Influenza Vaccination and Vitamin K Antagonist Treatment

A Placebo-Controlled, Randomized, Double-blind Crossover Study

Alfonso Iorio, MD; Michela Basileo, Biol D; Maura Marcucci, MD; Francesco Guercini, MD; Barbara Camilloni, Biol D; Elisa Paccamiccio, MD; Maria Vecchioli, MD; Anna Maria Iorio, Biol D

Background: Among millions of persons vaccinated against influenza virus each year, many are older patients treated with several drugs, including vitamin K antagonists (VKAs), among which warfarin is the most commonly used. Due to high interpatient and intrapatient variability, the therapeutic dose of VKA has to be individualized by monitoring of international normalized ratio (INR) values. The objectives of this study were to evaluate variation in the INR and warfarin weekly dose variation after influenza vaccination administration and to follow up patients for related hemorrhagic and thrombotic events to evaluate the safety of the influenza vaccine and to assess the immunogenicity of the influenza vaccination in patients receiving VKAs.

Methods: One hundred four patients on a stable VKA regimen and with an indication for influenza vaccination were randomized to receive influenza vaccination and subsequent placebo administration, or vice versa. All patients were tested for coagulation variables, clinical events, and antibody response against vaccine components.

Results: Similar mean prothrombin times, expressed as the INR and VKA weekly dose, were found in patients after receiving vaccine or placebo. The absence of any vaccination effect on VKA treatment was confirmed using a linear mixed-effects model. The percentages of time that patients were in therapeutic range were 70.7% after receiving vaccine and 72.4% after receiving placebo ($P = .57$). There were no fatal or major bleeding events and 11 minor mucocutaneous hemorrhagic events. After vaccination, the percentage of seroprotected patients ranged from 92.0% to 100.0% depending on the vaccine antigen examined.

Conclusions: Influenza vaccination had no significant effect on INR values or warfarin sodium weekly doses. Close monitoring of INR values is not required after influenza vaccination in patients on stable long-term VKA regimens.

Trial Registration: clinicaltrials.gov Identifier: NCT00222638

Arch Intern Med. 2010;170(7):609-616

©2010 American Medical Association. All rights reserved.
of Chest Physicians in 2004, influenza vaccination can potentiate the pharmacokinetics of warfarin. In contrast, the 2008 guidelines state that influenza vaccination can inhibit the effects of warfarin.

The inconsistent results obtained in assessing the interaction of influenza vaccine with VKAs may be because few patients were studied. They may also reflect the multiplicity of biologic mechanisms potentially involved (coagulation, immunologic, and virologic) and highlight the need for further investigation.

The present study was a prospective trial using standard methods aimed at controlling bias in the assessment of the safety and efficacy of influenza vaccine in patients receiving VKAs. The primary objectives of this study were to evaluate variation in the INR and warfarin weekly dose after influenza vaccination and to follow up patients for related hemorrhagic and thrombotic events. The secondary objective of our study was to assess the immunogenicity of influenza vaccination in patients receiving VKAs.

### METHODS

**STUDY PATIENTS**

The study protocol was performed by the VKA monitoring service of Perugia, Italy. Eligible patients were 18 years or older, were receiving a stable VKA regimen, and had an indication for influenza vaccination. Stable VKA therapy was defined as treatment for longer than 6 months and a recent history of 3 consecutive INRs 3 weeks apart in the appropriate therapeutic range.

Patients were enrolled in the study if the INR was within ±0.6 U of the therapeutic range at week −2 and within ±0.5 U at week −1. The INR was expected to be within ±0.2 U of the therapeutic range at randomization (time 0). Patients with a time 0 INR within ±0.5 U of the therapeutic range were retested the following week and were randomized or not depending on the test results.

Patients were excluded from the study if they met any of the following criteria (Figure 1): sensitivity to the vaccine or its components, short life expectancy (<6 months), planned surgery within 6 months, or refused to sign an informed consent form. At every study time point, patients filled out a questionnaire recording ischemic or bleeding adverse effects or adverse reactions related to vaccination (according to guidelines by the Centers for Disease Control and Prevention).

