

Multiple False-positive Serologic Tests for HIV, HTLV-1, and Hepatitis C Following Influenza Vaccination, 1991

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Objective.—(1) To assess factors associated with the occurrence of multiple false-positive viral enzyme-linked immunosorbent assays (ELISAs) for human immunodeficiency virus (HIV), human T-cell lymphotropic virus type 1 (HTLV-1), and hepatitis C virus (HCV) among individual blood donors and (2) to determine the frequency and time course of this phenomenon.

Design.—Case-control study.

Setting.—A regional blood center.

Participants.—Blood donors found to have multiple false-positive viral ELISAs (case donors) and randomly selected seronegative controls (control donors) who donated between October 31, 1991, and December 15, 1991. An additional random sample of 262 donation records was reviewed to calculate the proportion of donors who received influenza vaccine.

Main Outcome Measures.—Multiple false-positive viral ELISAs, receipt of influenza vaccination formulated for the 1991-1992 influenza season, and follow-up ELISA results on serum samples obtained from case donors.

Results.—Among 17 941 donors, 10 case donors were identified. Nine of the 10 case donors received influenza vaccine, compared with three of 30 control donors (odds ratio [OR]=81; 95% confidence interval [CI], 6 to 3670; $P<.001$). Among nine case donors, the mean time between vaccination and blood donation was 26 days (range, 9 to 68 days). Follow-up ELISAs of serum samples from seven case donors obtained 52 to 130 days (mean, 75 days) after vaccination demonstrated reversion to HIV and HTLV-1 seronegativity in all but one specimen, with persistence of positive HCV ELISAs in four specimens. We estimate between 0.6% and 1.7% of blood donors who received influenza vaccine this season had multiple false-positive viral ELISAs.

Conclusions.—The occurrence of multiple false-positive viral ELISAs among blood donors was associated with influenza vaccination, but was infrequent among vaccinees. This phenomenon is of short duration for HIV and HTLV-1, but may persist longer for HCV. We recommend influenza vaccinees not be deferred from blood donation. Blood donors with multiple false-positive viral ELISAs should be considered for future reentry as blood donors.

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ON DECEMBER 18, 1991, Assistant Secretary for Health James Mason, MD, announced that approximately 90 blood donors nationwide had false-positive screening enzyme-linked immunosorbent assays (ELISAs) for two or more of the following viruses: human immunodeficiency virus (HIV), human T-cell lymphotropic virus type 1 (HTLV-1), and hepatitis C virus (HCV). A Food and Drug Administration press release stated "at least 60% of these blood donors had recently been vaccinated against the flu." While the Food and Drug Administration made it clear that the influenza vaccine was not a source of infection for HIV, HTLV-1, or HCV, little epidemiologic information was available regarding this phenomenon. It was not clear to blood donation centers or public health officials whether prospective blood donors who recently received vaccination against influenza should be temporarily deferred from donation to avoid unnecessary collection and discarding of units of blood and possible permanent deferral of donors with false-positive tests. We attempted to define the epidemiologic features of this phenomenon among blood donors to the American Red Cross Blood Services, Badger Region.

Materials and Methods

The Badger Region serves a population of 2.5 million people residing in portions of Wisconsin, eastern Minnesota, eastern Iowa, northern Illinois, and the upper peninsula of Michigan. The Badger Region has four permanent sites and six mobile donation units that collected 140 382 units of blood from 84 690

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Results of Enzyme-linked Immunosorbent Assay (ELISA) and Confirmatory Testing of Case Donors for Antibody to Human Immunodeficiency Virus (HIV), Human T-cell Lymphotropic Virus Type 1 (HTLV-1), and Hepatitis C Virus (HCV)

	HIV	HTLV-1	HCV
Sera Obtained at Time of Blood Donation			
Case donors, No.	10	10	10
Positive ELISA at donation	7	8	10
Negative confirmatory test	6*	5*	10†
Indeterminant confirmatory test	1*	3*	0
Sera Obtained During Follow-up‡			
Case donors, No.	6	6	7
Positive ELISA at follow-up‡	0	1	4

*Western blot assay.

†Recombinant immunoblotting assay.

