IMPACT OF NUCLEIC ACID AMPLIFICATION TECHNOLOGY (NAT) IN ITALY IN THE THREE YEARS FOLLOWING IMPLEMENTATION (2001-2003)

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The use of NAT technology to screen blood donations in Italy became mandatory on 28 June 2002, but had been available experimentally since 2001. During the transition period, an EIA test to detect hepatitis C core antigen (HCVcoreAg) had also been permitted. Considering the large number of blood transfusion centres in Italy, an initial reorganisation of the biological validation of blood units was necessary, with a partial centralisation of NAT testing. The Gruppo Italiano per lo Studio delle Malattie Trasmissibili con la Trasfusione (Italian Group for the Study of Transfusion-Transmissible Diseases) conducted a national survey evaluating NAT testing, based on an annual collection of data through a questionnaire sent to all centres. In the first three years of the investigation, 219 blood transfusion centres returned the questionnaires.

In the period between January 2001 and December 2003, 3 894 894 blood donations were investigated for HCV RNA and 2 186 468 for HIV RNA. Of these, 12 were found to be HCV RNA positive and four HIV RNA positive, with an observed NAT versus antibody-based assay yield of 3.1/10^6 donations for HCV and 1.8/10^6 donations for HIV, respectively. Five of the 12 HCV RNA positive and anti-HCV negative donors had abnormal ALT values and their donations would have been discarded even in absence of NAT testing. Thus the final NAT yield for HCV is 1.79/10^6. The residual risk for HCV or HIV transmission by blood transfusion after NAT implementation is currently estimated to be extremely low in Italy.

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Introduction

In Italy, the screening of blood for hepatitis C virus (HCV) RNA became mandatory in July 2002. Recommendations regarding the introduction of nucleic acid amplification testing (NAT), or, as an alternative, enzyme immunoassay (EIA) tests capable of shortening the HCV window period, had been available from the Ministry of Health since 2001. In fact, during the above period the HCV core antigene (HCVcoreAg) EIA assay had been employed in blood screening, because its efficacy is similar to that of NAT testing, and it is extremely easy to use in blood transfusion centres.

The obligation to employ NAT technology for blood screening was limited to HCV, because of this assay's ability to reduce the window period of this infection by almost 80% (from 70 to 12 days) [1]. Such reductions are lower in the case of other important transfusion-transmissible infections.

In practice, for a number of reasons - such as the wish to guarantee higher levels of safety in transfusion therapy, the combination of a number of assays in a single commercial kit, and the fact that health decisions are made at the regional government level - almost half of the blood units collected in Italy are also screened for HIV RNA and, in a lower number of cases, for hepatitis B virus (HBV) DNA as well.

The maintained availability of the previously employed EIA screening tests allowed a comparison of the EIA-based residual risk projections, calculated with mathematical models, with the effective yields of the new technology.

A national survey was therefore organised with the following aims:

1) to study the organisational aspects of the introduction of NAT testing in Italy;
2) to assess the incidence of transfusion-transmitted infections, as detected by new technologies;
3) to evaluate the national distribution of HIV RNA screening, which is currently not mandatory in Italy;
4) to compare the new values of residual risk with existing data derived from serological assays employed in Italy.

Methods

The survey was promoted by the Gruppo Italiano per lo Studio delle Malattie Trasmissibili con la Trasfusione (Italian Group for the Study of Transfusion-Transmissible Diseases), part of the Settore Ricerca & Sviluppo della Società Italiana di Medicina Trasfusionale e Immunoematologia (Research & Development Department of the Italian Society of Transfusion Medicine and Immunohaematology), SIMTI.

From 2001 and 2003, an annual questionnaire was sent to all 308 Italian blood transfusion centres.

Assay manufacturers (Roche Diagnostics (Roche Molecular System, Branchburg, NJ, USA), Chiron Corporation (Chiron, Emeryville, CA, USA) and, for the first year only when HCVcoreAg was used, Ortho Clinical Diagnostics (Ortho-Clinical Diagnostics, Raritan, NJ, USA)) were requested to collect the questionnaires through their commercial networks, and to pass the data on to the working group where it was collated.

Data collection for both NAT and non-NAT procedures started in 2001, the year of the first experimental employment of NAT testing for blood screening prior to the date of its introduction by law (28 June 2002). In the same period, several blood transfusion centres introduced both HBV DNA and HIV RNA testing as part of their screening policy.

