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Transfusion Medicine

Infectious disease markers in autologous blood donors and first-time volunteer blood donors: 14 years' experience in a blood center

The proportion of blood donors with positive infectious disease markers was statistically higher in our population of 3,614 autologous donors than in our population of 276,106 first-time volunteer donors ($p<0.005$). Our data suggest that our autologous donor population is not as safe as our first-time volunteer donor population.

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In Spain, it is a current legal requirement[1] that both autologous and homologous blood donors pass the same ordinary predonation blood donor interview. Moreover, all samples from both autologous and homologous donors are tested for the presence of antibodies to HCV (anti-HCV) and HIV viruses (anti-HIV), HBsAg and RPR. If one of these tests is positive, then the donor is deferred. Moreover, it has also been suggested that the infectious markers frequency in autologous and volunteer donors may vary geographically and should be determined locally.[2]

With these controversies in mind, we decided to collect data to calculate the frequency of positive infectious disease markers in our population of autologous and first-time volunteer donors.

We evaluated the presence of the infectious disease markers from January 1989 to December 2002 both in 3,614 autologous and in 276,106 first-time volunteer blood donors who were eligible for blood donation. Both groups of donors followed the same medical history screening procedure. Minimal hemoglobin level, however, was 105 g/L for autologous donors, 125 g/L for first-time female and 135 g/L for first-time male volunteer donors.

Screening (ELISA kit) and confirmatory (RIBA kit) tests to analyze for the presence of anti-HIV 1/2 and anti-HCV were implemented in 1986 and 1989, respectively. Screening (enzyme immunoassay kit) and confirmatory (neutralization kit) tests to analyze for the presence of HBsAg were implemented in 1971. RPR was performed with RPR-Carbon (BioSystems, Barcelona, Spain) and was

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Table 1. Infectious markers in autologous and first-time volunteer blood donors per year.

		Autologous donors											
		Positive HBsAg[1]		*Positive HIV*[2]		*Positive HCV*[2]		*Ind HCV*[3]		*Positive RPR*[1]		*ALT>88 IU/L*	
Year	N	n	%	n	%	n	%	n	%	n	%	n	%
1989	10	0	0	0	0	0	0	0	0	0	0	0	0
1990	10	0	0	0	0	0	0	0	0	0	0	0	0
1991	24	1	4.17	0	0	0	0	0	0	0	0	0	0
1992	82	0	0	0	0	2	2.44	2	2.44	0	0	1	1.22
1993	104	0	0	0	0	0	0	0	0	0	0	0	0
1994	276	2	0.72	0	0	17	6.16	4	1.45	0	0	1	0.36
1995	305	3	0.98	1	0.33	13	4.26	1	0.33	1	0.33	1	0.33
1996	248	0	0	0	0	7	2.82	3	1.21	1	0.4	0	0
1997	402	1	0.25	0	0	7	1.74	2	0.5	1	0.25	0	0
1998	232	1	0.43	1	0.43	6	2.59	2	0.86	0	0	1	0.43
1999	350	1	0.29	0	0	5	1.43	4	1.14	1	0.29	1	0.29
2000	461	2	0.43	0	0	10	2.17	0	0	1	0.22	0	0
2001	566	2	0.35	0	0	8	1.41	3	0.53	0	0	0	0
2002	544	5	0.92	0	0	8	1.47	2	0.37	1	0.18	0	0
All	3,614	18	0.5	2	0.06	83	2.3	23	0.64	6	0.17	5	0.14

Table 1. Infectious markers in autologous and first-time volunteer blood donors per year (continued).

		First-time volunteer donors											
		Positive HBsAg[1]		Positive HIV[2]		Positive HCV[2]		Ind HCV[3]		Positive RPR[1]		ALT >88 IU/L	
Year	N	n	%	n	%	n	%	n	%	n	%	n	%
1989	10,203	55	0.54	6	0.06	104	1.02	19	0.19	18	0.18	93	0.91
1990	10,055	64	0.64	4	0.04	183	1.82	13	0.13	13	0.13	165	1.64
1991	13,401	59	0.44	6	0.04	142	1.06	48	0.36	10	0.07	250	1.87
1992	16,765	83	0.5	7	0.04	141	0.84	72	0.43	14	0.08	301	1.8
1993	15,057	46	0.31	4	0.03	88	0.58	44	0.29	4	0.03	55	0.37
1994	19,066	48	0.25	3	0.02	100	0.52	30	0.16	5	0.03	27	0.14
1995	17,897	47	0.26	10	0.06	70	0.39	23	0.13	2	0.01	34	0.19
1996	18,416	45	0.24	3	0.02	59	0.32	33	0.18	8	0.04	118	0.64
1997	24,091	35	0.15	4	0.02	57	0.24	32	0.13	3	0.01	123	0.51
1998	19,260	40	0.21	2	0.01	48	0.25	24	0.12	4	0.02	148	0.77
1999	20,451	34	0.17	2	0.01	43	0.21	21	0.1	8	0.04	172	0.84
2000	24,777	36	0.15	2	0.01	53	0.21	21	0.08	2	0.01	174	0.7
2001	29,265	39	0.13	5	0.02	71	0.24	23	0.08	8	0.03	258	0.88
2002	37,402	81	0.22	8	0.02	87	0.23	26	0.07	8	0.02	281	0.75
All	276,106	712	0.26	66	0.02	1,246	0.45	429	0.16	107	0.04	2,199	0.8

