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An estimate of the current risk of transmitting blood-borne infections through blood transfusion in Italy

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Summary. We conducted a retrospective cohort study to estimate the incidence of major blood-borne agents among Italian blood donors and calculated the risk of infection among blood recipients using the 'incidence/window period model'. The study was conducted among 46 180 blood donors enrolled in six blood centres between 1994 and 1999. During follow-up, seven new infections were confirmed: three donors seroconverted for anti-human immunodeficiency virus (HIV); two for anti-hepatitis C virus (HCV); and two showed hepatitis B surface antigen (HBsAg) reactivity; no cases of syphilis were observed. The incidence rates per 100 000 person/years were: 4·06 (95% CI: 0·82–11·85) for HIV; 2·41 (95% CI: 0·29–8·70) for HCV; and 2·70 (95% CI: 0·32–9·77) for HBsAg; the incidence for total hepatitis B virus (HBV) infection was 9·77 per 100 000 person/years (95% CI: 1·16–35·36). The estimated risk of an infectious blood unit not being detected was: 2·45 (95% CI: 0·13–12·33) per 1 million units for HIV; 4·35 (95% CI: 0·30–22·39) for HCV; and 15·78 (95% CI: 1·16–84·23) for HBV. Overall, an estimated 22·58 per 1 million units are infected. In Italy, the risk of transfusion-transmitted infections is low and is similar to that in other western countries. The introduction of new more sensitive screening tests could reduce the residual risk of transfusion-transmitted infection by 40–80%.

Keywords: blood transfusion, residual risk of infection, HIV, HBV, HCV.

The risk of acquiring infection with viral hepatitis and human immunodeficiency virus (HIV) through blood transfusion has drastically decreased since the introduction of sensitive blood-screening tests and of restrictive criteria for selecting blood donors. Nevertheless, there continue to be cases of transfusion-transmitted infections (Mele *et al*, 1995), the vast majority of which can be attributed to infected blood collected before the appearance of serological markers of infection (i.e. during the serological window period) (Busch *et al*, 2000). Thus, the risk of acquiring a transfusion-transmitted infection depends not only on the incidence of the infection among blood donors but also on the length of the specific window period. To address this problem, the 'incidence/window period' model has been developed. This mathematical model allows the current risk of acquiring transfusion-transmitted infections to be estimated. Although the results obtained with this model have been reassuring, in that they indicate that there is a low risk of transmitting viral hepatitis and retroviral infections through transfusion, to the best of our knowledge the model has only been applied in the United States and France (Couroucé & Pillonel, 1996; Schreiber *et al*, 1996).

In Italy, the estimate of the level of transfusion safety is mainly based on data from a national surveillance system of type-specific acute viral hepatitis (known as 'SEIEVA') (Mele *et al*, 2000; Stroffolini *et al*, 2000) and from longitudinal studies on the rate of serconconversion for anti-hepatitis C virus (HCV) and anti-HIV among chronic transfusion recipients of the Cooleycare cohort (Prati *et al*, 1998a,b). However, the data provided by these sources may represent overestimates because they do not exclude cases of nosocomial infection. Furthermore, the risk of hepatitis B virus (HBV) infection cannot be reliably measured, given the high vaccination coverage among chronic transfusion recipients.

The objective of the present multicentre study was to calculate the incidence of major blood-borne agents in a

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cohort of Italian blood donors and to estimate the current risk of acquiring transfusion-transmitted infections for blood recipients, using the incidence/window period model. We also considered the potential improvements in blood safety resulting from the introduction of new blood-screening tests.

PATIENTS AND METHODS

Study population. The present study was based on a retrospective cohort of donors who donated blood between January 1, 1994 and December 31, 1999 at six blood centres located in northern and central Italy and on the island of Sardinia. Prior to blood donation, all donors answered and signed a psycho-social questionnaire on risk factors for blood-borne infections, including invasive medical or surgical procedures, at-risk sexual contact, and drug use. Those donors who reported such risks were temporarily or permanently deferred from donating blood. We included in the study population all the repeat blood donors who donated whole blood or fractionated components by apheresis at least twice during the study period and who tested negative for all serological screening markers at the first blood donation.

