up testing, one patient was traced coincidentally after more than 500 days, while three patients were lost to follow-up (patients 28–30, Table 1). Twenty patients seroconverted in the second available sample (patients 1–20, Table 1). As shown in Table 1, the interval between the first HCV RNA positive sample and the first available anti-HCV positive sample was between 17 (patient 1) and 517 days (patient 20). However, one should keep in mind that these intervals do not represent the time to seroconversion, merely the time in which particular patients responded to the invitation for follow-up testing. Patient 20, with the longest interval between the first HCV RNA positive sample and the first anti-HCV positive sample, did not actually respond to follow-up testing but was tested 517 days after initial testing when his sample was coincidentally sent to our laboratory for HIV testing. Six and one anti-HCV negative/HCV RNA positive patients seroconverted in the third (patients 21–26, Table 1) and fourth available sample (patient 27, Table 1), respectively.

In anti-HCV negative/HCV RNA positive samples recognized by 24 mini-pool screening, the HCV RNA viral load was determined using a RealTime HCV assay with the m2000sp system and 0.2 ml sample preparation procedure (Abbott Molecular, Des Plaines, IL), as described previously (Halfon et al., 2006). The median HCV RNA viral load in the first anti-HCV negative/HCV RNA positive samples was 5.70 $\log_{10}$ IU HCV RNA/ml (range 3.39–7.73 $\log_{10}$ IU HCV RNA/ml) (Table 1).

The HCV genotypes were determined by a line probe assay INNO-LiPA HCV II (Innogenetics, Ghent, Belgium), following the manufacturer's instructions. Thirteen anti-HCV negative/HCV RNA positive patients were infected with HCV genotype 3, 12 with genotype 1 (seven with subtype 1a, four with subtype 1b and the subtype could not be determined for one) and one patient with genotype 2 (Table 1). This genotype distribution is consistent with the results of a recent national HCV genotyping study performed on 1504 patients in which HCV genotype 3 was identified as the most frequent genotype among intravenous drug users, followed by HCV genotype 1 and HCV genotype 2 (Seme et al., 2009).

The great majority of detected anti-HCV negative/HCV RNA positive patients were intravenous drug users and 80% responded to the invitation for follow-up testing, which enabled them to obtain timely and appropriate medical evaluation and counselling, with significant individual and community benefits. The detection of hepatitis C in the first few months after infection opens a unique window of therapeutic opportunity, enabling initiation of potent antiviral treatment, with a sustained virologic response rate of at least 82.5% (Corey et al., 2010). In the present study, five out of 27 patients with follow-up spontaneously cleared HCV RNA and the rest remained HCV RNA positive. Twelve patients received antiviral treatment and eight had already achieved sustained viral response at the time of manuscript submission.

In order to gain an insight into the cost effectiveness of the 24 mini-pool HCV RNA screening strategy, a rough calculation of the additional costs of such a strategy in comparison with a routine screening approach (anti-HCV only) was performed. The cost of detecting a single anti-HCV negative/HCV RNA positive patient increased slightly in comparison to the previous two-year pilot study (Seme et al., 2007) and amounted to approximately 1227 Euros. Considering a significantly higher sustained virologic response rate to timely treatment of acute hepatitis C in comparison to standard treatment of chronic hepatitis C and consequent cost savings from the prevention of future chronic hepatitis C related complications, the screening strategy used in the present study can be regarded as cost effective, although a formal cost effectiveness analysis was not performed. Due to the relatively high viral load in all anti-HCV negative/HCV RNA positive samples (Table 1), the number of samples in each pool could be even further increased and, consequently, the costs could be additionally reduced. However, the cost effectiveness of such screening strategy could be substantially different in laboratories offering screening for hepatitis C to a population with an epidemiological background significantly different from that in the present study.

In conclusion, the six-year prospective study showed that combined anti-HCV and 24 mini-pool HCV RNA screening is a useful and cost effective strategy not only in blood-transfusion settings but also in a routine clinical virology laboratory at which a significant proportion of the tested population belongs to a high-risk population.

Acknowledgments

The following clinical colleagues are acknowledged for their help in retrieving patient's case records Anica Berginc-Dolenšek, Brankica Bilič-Petrovič, Daša Cerar, Branka Čelan-Lucu, Miha Derganc, Ksenija Ekart-Koren, Tamara Fras-Stefan, Ljiljana Gajič, Andrej Kastelic, Matjaž Košorok, Alenka Kralj-Odar, Irena Lasič, Andreja Murnik-Rauh, Janja Prah, Natalija Planinc-Strunjaš, Aleksandra Todorovič and Milena Vidmar-Romič.

References

Alter, M.J., Seeff, L.B., Bacon, B.R., Thomas, D.L., Rigsby, M.O., Di Bisceglie, A.M., 2004. Testing for hepatitis C virus infection should be routine for persons at increased risk for infection. Ann. Intern. Med. 141, 715–717.

Busch, M.P., Glynn, S.A., Stramer, S.L., Strong, D.M., Caglioti, S., Wright, D.J., Pappalardo, B., Kleinman, S.H., NHLBI-REDS NAT Study Group, 2005. A new strategy for estimating risks of transfusion-transmitted viral infections based on rates of detection of recently infected donors. Transfusion 45, 254–264.

Chevaliez, S., Pawlotsky, J.M., 2009. Virological techniques for the diagnosis and monitoring of hepatitis B and C. Ann. Hepatol. 8, 7–12.

Corey, K.E., Mendez-Navarro, J., Gorospe, E.C., Zheng, H., Chung, R.T., 2010. Early treatment improves outcomes in acute hepatitis C virus infection: a meta-analysis. J. Viral Hepat. 17, 201–207.