[1]Screening and confirmatory tests were positive; [2]ELISA +/RIBA +; [3]Indeterminate result of HCV test: ELISA +/RIB –.

Table 2. OR (95% CI) of positive disease markers between autologous and first-time volunteer blood donors.

	Autologous n (%)	First-time volunteer n (%)	OR (95% CI)	P
Positive HBsAg[1]	18 (0.5)	712 (0.26)	0.52 (0.32-0.85)	0.004
Positive HCV[2]	83 (2.3)	1,246 (0.45)	0.19 (0.15-0.24)	<0.00001
Ind HCV[3]	23 (0.64)	429 (0.16)	0.24 (0.16-0.38)	<0.00001
Positive HIV[2]	2 (0.06)	66 (0.02)	0.43 (0.10-2.55)	0.2
Positive RPR[1]	6 (0.17)	107 (0.04)	0.23 (0.10-0.59)	0.0001
ALT >88 IU/L	5 (0.14)	2,199 (0.8)	5.79 (2.33-15.77)	<0.00001
Number of donors tested	3,614	276,106		

[1]Screening and confirmatory tests were positive; [2]ELISA + / RIBA +; [3]Indeterminate result of HCV: ELISA + / RIBA –.

confirmed with FTA and TPH-ABS. ALT values were assessed on Cobas Integra 700 (Roche, Branchburg, NJ, USA).

The upper limit of the value of ALT is 44 IU/L for men and women in our laboratory and we defer the blood donation when ALT is higher than 88 IU/L.

In this study, we considered a positive result for HBsAg, HIV, HCV and RPR if both screening and confirmatory tests were positive. A HCV result was considered indeterminate when the ELISA was positive and the RIBA negative The risk of a positive disease marker was estimated by the odds ratio (OR) with its 95% CI. χ^2 test (or Fisher's exact test when appropriate) was used to determine the differences between proportions of autologous and first-time volunteer blood donors with a positive infectious disease marker. A p value less than 0.05 was considered statistically significant. Statistical analysis was carried out with software (SPSS for Windows package, release 11.0, SPSS, Chicago, IL, USA).

Autologous donors were 1,359 (38%) men and 2,255 (62%) women with a median age of 56 years (range 16-75). First-time volunteer donors were 168,424 (61%) men and 107,682 (39%) women with a median age of 44 years (range 18-65).

The results of the infectious disease markers are shown in Tables 1 and 2. The proportion of donors with positive markers for HBsAg, anti-HCV and syphilis was statistically higher in the autologous donor group than in the first-time volunteer donor group (p<0.005). The proportion of donors with an indeterminate result for anti-HCV was also significantly higher in the autologous donor group than in the first-time volunteer donor group (p<0.00001). In contrast, the proportion of donors with elevated ALT values was higher in the first-time volunteer donor group than in the autologous donor group (p<0.00001). Finally, the proportion of donors with a positive result for anti-HIV was similar in the two groups (p=0.2).

Our data suggest that our autologous donors present greater risk than first-time volunteer donors in terms of their likelihood of transmitting a transfusion-related infection. One of the reasons could be that the two blood donor groups are different in sex distribution and age. Proportionally, more women were seen in the autologous donor group compared with first-time volunteer donor group. The median age was also higher in the autologous donor group. This reflects the population undergoing elective surgery, as has been reported previously.[3,4] Another reason could be that autologous donors may have medical conditions or are receiving therapy that

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would exclude them as homologous donors.[4] In fact, we showed in a preliminary study that autologous donors had surgery or received blood components more frequent than first-time donors.[5]

In that study, we saw that 67 (81%) out of 83 of autologous donors had previous surgery compared with 41 (6.6%) out of 622 first-time volunteer donors. We also saw that 56% of autologous donors had transfusion history compared with 10.6% of first-time volunteer donors. This observation is also supported by Starkey *et al.*[6] who reported a high risk ratio in units from autologous donors who were not candidates for crossover by donor history and hematocrit (range, 1.8 for elevated ALT to 8.9 to positive anti-HIV-1).