**MAIN STUDY END POINTS**

There were 3 primary end points of the study. These include the following: INR variation, warfarin weekly dose variation, and patient follow-up for treatment-related clinical events.

### INR Variation

We studied the time course of INRs during 28 days after influenza vaccination or placebo administration. The INRs were analyzed as raw data, as a proportion of results within the therapeutic range (within ±0.5 and ±1 U), and as the percentage of time in the therapeutic range.

### Warfarin Weekly Dose Variation

The time course of warfarin weekly doses were assessed during 28 days after influenza vaccination or placebo administration.

### Patient Follow-up for Treatment-Related Clinical Events

We studied clinically overt adverse effects related to VKA treatment (ie, hemorrhagic or thrombotic events) during the study period and within 6 months of the subsequent follow-up. Bleeding events were considered major if they met any of the following criteria: (1) a bleeding event was fatal, clinically overt, or involved a critical site (eg, intracranial, retroperitoneal, intraocular, spinal, or pericardial bleeding); (2) if a bleeding event was clinically overt and was associated with a decrease in hemoglobin level of at least 2 g/dL (to convert hemoglobin level to grams per liter, multiply by 10.0) or with a need for transfusion of at least 2 U of blood; or (3) if permanent cessation of the study treatment was warranted. All other bleeding events were considered minor.

### SECONDARY STUDY END POINT

The secondary objective of the study was to evaluate the efficacy of influenza vaccination according to immunologic criteria (antibody titer to establish the immune reaction). Laboratory methods to assess the efficacy of influenza vaccination in patients receiving anticoagulation therapy are described in greater detail elsewhere.

### STUDY PROCEDURE

All patients were immunized in the deltoid muscle with a single injection of commercially available trivalent subunit MF-59 ad-
juvanted influenza vaccine (FLUAD; Novartis, Siena, Italy). The viral components were Fujian/411/02 (influenza A[H3N2]), New Caledonia/20/99 (influenza A[H1N1]), and Shanghai/361/02 (influenza B). All patients, irrespective of randomization, underwent influenza vaccination at least 1 month before the expected time of influenza virus circulation. An identical injection syringe filled with normal saline was used for placebo administration. Empty sterile syringes identical to those containing vaccine were provided by the vaccine manufacturer, and normal saline was aspirated by a nurse immediately before injection.

Using a computer-generated random series blocked by 4 and sealed envelopes, patients were randomized into the following 2 groups: vaccine-placebo sequence (VP) and placebo-vaccine sequence (PV). In period 1, patients assigned to VP received influenza vaccine, and patients assigned to PV received placebo (Figure 1B, time 0 to time 28). A washout period of 14 days was included to reduce the risk of carryover. Thereafter, the patients crossed over and received the alternate treatment in period 2 (Figure 1B, time 0 to time 28). Patients, the physician in charge of the VKA monitoring service (E.P.), clinicians at the follow-up visits (F.G.), and technicians performing laboratory testing were unaware of patients’ treatment assignment. The nurse administering study treatments was the only individual who knew if the preparation was vaccine or placebo, and the nurse had no other role in the study.

**BLOOD SAMPLING AND LABORATORY ASSAYS**

The INR was checked by capillary sampling at baseline (time 0) and at 7, 14, and 28 days after influenza vaccination or placebo administration. At time 0 and time 28 in each period, patients were tested for influenza antigens and for INR by venous puncture. Capillary blood was collected by fingertip puncture and was analyzed using a commercially available system (ProTime; Instrumentation Laboratories, Milan, Italy). Blood for venous coagulation testing was drawn in a tube containing 0.109mM trisodium citrate (1:9 [vol/vol]) using a 19-gauge needle. The INR was measured in fresh plasma within 2 hours of venipuncture using a 1-stage prothrombin time method (RecombiPlastin on an ACL1000 coagulometer, IL). Titers of hemagglutination-inhibiting antibodies to the 3 influenza vaccine
antigens were determined simultaneously for all serum samples obtained from the same patient by a standard microtiter test using 0.5% turkey erythrocytes and egg-grown influenza antigens. The immunogenicity of influenza vaccine was evaluated according to the criteria of the Commission of the European Communities for influenza vaccination in older persons.\(^1^4\)

