Vancomycin Prophylaxis of Surgical Site Infection in Clean Orthopedic Surgery

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ABSTRACT

Community-acquired methicillin-resistant Staphylococcus aureus (MRSA) has been recognized as a public health concern since the mid-1990s. Because of the increase in reports of this pathogen, it has become increasingly tempting for clinicians to provide prophylaxis against this entity using antibiotics known to be effective against MRSA. The goal of this study was to assess the use of MRSA prophylaxis to determine whether it is safe and effective. A systematic search of the literature was performed to identify articles that examined the use of vancomycin in clean orthopedic surgery.

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orthopedic surgery. Infection rates and adverse events were extracted, and the data were aggregated and analyzed using a DerSimonian and Laird random effects model. Publication bias and study quality were also assessed. No benefit of parenteral administration of vancomycin was identified. Local, vancomycin-impregnated cement and powder are associated with lower infection rates. Few adverse events occurred, and most of those that occurred involved infusion rate.

Cost, resistance, and side effects are concerns in using vancomycin therapy in addition to standard antibiotic prophylaxis. Given the lack of efficacy of intravenous vancomycin, the authors do not recommend its routine use in clean orthopedic surgery. However, local administration appears to be safe and effective. The data are most compelling in orthopedic spine surgery in which a patient without prophylaxis is more than 4 times as likely to have a deep postoperative wound infection compared with a patient who received local vancomycin. The authors recommend the use of local antibiotics when possible in clean orthopedic surgery.

The use of prophylactic antibiotics in orthopedic surgery is a well-accepted practice. First-generation cephalosporins have been shown to be safe, effective, and superior to placebo in terms of reduction in surgical site infections.\(^1,2\) Nafcillin has also been shown to be an effective agent for prophylaxis, although *Staphylococcus aureus* organisms from the same series were found to be sensitive to penicillin.\(^3\) Since the first recognized case of community-acquired methicillin-resistant *S aureus* (MRSA) in the 1990s, an increase in the reports of these infections has been noted.\(^4\) This has led some clinicians to routinely use vancomycin as prophylaxis against surgical site infections. This practice is controversial because community-acquired MRSA differs from hospital-acquired MRSA in its transmission and mode of antibiotic resistance.\(^4\)

Although the use of vancomycin prophylaxis has seemed to increase, debate remains about whether the drug achieves adequate penetration of relevant tissues to justify prophylaxis. Parenteral vancomycin has variable absolute bone concentrations and lower bone:serum concentration ratios in uninfected bone than most other antistaphylococcal agents.\(^5\) In addition, recent data have shown a concerning trend toward higher infection rates when using vancomycin to prevent infections in the absence of the carrier state.\(^6\)

Although first-generation cephalosporins have been vetted in clinical trials for prophylaxis of surgical site infections, no such literature exists for vancomycin. Despite this, vancomycin has been shown to be safe in the treatment of infections.\(^7\) Incidence rates for adverse reactions consist of the following: an anaphylactoid reaction known as red man syndrome (5%-50%), thrombophlebitis (approximately 40%), rash (4%-6%), nephrotoxicity (7%-8%), proteinuria (approximately 2%), hepatotoxicity (approximately 2%), and ototoxicity (4%-10%).\(^2,10\) Although antihistamines are used to treat these reactions, anaphylaxis, although exceedingly rare, does not respond to this therapy.\(^10,11\)

Vancomycin prophylaxis in clean orthopedic surgery may be administered either locally or parenterally. Parenteral vancomycin is given prior to incision, similar to standard antibiotic prophylaxis. Local forms consist of antibiotic-impregnated cement typically used in adult reconstruction, and powder forms placed in bone grafts are primarily used for spine surgery. The elution and concentration of vancomycin following its use in the form of impregnated bone cement has been studied in vivo and was shown to have variable bioactivity against *Staphylococcus epidermidis*, as well as variable elution properties.\(^12\) Eshkenazi et al\(^13\) reported that patients who had been given vancomycin prophylaxis intravenously had effective serum and synovial vancomycin levels intraoperatively and in the knee for more than 20 hours postoperatively.

The purpose of the current systematic literature review was to determine whether evidence exists supporting the routine use of vancomycin prophylaxis in the face of increasing bacterial resistance in orthopedic surgery. In addition, the study aimed to determine which mode of administration, if any, was effective, and what safety profile vancomycin has as a prophylactic drug in clean orthopedic surgery.

**Search Strategy and Methods**

Medline and EMBASE databases were searched for articles containing the terms *vancomycin AND prophylaxis AND surgery* and *vancomycin AND prophylaxis AND orthopedics*. References from the articles were reviewed to identify additional studies of interest. Subspecialty abstract books from the past 3 years available online were searched to assure that the most recent data were obtained. Abstracts for the Scoliosis Research Society, the American Association of Hip and Knee Surgeons, the Orthopaedic Trauma Association, the European Paediatric Orthopaedic Society, the Pediatric Orthopaedic Society of North America, and the American Academy of Orthopaedic Surgeons from 2009 to 2011 were also searched. The abstract book for the American Orthopaedic Association was available for 2011 only, North American Spine Society abstract books were not available, and the Musculoskeletal Infection Society had abstracts for 2010 and 2009, but the 2011 abstracts were not posted at the time of this review, so the authors were unable to use these references. Finally, the tables of contents of 13 major orthopedics journals from the past 6 months were reviewed. These reviews were performed because this topic is becoming more popular, and many of the data are recent.