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Key words: blood transfusion, infectious disease markers, autologous blood donors, first-time blood donors.

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Blood Doping

Strengths and weaknesses of established indirect models to detect recombinant human erythropoietin abuse on blood samples collected 48-hr post administration

We studied indirect detection models of erythropoietin abuse (EPO) on blood samples collected 48-hr after administration of the drug during 6 weeks of recombinant human erythropoietin (rHuEPO) treatment. Although the efficiency of OFF-models was preserved, we found a loss of sensitivity of ON-models. This study also revealed an increased percentage of stomatocytes in athletes receiving rHuEPO.

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Recently, Gore *et al.*[1] have developed new, sensitive, mathematical models to detect current (ON-models) and recent (OFF-models) rHuEPO abuse. With ON-models, they found a better sensitivity when blood samples were collected 24hr post injection than with unstandardized protocols (25 min to 72 hr between injection and blood sampling). As the half-life time of rHuEPO is very short,[2] we hypothesized that ON-models might fail to detect subjects abusing rHuEPO when injection and blood sampling are performed 48hr apart. We also examined blood smears for abnormally shaped red blood cells (RBC), particularly stomatocytes, because EPO may affect the synthesis of some membrane proteins involved in RBC morphology.[3]

In brief, we studied scores and sensitivity of indirect detection models and stomatocyte counts in athletes receiving moderate doses of rHuEPO.

Fourteen endurance-trained athletes were randomly assigned to receive either EPO or placebo. The EPO group received subcutaneous injections of rHuEPO (Eprex® Janssen-Cilag, France) 3 times a week for 6 weeks as follows: 50 U/kg during the first 4 weeks (*acceleration phase*) and 20 U/kg the next 2 weeks (*maintenance phase*). The PLA group received subcutaneous injections of NaCl (0.9%). The time between injections and blood sampling was 48 hours. Blood samples were taken before any injection (day 0), during both the acceleration and maintenance phases, and then over the following 3 weeks (*wash-out phase*). Hematocrit (Hct), hemoglobin concentration (Hb) and percent of reticulocytes (%Rets) were determined in blood samples collected in EDTA tubes (PENTRA 120 Retic Hematology Analyzer). The coefficients of variation (CV) were 1.18%, and 14.8% for Hb and %Rets measurements, respectively. EPO and serum transferrin receptor (sTfr) levels were measured in serum (Quantitative IVD human EPO and Quantitative IVD human sTfr Elisa kits, R&D System, Inc.). Intra and inter-assay CV were 4.4% and 6.5% for EPO and 5.7% and 5.8% for sTfr. Blood samples were taken between noon and 2 PM from the athletes in a supine position. Hb, %Rets, EPO and sTfr concentrations were used to calculate the scores of the different models (he and hes On-model or hr and hre Off-model). ON-models scores were calculated during the acceleration (day 11 and day 25) and maintenance (day 32) phases and OFF-models scores during the wash-out phase (day 54 and day 61). Values from the placebo group were then used to establish the mean score, the standard deviation (SD) and the 95% confidence interval for each model. Scores greater than the mean of placebo group ± 1.96*SD indicated a probable intake of rHuEPO. Blood smears were prepared using the glass slide method and examined by light microscopy. Manual counting of stomatocytes (%Stom)[4] was performed by two investigators. ANOVA for repeated measurements was used to compare results from the two groups. The rates of detection with ON-models ranged from 13 to 63% during the acceleration and maintenance phases (Table 1). Detection was excellent (100%) for OFF-models 14 days after the end of treatment (day 54) and low at day 61 (38 to 50%). During rHuEPO treatment, Hb and sTfr concentrations, as well as %Rets (except at day 32) were significantly higher than basal and placebo values (Table 2). By contrast, EPO levels were similar to basal (except at day 11) and placebo values. During the wash-out period, Hb, %Rets and EPO values were significantly different from those at the basal measurement while sTfr was not statistically different. %Stom increased with rHuEPO injections and remained elevated until the end of the wash-out phase in 8/9 athletes.

The results suggest that ON-models may fail to detect EPO abuse when blood sampling is performed 48 hr after injec-

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