**STATISTICAL ANALYSIS**

The sample size for the study was calculated based on a mean (SD) INR of 2.61 (0.51) in the VKA monitoring service population, with a mean variation of ±0.5 at any follow-up time considered a clinically significant result. Assuming a 0.25 SD, 34 patients were needed to reach 80% study power at \(\alpha = 0.05\). Because the true standard deviation of the difference was unknown and assuming a standard withdrawal rate of 20%, we multiplied the sample size by 4, resulting in a population of at least 100 patients, which is usually considered adequate for immunogenic evaluation.

The main outcomes of the study were analyzed using a linear mixed-effects model for multilevel longitudinal data; INR and warfarin weekly dose measurements were considered separately as dependent variables.\(^1^5\) In the fixed-effects component of the model, the following sources of variation were investigated: treatment (vaccine or placebo), sequence (VP or PV), period (before or after crossover), time, and their interactions. Planned covariates included baseline characteristics that significantly differed between the 2 randomized groups. The random-effects component of the model allowed for between-patient random heterogeneity in the effects of treatment and of the interaction between treatment and time. The model estimates the regression coefficient (a measure of the mean effect) with adjusted 95% confidence intervals and a "random coefficient or slope" (representing deviation from the mean [ie, the regression coefficient for each patient]) for any variable in the fixed or random components of the mixed-effects model. For all patients, the model summarizes random coefficients with their standard deviations, which represent a measure of interindividual variability. The overall significance of the introduction of the random component of the model was evaluated using the log-likelihood ratio test. In a crossover design, this statistical approach also obviates preliminary testing for carryover. The models were fitted using maximum likelihood estimation.

Plots of individual predicted response profiles for patients treated with vaccine or placebo in period 1 and period 2 were obtained after estimation to help in appraisal of statistical results. The proportions of patients showing INR changes exceeding 0.5 or 1 U or adverse effects were compared using \(\chi^2\) statistics after pooling patients administered vaccine or placebo in the 2 randomized sequences. Correlation between capillary and venous INRs was tested using Passing-Bablock regression analysis and Bland-Altman plot analysis.\(^1^6\)

### Table 2. Baseline Demographic and Clinical Characteristics of the Study Population

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>PV Sequence (n=51)</th>
<th>VP Sequence (n=53)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male to female ratio(^a)</td>
<td>0.82</td>
<td>1.79</td>
</tr>
<tr>
<td>Age, mean (SD), y</td>
<td>70.8 (8.3)</td>
<td>71.8 (10.1)</td>
</tr>
<tr>
<td>BMI, mean (SD)</td>
<td>26.3 (3.7)</td>
<td>26.7 (4.1)</td>
</tr>
<tr>
<td>Atrial fibrillation, No.</td>
<td>28</td>
<td>28</td>
</tr>
<tr>
<td>Aortic prosthesis, No.</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Mitral prosthesis, No.</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Mitroaortic prosthesis, No.</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Venous thromboembolism, No.</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>Dilative cardiomyopathy, No.</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>INR, mean (SD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time 2</td>
<td>2.55 (0.67)</td>
<td>2.62 (0.74)</td>
</tr>
<tr>
<td>Time 1</td>
<td>2.51 (0.67)</td>
<td>2.64 (0.66)</td>
</tr>
<tr>
<td>Time 0</td>
<td>2.59 (0.60)</td>
<td>2.62 (0.60)</td>
</tr>
<tr>
<td>Warfarin sodium weekly dose, mean (SD), mg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time 1</td>
<td>30.00 (11.82)</td>
<td>31.93 (13.39)</td>
</tr>
<tr>
<td>Time 0</td>
<td>30.12 (11.96)</td>
<td>31.82 (12.85)</td>
</tr>
</tbody>
</table>

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); INR, international normalized ratio; PV, placebo-vaccine; VP, vaccine-placebo.

\(^a\)Significant at \(\alpha = 0.05\).
Statistical computations were performed using commercially available software (STATA, version 9.2; StataCorp LP, College Station, Texas). The study protocol was approved by the ethics committee of the Umbria region of Italy.

