

## Screening for HLA antibodies in plateletpheresis donors with a history of transfusion or pregnancy

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**BACKGROUND:** Transfusion-related acute lung injury (TRALI) is known as a life-threatening complication of transfusion. HLA and HNA antibodies have been associated with the immune pathway of TRALI. Since donors with a history of transfusion and/or pregnancy are presumed to have an increased risk of carrying such antibodies, we investigated the association of a history of transfusion or pregnancy with the occurrence of HLA alloimmunization in our donor population.

**STUDY DESIGN AND METHODS:** A total of 1018 female plateletpheresis donors and male plateletpheresis donors with a history of transfusion were enrolled in the study. Included donors were systematically screened, using Luminex technology, for anti-HLA Class I and II. The association of donor history with HLA alloimmunization status was analyzed.

**RESULTS:** The overall alloimmunization rate was 20.2%. In 0.0% of the nulliparous transfused female donors and in 1.3% of the transfused male donors, anti-HLA were detected. Thirty-one percent of the parous women versus 4.2% of the nulliparous women screened positive for anti-HLA. The rate of HLA alloimmunization increased with parity.

**CONCLUSION:** Our data indicate that a history of transfusion is a minor risk factor for immunization against HLA antigens. In contrast, former pregnancies constitute a major risk factor for the development of HLA antibodies. Since HLA alloimmunization rate increases with parity, TRALI risk reduction measures should focus on this particular donor population. Repeated testing of female plateletpheresis donors after each pregnancy is implemented in our blood service.

**T**ransfusion-related acute lung injury (TRALI) is a type of noncardiogenic pulmonary edema characterized by acute respiratory distress that can occur as a complication after transfusion.<sup>1</sup> Although the pathogenesis of TRALI is only partially resolved, an increasing number of studies have confirmed the role of antibodies against HLA and HNA antigens in the development of TRALI.<sup>2-6</sup> Recent case control studies have shown that HLA Class I antibodies are weak triggers of TRALI compared to HLA Class II and HNA antibodies,<sup>7-9</sup> and a smaller study even debated the involvement of HLA antibodies at all.<sup>10</sup>

TRALI accounted for 47% of the transfusion-related fatalities reported to the US Food and Drug Administration from 2005 through 2010.<sup>11</sup> Although all blood components have been implicated in TRALI, products containing large amounts of plasma impose a higher risk.<sup>12</sup> The Serious Hazards of Transfusion hemovigilance program conducted from 1996 to 2002 observed that plasma from female blood donors with white blood cell antibodies was the component most often implicated in TRALI.<sup>13</sup> A passive surveillance study of TRALI cases reported to the American Red Cross came to similar conclusions.<sup>14</sup>

Since then, several donor management strategies, for example, exclusion of female donors from single-unit fresh-frozen plasma production, have been proposed and implemented worldwide.<sup>13,15-17</sup> Even with modern TRALI

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risk reduction strategies, TRALI remains an important cause of transfusion-related morbidity and mortality and is estimated to occur in approximately one in 12,000 transfused blood components.<sup>8</sup>

At our institution, methylene blue-inactivated plasma has been, since 2004, produced originating only from male donors without a history of transfusion. In platelet (PLT) concentrates—both buffy coat derived and from apheresis—plasma content is significantly reduced by using PLT additive solutions (ASs). Before implementing additional measures for plateletpheresis donors, a pilot study was undertaken in our blood service to analyze the HLA immunization status in female plateletpheresis donors and in male plateletpheresis donors with a history of transfusion. Based on the outcome of this study, an HLA alloimmunization screening strategy was developed.

## MATERIALS AND METHODS

### Donor selection

Plateletpheresis donors are voluntary, nonremunerated donors between the ages of 18 and 65 years. In accordance with the European Directive 2002/98/EC and Belgian law they are selected and allowed to give PLTs 24 times a year with a minimum interval of 2 weeks between two subsequent donations. In addition to this legislation, plateletpheresis donors must test negative for red blood cell (RBC) alloantibodies at each donation. The screening of RBC alloantibodies is performed on each individual donation in first-time donors and after a history of transfusion and/or pregnancy. In all other situations, testing is performed on serum pools of 50 donations. If the pool tests positive, each individual donation is retested.