Laboratory methods. Screening tests for all the blood units were carried out using third generation enzyme-linked immunosorbent assays (ELISA) for detecting antibodies to HIV types 1 and 2, antibodies to HCV, and hepatitis B surface antigen (HBsAg). Antibodies to *Treponema pallidum* or to cardiolipine were detected by venereal disease research laboratory (VDRL) or by *T. pallidum* haemoagglutination assay (TPHA). These methods were licensed by the Istituto Superiore di Sanità (the Italian National Institute of Health) for the mandatory screening of blood units. In accordance with Italian law, alanine aminotransferase levels were also measured at each blood donation.

Samples that were repeatedly reactive to anti-HIV 1–2 ELISA were tested with Western blot (WB) and, when required, reverse transcription polymerase chain reaction (RT–PCR) analysis. Samples reactive to anti-HCV ELISA were subjected to third generation recombinant immunoblot assay (RIBA 3·0) and, when reactive to at least one band, were tested for the presence of serum HCV RNA by RT–PCR. Samples reactive to HBsAg were confirmed by neutralizing assay and/or by follow-up investigation with other HBV serological markers [antibodies to hepatitis B core (anti-HBc); antibodies to surface antigen (anti-HBs); antibodies to envelope antigen (anti-HBe); and envelope antigen (HBeAg)]. Samples reactive to VDRL or TPHA were subjected to anti-T. pallidum IgG and IgM ELISA for confirmation.

Statistical methods. The incidence of seroconversion for HIV, HCV, HBV (HBsAg) and syphilis was measured considering, for each study participant, the time that had elapsed between the first donation and the last donation. Incidence rates were expressed as the number of infections per 100 000 person/years of observation. The 95% confidence interval (CI) was calculated based on Poisson distribution.

For HBV infection, the crude incidence was adjusted for transient antigenaemia, according to Schreiber *et al* (1996). The adjustment was made considering that only a fraction of total HBV infections are detected by the HBsAg assay: in particular, 70% of total HBV infections have a transient antigenaemia (estimated duration = 63 d), and the HBsAg assay identifies only a fraction of these cases (the fraction can be obtained by dividing 63 d by the observed median interval between donations among seroconverted individuals). Twenty per cent have a primary antibody response but no detectable antigenaemia, and none of these donors is identifiable by HBsAg. Finally, 5% become long-term carriers, all of whom are detectable by HBsAg screening (Hoofnagle *et al*, 1978).

Incidence rates were used for estimating the residual risk of transfusion-transmitted infection. According to the method described by Schreiber *et al* (1996), for each infection, the risk was calculated by multiplying the incidence rate in blood donors by the length of the window period (expressed as a fraction of the year). To estimate the probable ranges of residual risk, we multiplied the confidence intervals of the incidence rates by the confidence intervals of the window periods. For each infection, the length of the window period was established according to current knowledge (Manns *et al*, 1992; Mimms *et al*, 1993; Couroucé *et al*, 1994; Busch *et al*, 1995).

The possible impact of false negative test results on the estimate of transfusion risk was not taken into consideration in the present study because of the low incidence of HIV, HCV and HBV infections in the Italian general population (D'Amelio *et al*, 1994; Kondili *et al*, 2001), and the high sensitivity of the current generation screening tests (very close to 100%).

RESULTS

During the study period, 46 180 donors who had no medical or behavioural contraindication to blood donation were enrolled at the six participating blood centres. The median age of the donors was 35 years (range: 18–68 years); 71·8% were men and 28·2% were women. The median follow-up was 1·9 years (range: 1 month to 5 years). During follow-up, donors gave blood a median of 3 times (range: 2–25). The total person/years of follow-up was 83 140 for anti-HCV and nearly 74 000 person/years for each of the other infections (one blood centre only provided data on HCV infection).