PATIENT DISPOSITION AND CHARACTERISTICS

As shown in Figure 1A, 278 patients had an indication for influenza vaccination and were referred to the VKA monitoring service. After screening for inclusion and exclusion criteria, 104 patients (57 male and 47 female [1.21 male to female ratio]) were randomized to the study. Four patients withdrew from the study because of nonadherence to the protocol. Baseline demographic and clinical characteristics of 51 patients randomized to PV and 53 patients randomized to VP are given in Table 2. No significant differences between the groups were found at baseline except for the male to female ratio, and sex was consequently included as a covariate in the mixed-effects model. Patients enrolled in the study were representative of the entire population of the VKA monitoring service in terms of sex distribution and indications for treatment.

TIME COURSES OF INRs AND WEEKLY DOSES

We preliminarily verified the correlation between capillary and venous INRs by comparing the values obtained by both methods at time 0 and time 28 in each period. Passing-Bablock regression analysis showed statistically significant agreement between the methods, and no significant bias existed between the methods by Bland-Altman plot analysis (Figure 2).

The time courses of INRs and warfarin weekly doses are shown in Figure 3. The INR means were similar between the 2 treatments in the 2 study sequences. For VP, the mean (SD) INRs were 2.63 (0.75) in period 1 and 2.67 (0.70) in period 2; for PV, they were 2.63 (0.73) in period 1 and 2.53 (0.64) in period 2 (Figure 3A).

In VP, the mean (SD) warfarin weekly dose was 31.91 (13.33) mg in period 1 and 31.15 (13.18) mg in period 2 (Figure 3B). In PV, the mean (SD) warfarin weekly dose was 29.88 (11.78) mg in period 1 and 30.27 (11.49) mg in period 2. Compared with the doses administered before enrollment, there were no significant changes in weekly doses during the study period irrespective of the type of initial treatment assigned.

As shown in Figure 3C for both groups, the percentages of time that patients were in the therapeutic range were 70.7% after vaccination and 72.4% after placebo administration (P = .57), and the percentage of time above or below the therapeutic range was about 15.0% after administration of vaccine or placebo. Among all patients, 97.4% remained within ±1 U of the therapeutic range after vaccination and 95.8% after placebo administration; 89.4% remained within ±0.5 U of therapeutic range after vaccination and 87.8% after placebo administration.

These differences were not statistically significant at α = .05.

LINEAR MIXED-EFFECTS MODEL ANALYSIS

Analysis of the same data using a linear mixed-effects model for multilevel longitudinal data confirmed that INR and warfarin weekly dose variation was independent of vaccination and was completely due to interpatient and intrapatient variability. The regression coefficients of the covariates included in the fixed-effects component of the model are given in Table 3. No covariate significantly affected INR (P = .30) or warfarin weekly dose (P = .21). Compared with the fixed-effects model, the inclusion of covariates allowing for between-patient random heterogeneity enabled the model to explain greater INR and warfarin weekly dose variability by log-likelihood ratio test (χ² = 113.01 for INR and χ² = 2601.69 for warfarin weekly dose; P < .001 for both). In the mixed-effects model, the standard deviation of the random coefficients for treatment and treatment × time interaction were large com-
pared with the corresponding means. The standard deviations were 81.0% greater than the means for treatment and greater than 100.0% for treatment × time when INR was analyzed, and they were greater than 100.0% for both covariates when warfarin weekly dose was analyzed.

In the analysis that included sex as a covariate, we found no effect of sex on INR or warfarin weekly dose. Figure 4 shows predicted INR and warfarin weekly dose response profiles for patients administered vaccine or placebo in period 1 and period 2.

**Clinically Overt Adverse Effects Related to VKA Treatment**

There were no fatal events among patients in the study. One episode of recurrent venous thromboembolism occurred in a patient with cancer and a central vein catheter. No major bleeding events were noted. Table 4 lists 11 minor mucocutaneous hemorrhagic events that occurred. Nine events (1 traumatic and 8 spontaneous) were recorded among VP patients, and 2 events were recorded among PV patients. Five events occurred within 28 days of placebo administration, and 6 events occurred within 28 days of vaccination. One patient receiving placebo had a high 6.9 INR at baseline, while the other 10 patients were in the therapeutic range. No patients reported use of aspirin or nonsteroidal anti-inflammatory drugs.