Studies were included if they met the following criteria: (1) English language;
(2) orthopedic patients only or orthopedic patients separable in the body of the text or in tables; (3) vancomycin used as surgical prophylaxis in intravenous, cement, or powder forms; (4) clean surgeries (ie, no vancomycin used for treatment of infection or for prophylaxis in the case of open fractures or traumatic arthrotomies); and (5) studies with levels of evidence of I through IV. Studies were excluded if they met any of the following criteria: (1) inclusion criteria were not met; (2) antibiotics being used for current infection; (3) basic science study; (4) animal model only; (5) editorial, opinion, review, or commentary; or (6) combination of vancomycin and another antibiotic used in a local form for prophylaxis. Search details are shown in Figure 1.

Study quality was graded using the systematic quality assessment described by Zaza et al. Each study was graded independently by 2 authors of the current study (W.W.K., K.D.B.).

Overall, 8 studies and 4 abstracts met the criteria for analysis (Table 1). The studies and abstracts included a total of 10,889 patients. A total of 6630 patients received vancomycin prophylaxis, and 4259 were controls. A total of 3180 patients were treated with intravenous vancomycin, and 4259 were controls.

Results

Deep and Superficial Infection Rates

Intravenous vancomycin added to typical gram-positive coverage was reported in 3 studies of patients undergoing total joint arthroplasty (Figure 2). One case control study reviewed infection rates in spine surgery before and after institution of a vancomycin prophylaxis regimen and found no difference in infection rates. No significant difference was found between those treated with intravenous vancomycin and those treated with standard prophylaxis (P=.380).

Five studies examined deep infections in spine surgery when using a vancomycin-impregnated powder. These studies showed in the aggregate that a patient was more than 4 times as likely to have a...
deep infection without powder prophylaxis than with prophylaxis \((P<.001)\) (Figure 3). O’Neill et al\(^{19}\) examined superficial infections in spine surgery patients but reported no difference with or without prophylaxis \((P= .239)\).

Two studies used vancomycin cement, but only 1 was comparative.\(^{17}\) This study found a significant decrease in deep infections but not in superficial infections \((P= .013\) and \(.492, \) respectively).\(^{17}\) One study used a mixture of vancomycin in the hydroxyapatite layer of noncemented TKA and reported no difference in deep or superficial infections \((P= .249\) and \(.999, \) respectively).\(^{15}\)

### Safety

Incidence rates for adverse reactions associated with vancomycin have been well documented in the scientific literature and include the following: an anaphylactoid reaction known as red man syndrome \((5\%-50\%)\), thrombophlebitis \((4\%-6\%)\), nephrotoxicity \((7\%-8\%)\), proteinuria \((approximately 2\%)\), hepatotoxicity \((approximately 2\%)\), and ototoxicity \((4\%-10\%)\).\(^{7-10}\) Its use as prophylaxis warrants careful precautions to avoid these complications.

Only 1 of the 12 studies reported the presence of these complications.\(^{20}\) Ritter et al\(^{20}\) reported elevated creatinine levels in 8 \((4\%)\) of 201 patients that returned to baseline level postoperatively and minor drug infusion reactions in 40 \((20\%)\) patients: red man syndrome \((2\%)\), itching \((15\%)\), rash \((1\%)\), and itching and rash \((2\%)\). All complications responded to antihistamine therapy and none required cessation of drug infusion. The 5 studies that used local vancomycin powder for spine surgeries reported no adverse effects attributed to the local application of vancomycin, specifically no episodes of hypotension or renal toxicity.\(^{5,19,21,24,25}\)

### Vancomycin Levels

Vancomycin levels were measured in the serum of articular fluid in 3 stud-

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**Table 1: Study Demographics**