During a 1-year time period, plateletpheresis donors were recruited for this study. All female donors and male donors with a transfusion history registering for PLT donation were asked to participate in the study.

The involved donors were informed about the aim of the study by the physician at the collection site, signed an informed consent, and were asked to complete a short questionnaire containing the following questions:

- Female donors: pregnancies including miscarriages?
  - o If yes: the number of pregnancies, the year of the partus or abortion
  - o If more than one pregnancy: the involvement of one or more biologic fathers
- Male and female donors: transfusion history?
  - o If yes: number and year of transfusion(s)

Two additional blood samples, one serum sample for HLA antibody-screening and identification and granulocyte agglutination test and one EDTA sample for future DNA typing of HLA and/or HNA, were collected.

### Detection of HLA antibodies

HLA antibodies were screened using a commercial platform (Luminex, LIFECODES LifeScreen Deluxe, Immucor, Norcross, GA). This method is an adaptation of standard flow cytometry using fluorescent antigen-coated microbeads. For screening, these beads are coated with a wide range of HLA antigens of multiple individuals. For identification, the beads are coated with HLA antigens of one individual (LIFECODES Class I ID and Class II ID, Immucor) or with single recombinant antigens (LIFECODES LSA Class I and Class II, Immucor). This method detects HLA Class I and HLA Class II antibodies separately in one assay and is known from clinical studies to be sensitive and specific.<sup>18</sup> A result was defined as positive following the algorithm proposed by the manufacturer. Positive results were classified as weak positive if both mean fluorescence intensity and adjusted value were, respectively, less than 2000 and 10. This is an operational definition to select sera that needed further testing with the single-antigen identification test to confirm the specificity of HLA antibodies and to exclude nonspecific reactions in borderline positive samples.

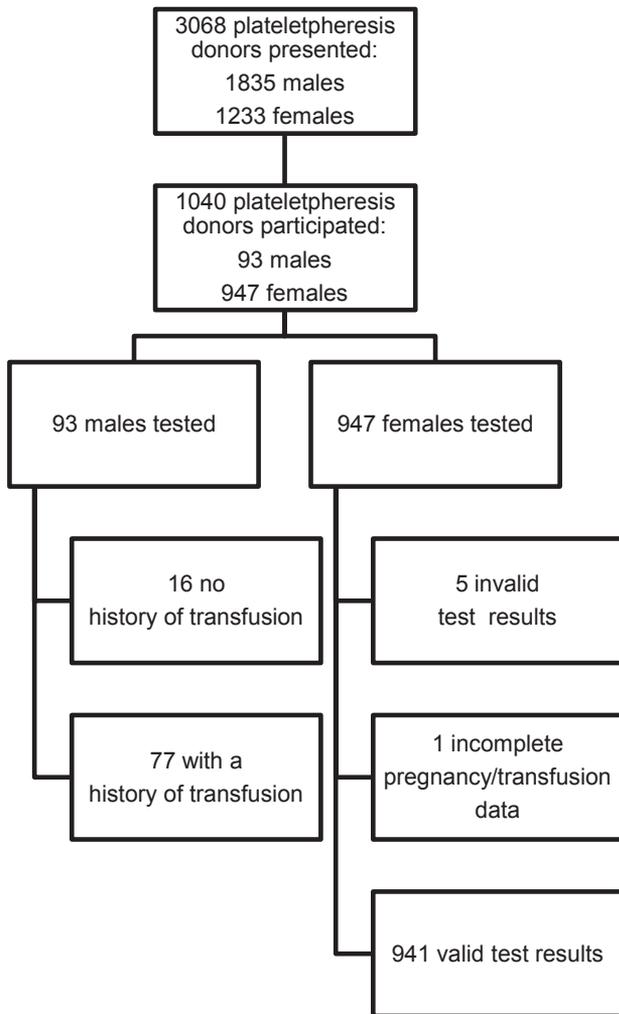
### Statistical analysis

Two-sided Fisher's exact tests were performed to assess associations between two variables: "history of pregnancy" and "alloimmunization," "history of transfusion" and "alloimmunization," "one versus more biologic fathers in multiparous women" and "alloimmunization," and "transfusion and pregnancy at the same time versus transfusion and pregnancy separated in time in parous women" and "alloimmunization." *p* values of less than 0.05 were considered significant. The association of "time since last pregnancy" with "alloimmunization" was examined by chi-square test and the association of "number of pregnancies" with "alloimmunization" was performed using a correlation model (95% confidence interval [CI]).