During the three-year investigation period, 219 blood transfusion centres returned the questionnaires. During this time, 3 894 894 blood donations (representing approximately 80% of blood donations

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collected in Italy in the same period) were investigated for HCV RNA. A total of 1 798 693 units in 29 blood transfusion centres were tested by Chiron TMA HIV/1-HCV and additional 2 096 201 units in 81 Blood transfusion centres were tested with Cobas Ampliscreen HCV Roche, on 10-24 sample minipools.

In 2001, an additional 850 080 units were tested in 109 blood transfusion centres by using Ortho HCVcoreAg instead of NAT.

In 2002 and 2003 HIV RNA screening data were collected as well. 2 186 468 units were examined, of which 1 640 278 units in 29 blood transfusion centres were tested by Chiron TMA HIV/1-HCV and 546 190 units in 37 blood transfusion centres, by Cobas Ampliscreen HIV Roche.

The blood units tested by the Roche assay were minipools ranging from 10 to 24 samples. The Chiron system was applied to single samples in 80% of blood donations and to 8-sample pools in the remaining 20%.

Before the introduction of NAT, the predicted residual risk for HCV and HIV transmission was calculated by applying the incidence/window period model [2,3] on the basis of serological tests performed on approximately four million blood units collected in Lombardia (northern Italy) between 1996 and 2003 [4,5]. The blood units collected in Lombardia amount to the 20% of the total number of blood donations in Italy each year.

Results

Table 1 illustrates the overall study data collection setting, including the number of participating centres, number of units examined by each test, and average number of tests performed per centre.

TABLE 1

Number of participating centres, number of units examined by each test and average number of tests performed per centre, Italy, 2001-2003

Year	Number of centres	Number of units examined by NAT	Number of units examined by HCVcoreAg	Number of units examined/centre
2001	167	368 953	850 080	7299
2002	94	1 537 706	-	16 358
2003	93	1 988 235	-	21 379

In 2001, when HCVcoreAg was performed as an alternative to NAT testing, 167 centres examined a total of 1 219 033 units. During 2002 and 2003, when only NAT testing was allowed, 94 centres provided results for 3 525 941 units.

TABLE 2

Number of units tested and number of centres by method employed, Italy

	Chiron TMA N	Roche PCR N	Total N
Centres providing HCV RNA data	29 (26.4%)	81 (73.6%)	110
Units tested for HCV RNA	1 798 693 (46.2%)	2 096 201 (53.8%)	3 894 894
Centres providing HIV RNA data	29 (44%)	37 (56%)	66
Units tested for HIV RNA	1 640 278 (75%)	546 190 (25%)	2 186 468

Table 2 shows the numbers of units tested, as well as the number of centres, by the method employed.

Of the 850 080 units tested by HCVcoreAg assay, none resulted positive with negative HCV antibodies.

Of the 3 894 894 units tested for HCV RNA, 616 tested positive for both HCV RNA and anti-HCV and 12 tested positive for HCV RNA alone.

Of the 2 186 468 units tested for HIV RNA, 59 resulted positive for HIV RNA and anti-HIV, and four for HIV RNA alone.

Of the 12 HCV-infected donors detected during the window phase, seven had normal ALT levels, and five had abnormal values.

All donors who had been HCV or HIV NAT positive but antibody negative at the time of donation seroconverted during the follow-up period. The lower levels of viral load detected during screening were approximately 100 000 IU/ml and 21 000 copies/ml for HCV and HIV, respectively.

One HCV RNA positive/anti-HCV negative donor and one HIV RNA positive/anti-HIV negative donor were first time donors.

The risk factors reported were: a positive sexual partner (2 HCV- and 4 HIV-infected donors), drug use (n=1), surgery (n=1), and unidentified (n=8).

Table 3 illustrates the residual risk of transmitting HCV and HIV based on serological testing, the projected yield and the estimated residual risk after NAT implementation, and the number of infectious units detected by NAT in the window phase during the period 2001-2003.

TABLE 3

Residual risk of transmitting HCV and HIV based on serological testing, projected yield and estimated residual risk after NAT implementation and units found positive in the window phase by NAT x 10^6 donations, Italy, 2001-2003

	Residual risk/10^6 donations with EIA test (CI 95%)	Projected yield after NAT introduction /10^6 donations (CI 95%)	Estimated residual risk based on EIA and NAT testing /10^6 donations (CI 95%)	Units found positive by NAT during the window phase /10^6 donations
HCV	**2.7** (1.1-4.2)	**2.2** (0.9-3.5)	**0.5** (0.1-0.9)	**1.79**
HIV	**2,2** (1.4-2.9)	**1.1** (0.7-1.4)	**1.1** (0.7-1.4)	**1.8**

Discussion

The introduction in Italy of NAT screening for blood safety determination was complicated by several organisational difficulties, including the large number of blood transfusion centres authorised to perform the biological validation of donated blood. In fact, only a minority of centres (107 by the end of 2003) was authorised to screen blood using NAT methods, thus promoting a departmental reorganisation of blood transfusion centres.