During follow-up, a total of seven new infections were confirmed. In particular, three donors seroconverted for anti-HIV; two for anti-HCV; and two showed HBsAg reactivity. The corresponding incidence rates per 100 000 person/years were 4·06 (95% CI: 0·82–11·85) for HIV; 2·41 (95% CI: 0·29–8·70) for HCV; and 2·70 (95% CI: 0·32–9·77) for HBsAg. The estimated total HBV incidence was 9·77 per 100 000 person/years (95% CI: 1·16–35·36). No cases of syphilis were observed (Table I). Two persons, one reactive for anti-HCV and one for HBsAg ELISA, were excluded from the analysis because they did not return to the blood centre for confirmatory tests. If

Table I. Number of seroconversions and incidence of viral infections among 46 180 blood donors; Italy 1994–1999.

Markers	No. of seroconversions	No. of person/years	Incidence per 100 000 person/years (95% CI)
HIV	3	73 979	4·06 (0·82–11·85)
Syphilis	0	73 979	0·00 (0·00–4·99)
HCV	2	83 140	2·41 (0·29–8·70)
HBV			
HBsAg	2	73 978	2·70 (0·32–9·77)
Total HBV	–	–	9·77 (1·16–35·36)

HIV, human immunodeficiency virus; HCV, hepatitis C virus; HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen.

these persons had been included as positive for anti-HCV and HBsAg, the resulting incidence would have been 3·61 per 100 000 person/years (95% CI: 0·73–10·55) for HCV and 4·06 per 100 000 person/years (95% CI: 0·82–11·85) for HBsAg.

Most of the donors who seroconverted were men (85·7%), and their median age was 36 years (range: 32–58 years). The median number of donations before seroconversion was 4 (range: 2–9).

On the basis of the incidence data, and considering the length of the specific window periods, we estimated the risk of not detecting an infectious blood unit through serological screening. The data are summarized in Table II. The estimated risk was 2·45 (probable range: 0·13–12·33) per 1 million units for HIV; 4·35 (probable range: 0·30–22·39) for HCV; and 15·78 (probable range: 1·16–84·23) for total HBV. Overall, 22·58 per 1 million blood units were estimated to have been infected: 89% of this risk was attributed to hepatitis viruses (70% to HBV and 19% to HCV), whereas 11% was owing to HIV.

As this risk was derived from data collected among repeat donors, the estimate was adjusted to include first-time donations, which account for 9·7% of the total annual blood donations in Italy (Ghirardini *et al*, 2000). As the risk of blood-borne infection is about two times higher among first-time donors than among repeat donors (Gunson & Rawlinson, 1988; Lackritz *et al*, 1995), the overall risk was 24·77 per 1 million units. The expected decreases in the transfusion risk for different agents following the introduction of nucleic acid amplification testing (NAT) (Busch, 2000) are provided in Table III.

DISCUSSION

In the present study, the current risk of acquiring major transfusion-transmitted infections (i.e. HIV, HBV and HCV) in Italy was similar to the risk calculated using the same statistical model in other western countries, including the United States (Lackritz *et al*, 1995; Schreiber *et al*, 1996; Glynn *et al*, 2000) and France (Couroucé & Pillonel, 1996). Overall, for each 1 million units donated, 22·58 were expected to be infectious. These data confirm that the risk of acquiring a transfusion-transmitted infection is considerably lower than the risk of having adverse outcomes as a consequence of other medical procedures. For example, the incidence of iatrogenic infections in an intensive care unit was as high as 70–80 cases per 1000 patients (Doebbeling *et al*, 1992), and the risk of death among women taking

Table II. Length of window period and residual risk of viral infection per 1 million blood units; Italy 1994–1999.

Markers	Length of window period (d)		Residual risk per 1 million units (probable range)
	Estimate	Range	
HIV	22	6–38	2·45 (0·13–12·33)
HCV	66	38–94	4·35 (0·30–22·39)
HBV			
HBsAg	59	37–87	4·36 (0·32–23·27)
Total HBV	–	–	15·78 (1·16–84·23)

HIV, human immunodeficiency virus; HCV, hepatitis C virus; HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen.