## RESULTS

### Donor selection

During a 1-year enrollment period, 3068 plateletpheresis donors presented to the blood center (Fig. 1). A total of 1040 (947 females and 93 males) plateletpheresis donors consented to participate in the study and answered the questionnaire regarding pregnancies and transfusion history. Sixteen male donors had no history of transfusion and were excluded from the study. Samples of five female donors gave invalid test results and these donors were also excluded from the study. In seven of the 1019 donors, questionnaires were not completed for history of transfusion or history of pregnancy. Six donors were contacted successfully. The one remaining donor with incomplete questionnaire was excluded from the study. This resulted



**Fig. 1. Flow chart of the study population. Of the 3068 plateletpheresis donors who presented to the blood center, 1040 participated in the study and serum samples were collected for testing. Only 1019 (98%) of the 1040 donors were accepted for further analysis (77 male donors with a history of transfusion and 942 female donors). One positive tested female donor who gave incomplete pregnancy and transfusion data was unavailable and was excluded from the study. This resulted in a final sample of 1018 donors for analysis consisting of 941 female and 77 male donors who received transfusions.**

in a final sample of 1018 donors, 941 female and 77 transfused male donors for further study. In two donors, time interval since transfusion was not available. These donors were not excluded from the study.

**Frequency of HLA immunization in the study population**

Table 1 summarizes the results obtained by screening for HLA antibodies in the studied population. The overall

number of positive tested donors (HLA I and/or II) was 206 (20.2%): one male and 205 female. Overall, 59 donors had a positive screening test for HLA Class I, 75 for HLA Class II, and 72 for both HLA Class I and HLA Class II. Of the 206 positive screened donors, 40 were considered as weak positive (one male and 39 female). Identification of the antibody revealed 25 clear-cut positive results (also directed against HLA haplotypes); in 15 no HLA alloantibody specificity could be defined. Thirty-nine of the 40 weak positive results were determined in donors with history of transfusion and/or pregnancy.

**History of previous pregnancies or transfusions**

A total of 941 female donors answered the transfusion history and pregnancy history questions. A total of 619 (65.8%) of these donors reported previous pregnancies. A total of 106 donors reported a combined history of pregnancy and transfusion. Only 12 of 322 female donors without a history of pregnancy reported previous blood transfusions (Table 2).

**Impact of transfusion history**

Of the 77 male donors with history of transfusion, only one donor screened positive, classified as weak positive, for HLA antibodies (Table 1). As shown in Table 2, 12 of 322 nulliparous female donors had a history of transfusion. In none of these female donors were HLA antibodies detected. In addition, the frequency of HLA antibodies was similar in parous women with a history of transfusion and parous women without a history of transfusion.

**Impact of history of pregnancy**

A total of 192 of 619 (31.0%) female donors who reported a previous pregnancy versus 13 of 322 (4.0%) of the nulliparous women tested positive for one or both classes of HLA antibodies. Regardless of number of pregnancies, a “history of pregnancy” was found to be related to HLA alloimmunization ( $p < 0.0001$ ; Table 3).

In two of the 106 female donors with a history of both transfusion and pregnancy, information on the year of transfusion was not available. For 65 of the remaining 104 donors, pregnancy and transfusion occurred in the same year. In 26 of 65 (40.0%) donors from this subgroup, HLA antibodies were found (Class I and/or II). Although the frequency of HLA antibodies appeared lower in donors in which transfusion and pregnancy were more dispersed in time, namely, 11 of 39 (28.2%), this difference between both groups was not significant ( $p = 0.291$ ; Table 3).

The total number of pregnancies of parous female donors was 1483. Of the 65 donors with a history of pregnancy and transfusion in the same year, the total number

**TABLE 1. Results summary of all donors (male and female)\***

Antibodies	All donors, n = 1018 (100.0%)	Male (T > 0), n = 77 (7.5% of all donors)	Female, n = 941 (92.4% of all donors)
Anti-HLA positive	206/1018 (20.2)	1/77 (1.3)	205/941 (21.8)
HLA I positive only	59 (28.6)	0 (0.0)	59 (28.8)
HLA II positive only	75 (36.4)	1 (100.0)	74 (36.1)
HLA I and II positive	72 (35.0)	0 (0.0)	72 (35.1)
Anti-HLA negative	812/1018 (79.8)	76/77 (98.7)	736/941 (78.2)

\* Data are reported as number (%).