Coste, J., Reesink, H.W., Engelfriet, C.P., Laperche, S., Brown, S., Busch, M.P., Cuijpers, H.T., Elgin, R., Ekermo, B., Epstein, J.S., Flesland, O., Heier, H.E., Henn, G., Hernandez, J.M., Hewlett, I.K., Hyland, C., Keller, A.J., Krusius, T., Levicnik-Stezinar, S., Levy, G., Lin, C.K., Margaritis, A.R., Muylle, L., Niederhauser, C., Pastila, S., Pillonel, J., Pineau, J., van der Poel, C.L., Politis, C., Roth, W.K., Sauleda, S., Seed, C.R., Sondag-Thull, D., Stramer, S.L., Strong, M., Vamvakas, E.C., Velati, C., Vesga, M.A., Zanetti, A., 2005. Implementation of donor screening for infectious agents transmitted by blood by nucleic acid technology: update to 2003. Vox Sang. 88, 289–303.

Cox, A.L., Netski, D.M., Mosbruger, T., Sherman, S.G., Strathdee, S., Ompad, D., Vlahov, D., Chien, D., Shyamala, V., Ray, S.C., Thomas, D.L., 2005. Prospective evaluation of community-acquired acute-phase hepatitis C virus infection. Clin. Infect. Dis. 40, 951–958.

Dhumeaux, D., Marcellin, P., Lerebours, E., 2003. Treatment of hepatitis C. The 2002 French consensus. Gut 52, 1784–1787.

Ghany, M.G., Strader, D.B., Thomas, D.L., Seeff, L.B., American Association for the Study of Liver Diseases, 2009. Diagnosis, management, and treatment of hepatitis C: an update. Hepatology 49, 1335–1374.

Halfon, P., Bourliere, M., Penaranda, G., Khiri, H., Ouzan, D., 2006. Real-time PCR assays for hepatitis C virus (HCV) RNA quantitation are adequate for clinical management of patients with chronic HCV infection. J. Clin. Microbiol. 44, 2507–2511.

Khan, N., Aswad, S., Shidban, H., Aghajani, M., Mendez, R., Mendez, R., Comanor, L., 2004. Improved detection of HCV infection in hemodialysis patients using a new HCV RNA qualitative assay: experience of a transplant center. J. Clin. Virol. 30, 175–182.

Mine, H., Emura, H., Miyamoto, M., Tomono, T., Minegishi, K., Murokawa, H., Yamanaka, R., Yoshikawa, A., Nishioka, K., Japanese Red Cross NAT Research Group, 2003. High throughput screening of 16 million serologically negative blood donors for hepatitis B virus, hepatitis C virus and human immunodeficiency virus type-1 by nucleic acid amplification testing with specific and sensitive multiplex reagent in Japan. J. Virol. Methods 112, 145–151.

National Institutes of Health, 2002. National Institutes of Health Consensus Development Conference Statement: management of hepatitis C: 2002 – June 10–12, 2002. Hepatology 6 (5 Suppl. 1), S3–S20.

Roth, W.K., Weber, M., Buhr, S., Drosten, C., Weichert, W., Sireis, W., Hedges, D., Seifried, E., 2002. Yield of HCV and HIV-1 NAT after screening of 3.6 million blood donations in central Europe. Transfusion 42, 862–868.

Scott, J.D., Gretch, D.R., 2007. Molecular diagnostics of hepatitis C virus infection: a systematic review. JAMA 297, 724–732.

Seme, K., Poljak, M., Babič, D.Z., Močilnik, T., Vince, A., 2005. The role of core antigen detection in management of hepatitis C: a critical review. J. Clin. Virol. 32, 92–101.

Seme, K., Močilnik, T., Fujs, K., Babič, D.Z., Todorović, A., Fras-Stefan, T., Poljak, M., 2007. Twenty-four mini-pool HCV RNA screening outside a blood transfusion setting: Results of a two-year prospective study. J. Virol. Methods 140, 218–221.

Seme, K., Vrhovac, M., Močilnik, T., Matičič, M., Lešničar, G., Baklan, Z., Meglič Volkar, J., Rajter, M., Štepec, S., Lunar, M., Poljak, M., 2009. Hepatitis C virus genotypes in 1504 patients in Slovenia, 1993 to 2007. J. Med. Virol. 81, 634–639.

Simmonds, P., Kurtz, J., Tedder, R.S., 2002. The UK blood transfusion service: over a (patent) barrel? Lancet 359, 1713–1714.

Stramer, S.L., Glynn, S.A., Kleinman, S.H., Strong, D.M., Caglioti, S., Wright, D.J., Dodd, R.Y., Busch, M.P., National Heart, Lung, and Blood Institute Nucleic Acid Test Study Group, 2004. Detection of HIV-1 and HCV infections among antibody negative blood donors by nucleic acid-amplification testing. N. Engl. J. Med. 351, 760–768.

Velati, C., Romanò, L., Fomiatti, L., Baruffi, L., Zanetti, A.R., SIMTI Research Group, 2008. Impact of nucleic acid testing for hepatitis B virus, hepatitis C virus, and human immunodeficiency virus on the safety of blood supply in Italy: a 6-year survey. Transfusion 48, 2205–2213.

Vermeulen, M., Lelie, N., Sykes, W., Crookes, R., Swanevelder, J., Gaggia, L., Le Roux, M., Kuun, E., Gulube, S., Reddy, R., 2009. Impact of individual-donation nucleic acid testing on risk of human immunodeficiency virus, hepatitis B virus, and hepatitis C virus transmission by blood transfusion in South Africa. Transfusion 49, 1115–1125.