**Hemagglutination-Inhibiting Antibody Response to Influenza Vaccine Antigens**

Titers of hemagglutination-inhibiting antibody response at 28 days after influenza vaccination were not statistically different in VP vs PV. All patients had adequate immunogenicity to all 3 vaccine antigens based on criteria by the Commission of the European Communities for influenza vaccination in older persons. After vaccination, the percentages of seroprotected patients ranged from 92.0% (against influenza A[H1N1] vaccine antigen) to 100.0% (against influenza A[H3N2]). The mean fold increases of geometric mean titers ranged from 2.7 (against influenza A[H1N1]) to 8.9 (against influenza B). The percentages of seroconversion ranged from 33.0% (against influenza A[H1N1]) to 82.0% (against influenza A[H3N2]).

---

**Table 3. Effects of Covariates on International Normalized Ratio (INR) and Warfarin Sodium Weekly Dose**

<table>
<thead>
<tr>
<th>Covariate</th>
<th>Regression Coefficient (95% CI)</th>
<th>( \beta ) P Value</th>
<th>Regression Coefficient (95% CI)</th>
<th>( \beta ) P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sequence</td>
<td>0.073 (−0.148 to 0.293)</td>
<td>.52</td>
<td>1.241 (−3.493 to 5.975)</td>
<td>.61</td>
</tr>
<tr>
<td>Sequence × period</td>
<td>0.028 (−0.157 to 0.214)</td>
<td>.76</td>
<td>0.346 (−1.330 to 1.926)</td>
<td>.67</td>
</tr>
<tr>
<td>Time, wk</td>
<td>0.012 (−0.041 to 0.066)</td>
<td>.64</td>
<td>−0.056 (−0.176 to 0.064)</td>
<td>.36</td>
</tr>
<tr>
<td>Sequence × period × time</td>
<td>0.008 (−0.028 to 0.043)</td>
<td>.67</td>
<td>−0.041 (−0.123 to 0.041)</td>
<td>.33</td>
</tr>
<tr>
<td>Treatment</td>
<td>−0.095 (−0.253 to 0.064)</td>
<td>.24</td>
<td>0.228 (−0.902 to 1.357)</td>
<td>.69</td>
</tr>
<tr>
<td>Treatment × time</td>
<td>0.009 (−0.051 to 0.068)</td>
<td>.77</td>
<td>0.092 (−0.058 to 0.243)</td>
<td>.23</td>
</tr>
<tr>
<td>Constant</td>
<td>2.584 (2.425 to 2.743)</td>
<td>&lt;.01</td>
<td>30.130 (26.753 to 33.506)</td>
<td>&lt;.01</td>
</tr>
</tbody>
</table>

**Figure 4.** Individual response profiles for international normalized ratio (INR) (A) and warfarin sodium weekly dose (B) as estimated using the mixed-effects model of analysis of variance. No trend is evident on visual inspection.
This study investigated INR and weekly warfarin dose variation among patients receiving a stable long-term VKA regimen who were randomized to receive influenza vaccination and subsequent placebo administration, or vice-versa. The results showed no interaction between influenza vaccination and VKA treatment. In both sequences, the mean INR and warfarin weekly dose were similar after 28 days, and the percentage of time spent in therapeutic range was about 70.0%. No major bleeding or thrombotic event was observed. Minor mucocutaneous hemorrhagic events were balanced between VP and PV and were not more frequent than expected.

The results of the study are based on robust scientific evidence obtained using a controlled design in a randomized population of adequate size and are in accord with the findings of some previous studies but in contrast with others. The main reason for conflicting results may be the observational design of studies. Observational studies likely were prone to recall bias (more attention paid to follow up patients who received influenza vaccination or greater probability that vaccinated patients visited their physician because of symptoms), ascertainment bias (greater likelihood to ascribe an unrelated event to vaccination otherwise considered irrelevant in nonvaccinated patients), or selection bias (exclusion of patients with given characteristics from vaccination).