Since July 2002, HCV RNA testing has been routinely carried out as part of blood screening procedures in Italy, and the numbers of units screened for HIV RNA, and more recently for HBV DNA, are increasing.

HCVcoreAg has been completely abandoned.

The data collected in this survey, especially during its third year, covered approximately 80% of the entire 2.5 million units collected in Italy annually, and approximately 90% of the authorised centres provided data.

For the overall 2001-2003 period, 46% of blood donations were tested with Chiron assays and 54% with Roche assays: the number of centres using Roche technology was higher than those using Chiron assay, but the number of blood units tested was similar.

On the basis of serological data previously collected in Lombardia and taking into account the data collected during the years 2001-2003 (corresponding to the first period of NAT implementation), the estimated residual risk for transfusion-transmitted infection was 2.7×10^6 for HCV, and 2.2×10^6 for HIV infection [4]. Similar data has been reported by others in Italy [6].

From the implementation of NAT screening until the end of

2003, 3 894 894 units of blood were tested for HCV RNA and 2 186 468 for HIV RNA. Twelve HCV RNA-positive/anti-HCV antibody-negative, and four HIV RNA -positive/anti-HIV antibody-negative donors were detected, with an observed NAT versus antibody-based assay yield of 3.1 per 10^6 donations for HCV and 1.8 per 10^6 for HIV, respectively. Significantly, 5 of the 12 HCV RNA positive/anti-HCV negative donors had abnormal ALT. Since ALT testing is systematically performed in Italy, donations from such donors would have been discarded even in the absence of NAT results. Thus, the yield of NAT versus all mandatory tests for HCV is 1.79 per 10^6 donations.

The projected values (2.2 per 10^6 for HCV and 1.1 per 10^6 for HIV) were calculated on the basis of epidemiological data collected in the Lombardia region, which amounts to approximately one fifth of Italy's total number of blood donors and donations. Differences between the observed and the expected yields were not significant. This data indicates the satisfactory quality of both the surveillance system and the mathematical model.

So far, data on transfusion-transmitted HBV infection have not been collected at a national level, although there are plans to do so in the future. At present, the Ministero della Salute (Ministry of Health) is not planning to introduce HBV NAT testing for blood screening although the HBV predicted residual risk, calculated through mathematical modelling based on incident infections in donors screened in Lombardia during the period 1996 and 2003, is estimated to be 13.9 per 10^6.

Now that NAT has been implemented, the residual risk for transmitting HCV or HIV by blood transfusion in Italy is extremely low. The surveillance system described in this publication will be maintained to observe eventual shifts in the epidemiology of these infections, as well as the opportunity to introduce additional assays or to remove some of the currently performed tests.

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ORIGINAL ARTICLES

Surveillance report

INCIDENCE OF VIRAL MARKERS AND EVALUATION OF THE ESTIMATED RISK IN THE SWISS BLOOD DONOR POPULATION FROM 1996 TO 2003

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Among the well known transfusion-associated risks, the transmission of pathogenic viruses is regarded as one of the most serious. Over the past two decades, a series of overlapping safety procedures have been successively implemented to minimise this risk. It is now generally considered that the risk of transmitting viral infections via blood products is very low in developed countries. The present study analyses the incidence of the key infectious diseases HIV, hepatitis B virus (HBV) and hepatitis C virus (HCV) between 1996 and 2003 from 99% of voluntary repeat blood donors visiting the blood transfusion service of the Swiss Red Cross. Furthermore the estimated risk of these viral markers was calculated. From 1996 to 2003 the incidence rate for HCV decreased continuously, whereas no significant decrease in the incidence rate of HIV and HBV was observed. From 2001 to 2003, the last calculated period, the residual risk was estimated to be 1 in 1 900 000 for HIV, 1 in 2 200 0000 for HCV and 1 in 115 000 for HBV, respectively. This agrees with international studies, which have been shown that the estimated residual risk for HBV between 1996 and 2003 is higher than that of HCV and HIV.

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