T = history of transfusion.

**TABLE 2. Results summary of female donors (with or without pregnancy and/or transfusion)\***

Antibodies	All female donors, n = 941 (100.0%)			
	Female P > 0, n = 619 (65.8% of all female donors)		Female P = 0, n = 322 (34.2% of all female donors)	
	T = 0, n = 513 (82.9%)	T > 0, n = 106 (17.1%)	T = 0, n = 310 (96.3%)	T > 0, n = 12 (3.7%)
Anti-HLA positive	154/513 (30.0)	38/106 (35.8)	13/310 (4.2)	0/12 (0.0)
HLA I positive only	48 (31.2)	10 (26.3)	1 (7.7)	0 (0.0)
HLA II positive only	57 (37.0)	7 (18.4)	10 (76.9)	0 (0.0)
HLA I and II positive	49 (31.8)	21 (55.3)	2 (15.4)	0 (0.0)
Anti-HLA negative	359/513 (70.0)	68/106 (64.2)	297/310 (95.8)	12/12 (100.0)

\* Data are reported as number (%).

P = history of pregnancy; T = history of transfusion.

**TABLE 3. HLA antibody prevalence in different donor subgroups\***

Donor group	HLA antibodies	No HLA antibodies	p value†
Females (n = 941)			
Parous (n = 619)	192 (31.0)	427 (67.0)	p < 0.0001
Nulliparous (n = 322)	13 (4.0)	309 (96.0)	
Nulliparous females‡ (n = 322)			
Transfused (n = 12)	0 (0.0)	12 (100.0)	p = 1
Nontransfused (n = 310)	13 (4.2)	297 (95.8)	
Parous females§ (n = 619)			
Transfused (n = 106)	38 (35.8)	68 (64.2)	p = 0.2497
Nontransfused (n = 513)	154 (30.0)	359 (70.0)	
Multiparous females   (n = 486)			
Pregnancies from more than one biologic father (n = 20) (mean number of pregnancies, 2.75)	8 (40.0)	12 (60.0)	p = 0.62
Pregnancies from one biologic father (n = 446) (mean number of pregnancies, 2.77)	148 (33.2)	298 (66.8)	
Parous and transfused females (n = 104)			
Pregnancy and transfusion in the same year (n = 65)	26 (40.0)	39 (60.0)	p = 0.291
Pregnancy and transfusion not in the same year (n = 39)	11 (28.2)	28 (71.8)	

\* Data are reported as number (%).

† Two-sided Fisher's exact test, 95% CI, p = 0.05.

‡ Nulliparous = women with no pregnancy.

§ Parous = women with pregnancy.

|| Multiparous = women with two or more pregnancies.

of transfusions was 76. So the rate of transfusion in female donors per pregnancy was 5.1% (76/1483).

### Impact of number of pregnancies on the prevalence of HLA antibodies

The association of number of pregnancies with alloimmunization was performed using a correlation model (Fig. 2). Of the female donors, 619 of 941 (65.8%) had a history of pregnancy, 106 (17.1%) with and 513 (82.9%) without a history of transfusion. Parity ranged from zero to

nine pregnancies. With increasing parity, the likelihood of finding HLA antibodies increased with the number of pregnancies (p = 0.003; Fig. 2). The association with the number of pregnancies strongly remained even after excluding data from the transfused parous female donors (p = 0.002; Fig. 2). Our data confirm the findings of other studies that each pregnancy increases the likelihood of HLA immunization.<sup>19-22</sup>

A total of 310 of 941 female donors reported no history of pregnancy or transfusion. Nevertheless, 13 of