‡The mean duration between influenza vaccination and follow-up testing was 75 days (range, 52 to 130 days).

donors during 1991. Blood is donated 5½ days per week to permanent sites and 5 days per week to mobile units. All blood specimens were tested for alanine aminotransferase (ALT) (EPOS Systemate, EM Diagnostics, Gibbstown, NJ), hepatitis B surface antigen (Auszyme Monoclonal, Abbott Laboratories, Abbott Park, Ill), antibody to hepatitis B core antigen (Corzyme, Abbott Laboratories, Abbott Park, Ill), anti-HIV antibody (HIV-1 EIA, Abbott Laboratories, Abbott Park, Ill), anti-HTLV-1 antibody (HTLV-1 EIA, Abbott Laboratories, Abbott Park, Ill), and anti-HCV antibody (Recombinant C-100-3 HCV EIA, Abbott Laboratories, Abbott Park, Ill). Testing was accomplished the day following donation at the regional headquarters in Madison, Wis, and Duquaque, Iowa. Alanine aminotransferase levels less than 60 U/L were considered to be within normal limits. Specimens repeatedly ELISA-positive for HIV-1 underwent confirmatory testing at the American Red Cross National Reference Laboratory for Infectious Diseases using a licensed Western blot kit (Biotech-Dupont, Rockville, Md). Specimens repeatedly positive for HTLV-1 were tested using a standard Western blot assay¹ at the National Reference Laboratory for Infectious Diseases. Repeatedly reactive HCV ELISA specimens underwent supplementary testing using a recombinant immunoblotting assay (Chiron Corporation, Emeryville, Calif).

A case-control study was conducted to investigate risk factors associated with multiple false-positive viral ELISAs. We defined a case donor as a blood donor at a Badger Region site during the study interval October 31, 1991, through December 15, 1991, who had repeatedly reactive ELISA results on a single donated specimen for two or more of the following viruses—HIV, HTLV-1 and HCV—that were unconfirmed by supplementary testing. Thirty control donors were randomly selected from seronegative blood donors (for all

viral serologic tests) during the study interval. Data on age, gender, blood type, receipt of vaccination, type of vaccine received, site, date, and time of donation were abstracted from standard blood donation records. A Red Cross official interviewed case donors and control donors (January 13 through 21, 1992) and questioned them regarding receipt of influenza, tetanus, measles-mumps-rubella, hepatitis B, rubella (single antigen), polio, and pneumococcal vaccines during the 12-month period prior to the index blood donation. Study donors acknowledging receipt of a vaccine were questioned regarding date of vaccination.

To estimate the frequency of recent influenza vaccination among blood donors, a random sample of donation records of persons who had donated blood during the study interval was examined. Donors who acknowledge receipt of a vaccine on the donation record are routinely questioned by a nurse regarding the type of vaccine received and this information is recorded on the blood donation record. Information regarding age, gender, date of donation, and receipt of influenza vaccination during the 12-month period before donation were abstracted from these records. We calculated that a sample size of 215 donation records would be required to have 95% confidence that the percentage of donors who received influenza vaccine did not vary from $7.5\% \pm 3.5\%$ (mean \pm SD). We sampled 262 blood donation records to allow for systematic randomization.

To examine the kinetics of this phenomenon, serum samples were obtained from consenting case donors more than 30 days after their initial ELISA-positive donation and retested using ELISAs for HIV, HTLV-1, and HCV antibody.

We calculated odds ratios (ORs) and exact 95% confidence intervals (CIs) using conventional techniques² and used two-tailed Fisher's Exact Tests to test differences between variables. Standard

formulas were used to calculate the 95% CIs for the proportion of donors vaccinated against influenza during the study interval.³

Results

Among 17 941 blood donors during the study interval, 10 case donors were identified. Among case donors, the mean age was 47 years (range, 19 to 73 years), four were male, eight had previously donated blood, and six had donated during the 3 months prior to their ELISA-positive donation. The number of positive ELISA results for the 10 case donors is shown in the Table. Five case donors had positive results for all three viral ELISAs. The majority of positive ELISAs for HIV and HTLV-1 were confirmed to be negative by Western blot assay, with the remainder being indeterminate (Table). All case donors with ELISAs reactive for HCV were subsequently negative for anti-HCV antibody using a recombinant immunoblotting assay (Table). Nine of 10 case donors had normal ALT levels, while one had a mildly elevated ALT level of 106 U/L.