Table III. Hypothetical reduction in the residual risk of infection following the introduction of nucleic acid amplification testing (NAT) testing in donor screening.

	Estimated window period (d)*	Estimated residual risk per 1 million donations tested by NAT	Reduction in the estimated residual risk†
HIV	11	1·22	−50%
HCV	12	0·79	−82%
HBV	35	9·36	−41%

*Busch (2000).

†Compared to third generation enzyme-linked immunosorbent assay.

oral contraceptives is estimated to be 1 in 50 000 (McCullough, 1993).

The comparison of our data with those of two large surveys recently conducted in Italy by the Cooleycare Cooperative Group to calculate the rate of seroconversion among chronic transfusion recipients can contribute to understanding the epidemiology of viral infections in transfusion recipients. With regard to HIV, our estimate of the risk (2·45 cases per 1 million units) is of the same order of magnitude as the risk directly measured in blood recipients (6 cases per 1 million units) (Prati *et al*, 1998b). The slight difference can be explained by the tendency of the incidence/window period model to underestimate the actual risk (Schreiber *et al*, 1996). In any case, our data confirm that the current risk of acquiring transfusion-transmitted HIV infection is very low.

With regard to HCV infection, there is a substantial discrepancy between the estimated risk of 4·35 per 1 million units and the incidence among blood recipients (4·27 per 1000 person/years, which corresponds to a risk of 1 per 7100 units) (Prati *et al*, 1998a). This indicates that the transfusion of infected blood is actually responsible for only a fraction of the cases of HCV infection among transfusion recipients and that most new infections can be attributed to other modes of transmission. Given the key role played by iatrogenic transmission in circulating HCV infection in Italy (Guadagnino *et al*, 1997; Prati *et al*, 1997; Mele *et al*, 2000, 2001), it seems very likely that this route, more than blood transfusion, is important for the spread of viruses among hospital patients. Further improving the safety of medical procedures could greatly limit the number of new cases of HCV infection and eventually contribute to reducing the risk of transmission through transfusion. In fact, a more scrupulous application of prophylactic measures in health-care settings could explain the finding that the incidence of HCV infection in our study population was lower than that reported for Italian donors in the period from 1991 to 1995 (Prati *et al*, 1997) (1 per 10 000 person/years versus 2·4 per 100 000 person/years).

Based on our data, HBV is the agent most commonly involved in the residual cases of transfusion-transmitted viral infections. However, the infectivity of HBV-positive blood greatly depends on the level of HBV vaccination coverage among blood recipients, which has progressively increased since the development of effective vaccines. Furthermore, the outcome of transfusion-associated HBV infection is generally favourable, at least in immunocompetent hosts, with few cases of acute illness or chronic carriage of the virus (Busch, 1998). In light of these considerations, the clinical impact of transfusion-transmitted HBV infection is expected to be limited compared with that of HCV and HIV infections.

A recent study conducted in the United Kingdom, which reported no seroconversions for HIV, HBV or HCV among the recipients of 20 000 units of blood (Regan *et al*, 2000), merits consideration. The discrepancy between these data and our results may be owing to epidemiological differences between Italy and the United Kingdom: in particular, the prevalence of HBV and HCV infections among blood donors is considerably higher in Italy. However, this discrepancy could also be owing to the fact that a relatively small number of transfusion events were investigated in the British series.

We believe that our results could be useful in deciding whether or not to introduce new tests as part of blood donor screening in Italy. The recent availability of the mini-pool nucleic acid amplification test (NAT) and the development of sensitive assays for detecting antigens could potentially reduce the residual risk of infection with different agents by approximately 40–80% (Table III). Additional benefits could be provided by the introduction of effective viral inactivation procedures; however, according to our results, the additional reduction in risk would be negligible. On the other hand, public pressure to enhance the safety of transfusion and the great political and legal repercussions resulting from the notification of even a few cases of transfusion-transmitted infection should be taken into consideration. To make effective decisions in terms of public health policy, data need to be carefully interpreted and should also consider the cost–benefit relationship.

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