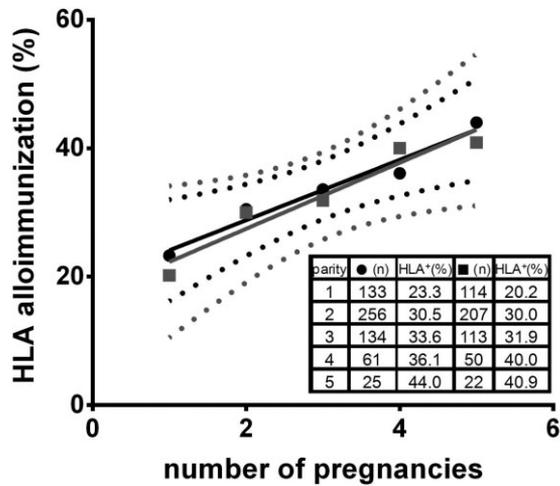


Fig. 2. Correlation model for the prevalence of HLA antibodies with increased parity. The dotted lines represent the 95% CI. (●) History of pregnancy and transfusion,  $p = 0.003$ ; (■) history of pregnancy only,  $p = 0.008$ .

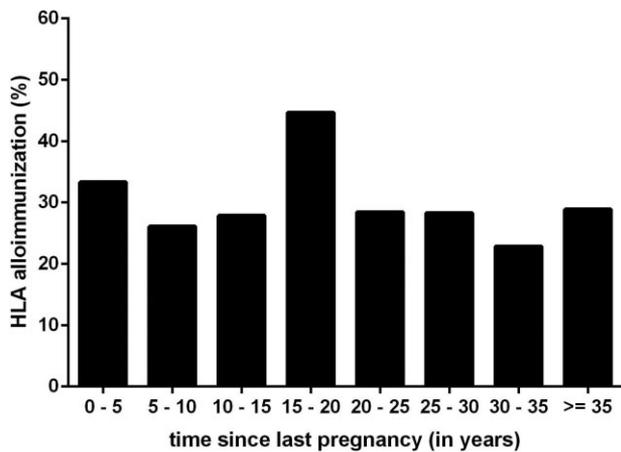


Fig. 3. Prevalence of HLA antibodies in parous females by time since last pregnancy.

them had clearly defined HLA antibodies directed against well-defined HLA antigens spreading over HLA AB DR-haplotypes, which may indicate missed abortions or intentional nondisclosure of abortions or pregnancies (Table 2).

**Impact of time since last pregnancy on the prevalence of HLA antibodies**

The influence of “time since the last pregnancy” on the occurrence of HLA antibodies is displayed in Figure 3. Time since last pregnancy was categorized in 5-year intervals. HLA alloimmunization is the highest if the interval of time since last pregnancy is between 15 to 20 years (44.7%). Overall, a small significance between time since last pregnancy and HLA antibody prevalence was found

( $p = 0.0351$ ), but no specific trend could be determined. We observed no abrupt decline in the prevalence of HLA antibodies as time since last pregnancy increased (from 30.0% [ $<10$  years] to 25.2% [ $>30$  years]) unadjusted for number of pregnancies, in contrast to Triulzi and coworkers<sup>20</sup> (from 31.3% [ $<10$  years] to 18.3% [ $>30$  years]). This difference might be caused by the high sensitivity of the Luminex assay.

**Impact of pregnancies from more than one biologic father in the occurrence of HLA antibodies**

In multiparous women, the involvement of more than one biologic father in the occurrence of HLA alloimmunization was investigated. Twenty donors reported more than one biologic father, 446 reported one biologic father, and 20 donors did not answer the question. The occurrence of HLA alloimmunization was not significantly different among both groups (Table 3).

**DISCUSSION**

In 2008, our blood service started reducing the volume of residual plasma by AS supplementation for all PLT products. This was, given the known relationship between transfusion of plasma and TRALI risk,<sup>12</sup> an important step in reducing the risk for TRALI induced by donor antibodies in PLT products. Whereas for virus-inactivated plasma, all female donors are deferred, a similar approach for plateletpheresis products would result in an unacceptable loss of volunteer donors as approximately 32.0% of the plateletpheresis donations are female in our blood service. The ultimate goal of this study was to develop a strategy that would further reduce the risk of TRALI without compromising the supply of PLT concentrates. We therefore evaluated the effect of a history of transfusion or pregnancy on HLA alloimmunization in our plateletpheresis donor population and studied the influence of time since last pregnancy, the number of pregnancies, and the involvement of different biologic fathers on the occurrence of HLA alloimmunization.