Case donors did not differ significantly from control donors regarding age, gender, date of donation, donation site, time of donation, and blood type when examined by analysis of variance (ANOVA). Among the 30 control donors, the mean age was 37 years (range, 17 to 67 years) and 14 were male. During the interview, nine of the 10 case donors said that they had received influenza vaccination the season prior to index donation, compared with only three of 30 controls (OR=81; 95% CI, 6 to 3670; $P < .001$, Fisher's Exact Test, two-tailed). Vaccination with tetanus toxoid or measles-mumps-rubella vaccine was not significantly associated with case-donor status when controlled for receipt of influenza vaccine. No individual in the study received hepatitis B, polio, rubella, or pneumococcal vaccines during the 12 months prior to donation.

We examined the kinetics of multiple positive viral screening test results among case donors who received influenza vaccine. Among the nine case donors who had received influenza vaccine, the mean time between influenza vaccination and blood donation was 26 days (range, 9 to 68 days); this was similar to that of the three control donors who received influenza vaccine (mean, 20 days; range, 8 to 35 days). Follow-up of seven case donors sampled a mean of 75 days (range, 52 to 130 days) after influenza vaccination demonstrated reversion to ELISA negativity among six of six tested for antibody to HIV and five of six tested for antibody to HTLV-1; however, ELISA results remained

reactive for antibody to HCV for four of seven case donors (Table).

Next, we investigated the risk of blood donors having multiple positive viral screening tests following influenza vaccination. Among 262 seronegative donors who were randomly selected from the study interval, 15 (5.7%; 95% CI, 2.9% to 8.5%) had noted receipt of influenza vaccination (for the 1991-1992 season) on the standard donor record. Based on these data, we estimate between 520 (2.9%) and 1524 (8.5%) of 17 941 blood donors during the study interval received influenza vaccine (formulated for the 1991-1992 season) prior to donation. Because nine case donors received influenza vaccine, we conclude that between 0.6% (9/1524) and 1.7% (9/520) of blood donors who received influenza vaccine had multiple (two or more) false-positive viral serologic tests for antibody to HIV, HTLV-1, and HCV.

Comment

Our study data revealed that influenza vaccination was associated with the occurrence of multiple false-positive viral ELISAs among blood donors. The proposed mechanism is believed to be due to an early nonspecific IgM response (oral and written communications, March 3, 1992, S. L. Stramer, PhD, Abbott Laboratories, Abbott Park, Ill); therefore, the association of this phenomenon with other vaccines is plausible, though not demonstrated in this small study. Although this phenomenon appears to be

of short duration for HIV and HTLV-1, the maximal duration of HCV false-positivity is yet unknown.

To avoid unnecessary collection and discard of false-positive units of blood, blood center officials may consider temporary deferral of potential blood donors who recently received influenza vaccine, but such a decision could result in a significant temporary decrease in the blood supply. Furthermore, the estimated low incidence of multiple false-positive viral ELISAs among recipients of this season's influenza vaccine suggests that influenza vaccinees need not be deferred from blood donation. We recognize that our review of blood donation records likely underestimates the number of blood donors who received influenza vaccine. This underestimation would lead to an overestimation of the risk of donors having multiple false-positive viral serologic tests following influenza vaccination. Given that this risk is already estimated to be very low ($\leq 1.7\%$), more precise estimates only further strengthen our recommendation that potential blood donors who recently received influenza vaccine not be deferred from blood donation.

Perhaps the most important issue among blood donation centers with regard to this phenomenon is the permanent loss of donors. Blood donations with positive ELISAs for HIV and HTLV that are negative by Western blot assay are destroyed and not transfused, but the donors are allowed to donate again and

are permanently deferred from donation only if the subsequent ELISA is positive.⁴ In contrast, due to the lack of a licensed confirmatory test for HCV, the US Public Health Service has recommended that blood donation centers indefinitely defer all donors who are repeatedly HCV ELISA reactive on a single donation.⁵ The presence of HCV false-positivity among our case donors has resulted in their exclusion from blood donation. Based on our findings, we recommend that case donors who received influenza vaccination and have been indefinitely deferred from donating blood be considered for reentry into the blood donor pool once reactivity to the specific screening tests has reverted to negative.

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