Moreover, 3 characteristics of our work are noteworthy in support of our novel results and in view of prior studies. First, the crossover design allowed us to actively treat all patients, eliminate most intrapatient variability effects, and limit the number of recruited patients. The 2 groups of randomized patients were comparable at baseline except for sex. Because of the crossover design, the chance baseline imbalance in sex distribution did not affect our results, as demonstrated by the nonsignificance of sex when it was included as a covariate in the mixed-effects model. Second, a 42-day follow-up period represented a compromise between the need to vaccinate all patients before the expected time of influenza virus circulation and the objective of assessing the effect of vaccination on VKA treatment. Most published studies have investigated the interaction between influenza vaccine and VKAs for 28 days. One study reported that reduction of anticoagulation intensity persists up to 3 months after vaccination but only among patients older than 70 years. Third, we preplanned in our research protocol to assess INR at fixed short-term intervals to discern any effect due to influenza vaccination. This is a critical difference when our study is compared with observational studies in which INR was measured in symptomatic patients at various time points after vaccination.

Three limitations of our study and research protocol merit discussion. First, we used a capillary method to monitor INR, as this is the routine method in our VKA monitoring service. Good correlation between capillary and venous determination of INRs has been previously found. Notwithstanding, we measured capillary and venous INRs at time 0 and at time 28. Our results showed good correlation between the 2 methods. Second, at the planning stage of our trial, we had incomplete data for sample size calculation. However, empiric augmentation of the sample and use of the mixed-effects model allowed us to reach a study power of 0.83. Third, we did not assess platelet count and function and did not investigate whether any observed bleeding event was secondary to platelet disorders caused by vaccination or viral infection, as previously shown by others.

In conclusion, influenza vaccination causes no variation in INR or warfarin weekly dose, and there is no interaction between influenza vaccination and VKA treatment. Therefore, influenza vaccination can be safely administered to patients on a stable VKA regimen, without need to increase the frequency of INR testing.

Table 4. Minor Mucocutaneous Hemorrhagic Events

<table>
<thead>
<tr>
<th>Day</th>
<th>Events in PV Sequence</th>
<th>INR</th>
<th>Events in VP Sequence</th>
<th>INR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Period 1</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>1 Nosebleed</td>
<td>2.9</td>
<td>1 Posttraumatic elbow hematoma</td>
<td>1.5</td>
</tr>
<tr>
<td>14</td>
<td>0</td>
<td>...</td>
<td>1 Gingival bleeding</td>
<td>2.3</td>
</tr>
<tr>
<td>28</td>
<td>1 Bruising</td>
<td>6.9</td>
<td>2 Nosebleeds</td>
<td>2.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1 Conjunctival hemorrhage</td>
<td>3.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1 Nosebleed</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Period 2</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>...</td>
<td>1 Nosebleed</td>
<td>...</td>
</tr>
<tr>
<td>14</td>
<td>0</td>
<td>...</td>
<td>2 Nosebleeds</td>
<td>3.0</td>
</tr>
<tr>
<td>28</td>
<td>0</td>
<td>...</td>
<td>2 Nosebleeds</td>
<td>2.6</td>
</tr>
<tr>
<td>Total</td>
<td>2</td>
<td>9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: Ellipsis, not applicable; INR, international normalized ratio; PV, placebo-vaccine; VP, vaccine-placebo.

COMMENT

Accepted for Publication: October 7, 2009.
Correspondence: Alfonso Iorio, MD, Department of Internal and Cardiovascular Medicine, University of Perugia, 06100 Perugia, Italy (iorioa@unipg.it).

Author Contributions: Study concept and design: A. Iorio, Guercini, and A. M. Iorio. Acquisition of data: Guercini, Paccamiccio, and Vecchioli. Analysis and interpretation of data: Basileo, Marcucci, and Camillon. Drafting of the manuscript: Basileo, Marcillon, and Vecchioli. Critical revision of the manuscript for important intellectual content: Guercini, Paccamiccio, and A. M. Iorio. Statistical analysis: Mar-
Financial Disclosure: None reported.

REFERENCES