The obtained data confirm that the frequency of HLA alloimmunization is higher in women with a history of pregnancy. Although donors that screened positive for RBC alloantibodies were already excluded in this cohort, 31.0% of the parous female donors screened positive for HLA antibodies. This percentage is equal to or even higher than the 25.4% of all female donors described in Powers and colleagues<sup>19</sup> and the 24.4% in parous female donors reported by Triulzi and colleagues.<sup>20</sup> In our study, a Luminex platform was used to perform the HLA screening. The high sensitivity of this method may explain the high percentage of HLA-positive parous women, even when testing was conducted decades after the last pregnancy.

The observation that even more than 30 years after the last delivery a significant association between pregnancy history and HLA immunization is detected supports that a strategy in which all parous female donors are tested for HLA alloantibodies irrespective of the time span since the last pregnancy makes sense and is in line with Powers and colleagues.<sup>19</sup>

The increasing frequency of HLA alloimmunization with increasing number of pregnancies indicates that each pregnancy may act as an additional immunizing event. Therefore, screening for HLA antibodies, comparably to RBC alloantibody screening should be repeated after each pregnancy. Although the observed frequency of HLA immunization appeared higher if multiple pregnancies were induced by more than one biologic father, study numbers were too low to demonstrate significant difference. Surprisingly, 4.2% of all female donors without previous pregnancies or transfusions were found positive in our study. These HLA alloimmunizations may be related to missed abortions or intentional nondisclosure of induced abortions or pregnancies.

Powers and colleagues<sup>19</sup> demonstrated the presence of HLA antibodies in 12.0% of male donors with a prior history of transfusion. Our study could not confirm these findings. Indeed, in our donor population, only one of 77 screened male donors with a history of transfusion had a (weak) positive result for HLA antibodies. In addition, none of the nulliparous women with a history of transfusion had HLA antibodies. Our data suggest that, compared to pregnancy, transfusion does not appear to be as potent for HLA immunization. In our blood service, leukoreduction of cellular blood components was implemented nationwide in 2005. This measure possibly decreased the risk for primary HLA alloimmunization after transfusion. Furthermore, the deferral of donors that screen positive for RBC alloantibodies could have lowered the percentage of donors sensitized to HLA antigens in the remaining active donor population. In general, our observations suggest that screening for HLA alloimmunization based on a history of transfusion has limited additional value in the era of leukoreduction. Future studies should confirm this finding.

The overall transfusion rate in our parous female donor population (5.1%) is high compared to transfusion rates in the general obstetric population in Flanders (Belgium; 1.0%-1.2% during the period 2002 and 2007)<sup>23</sup> and compared to transfusion rates reported in other countries.<sup>24-26</sup> In our donor population we observed a decreasing transfusion rate over time, namely, 6.7% from 1960 to 1969, 6.1% from 1970 to 1979, 5.5% from 1980 to 1989, 4.1% from 1990 to 1999, and 3.8% after 2000, suggesting the existence of a more liberal transfusion policy in national hospitals in previous decades. In addition, the high transfusion rate in our study population might also be explained in part by increased motivation of women

who themselves once needed transfusion to become a blood donor. The current study design tested male donors with a history of transfusion not including a control group that was never transfused; therefore, a comparison of HLA alloimmunization incidence between these two groups was not possible.

It should be pointed out that testing donors for HLA antibodies will never completely eliminate the risk of TRALI, especially as antibodies directed to neutrophil-specific antigens may be involved.<sup>7</sup> At present, additional testing for HNA antibodies is not performed as the tests are not available in our institute. When routine HNA testing on the Luminex becomes available, the study group of HLA antibody positive tested donors will be reanalyzed for this variable.

After completion of this study, donors who tested positive for HLA Class I and/or Class II antibodies were excluded from plateletpheresis donation. This resulted in a loss of approximately 21.0% of the female PLT donor population and approximately 10.0% of the entire PLT donor population.

Within our institution, screening for HLA antibodies is now routinely conducted for all new female plateletpheresis donors and for female plateletpheresis donors after each pregnancy. For male and female plateletpheresis donors, we currently continue to screen for HLA antibodies after each transfusion episode to obtain more data to confirm that transfusion of leukoreduced blood constitutes a minor risk factor.

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#### CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

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