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Journal</th>
<th>Vancomycin Medium</th>
<th>Surgery Type</th>
<th>Outcomes</th>
<th>LOE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rahman et al(^5)</td>
<td>2011</td>
<td><em>Scoliosis Research Society Annual Meeting</em></td>
<td>Powder</td>
<td>Adult spinal deformity</td>
<td>Deep infection</td>
<td>III</td>
</tr>
<tr>
<td>Assor(^15)</td>
<td>2010</td>
<td><em>Canadian Journal of Surgery</em></td>
<td>Powder</td>
<td>TKA (noncemented)</td>
<td>Superficial and deep infection</td>
<td>II</td>
</tr>
<tr>
<td>Buttaro et al(^16)</td>
<td>2010</td>
<td><em>Hip International</em></td>
<td>Cement</td>
<td>Revision THA for aseptic failure</td>
<td>Superficial and deep infection; short-term clinical and radiographic results</td>
<td>IV</td>
</tr>
<tr>
<td>Chiu &amp; Lin(^17)</td>
<td>2009</td>
<td><em>Journal of Bone and Joint Surgery, American Volume</em></td>
<td>Cement</td>
<td>Revision TKA (cemented)</td>
<td>Superficial and deep infection</td>
<td>I</td>
</tr>
<tr>
<td>Kekamp et al(^18)</td>
<td>1999</td>
<td><em>Journal of Spinal Disorders &amp; Techniques</em></td>
<td>Intravenous</td>
<td>Spinal surgery (various)</td>
<td>Superficial and deep infection</td>
<td>III</td>
</tr>
<tr>
<td>O’Neill et al(^19)</td>
<td>2011</td>
<td><em>The Spine Journal</em></td>
<td>Powder</td>
<td>Posterior spinal fusion for traumatic injuries</td>
<td>Superficial and deep infection; complications</td>
<td>II</td>
</tr>
<tr>
<td>Ritter et al(^20)</td>
<td>1989</td>
<td><em>Orthopedics</em></td>
<td>Intravenous</td>
<td>Bilateral/unilateral total joint arthroplasty</td>
<td>Superficial and deep infection; complications</td>
<td>IV</td>
</tr>
<tr>
<td>Sweet et al(^21)</td>
<td>2011</td>
<td><em>Spine</em></td>
<td>Powder</td>
<td>Thoracic and lumbar posterior instrumented spinal fusions</td>
<td>Superficial and deep infection; complications</td>
<td>II</td>
</tr>
<tr>
<td>Tyllianakis et al(^22)</td>
<td>2010</td>
<td><em>Journal of Arthroplasty</em></td>
<td>Intravenous</td>
<td>Primary TKA and THA</td>
<td>Superficial and deep infection</td>
<td>II</td>
</tr>
<tr>
<td>Smith et al(^23)</td>
<td>2011</td>
<td><em>American Association of Hip and Knee Surgeons Annual Meeting</em></td>
<td>Intravenous</td>
<td>Adult joint reconstruction</td>
<td>Deep infection</td>
<td>III</td>
</tr>
<tr>
<td>Molinari et al(^24)</td>
<td>2011</td>
<td><em>Scoliosis Research Society Annual Meeting</em></td>
<td>Powder</td>
<td>Adult spinal surgery</td>
<td>Deep infection</td>
<td>IV</td>
</tr>
</tbody>
</table>

**Abbreviations:** LOE, level of evidence; THA, total hip arthroplasty; TKA, total knee arthroplasty.
ies,15,20,21 Assor15 measured the intra-articular vancomycin concentration in 4 patients on postoperative day 3 or 4 when surgical drains were removed. The concentration was found to be at least 12 times the minimum inhibitory concentration in all patients. No long-term or serum measurements were performed in this study.

Ritter et al20 measured the serum vancomycin concentration in all patients over a 24-hour postoperative period. Peak values occurred at 1 hour postoperatively, with a mean value of 25.8 µg/mL. Mean concentration at 24 hours was 4.5 µg/mL, still greater than the minimum inhibitory concentration for all sensitive organisms.

Wound and serum vancomycin concentrations were measured in 178 patients in the study by Sweet et al.21 Wound drains were used as the source for the wound concentrations, which were a mean of 1457 µg/mL postoperative day 1 and 128 µg/mL at postoperative day 3. Serum levels were not detectable in 80% of the patients sampled and were less than 1.6 µg/mL in the remaining 20%.21

The remaining 8 studies did not measure serum or local vancomycin concentrations.

### Publication Bias

The current study’s funnel plots for the question of whether vancomycin was effective (all modalities) found significant publication bias (P = .02). The publication bias suggests that larger effect sizes were shown in smaller studies. However, a trim and fill analysis showed the overall odds of infection with vancomycin to be 20% of what it was without vancomycin; this result was statistically significant (P < .01).

When all superficial infections were examined, some evidence of publication bias was found, with smaller studies showing lower infection rates preferentially in the prophylaxis group compared with the nonprophylaxis group (P = .05). The significance of this finding is unclear because the identification of superficial infections was not a main aim of any study. The funnel plot for vancomycin powder in spine surgery was symmetric, with no evidence of publication bias (P = .63).

### Study Quality

The authors were unable to assess study quality on the 4 abstracts because not enough information existed. Two of these studies were Level III case controls, and 1 was a Level IV case series.

Of the remaining 8 studies, all adequately described the patient population and intervention being evaluated. The selection and screening criteria for patient inclusion were clearly described in all but 1 study.18 The entire population was used in all 8 studies, minimizing selection bias. In 1 of the studies, all treatment group patients were operated on by 1 surgeon, whereas all controls were operated on by different surgeons, which could bias infection results through surgical technique, operative time, or other factors known to influence infection.19 In another study, the treatment and control groups were separated by time and were not randomized.21

In no study were the observers blinded to the intervention. Furthermore, 3 studies did not exclude patients with significant comorbidities, which could allow confounding but potentially increase the external validity of their results.16,20,21 One study had longer operative time in the control group, which was not controlled for to minimize confounding.19

All but 1 study described the antibiotic regimens given to the patients pre-, intra-, and postoperatively (Table 3).18
Similarly, 1 study did not report precise diagnostic criteria for deep and superficial infections. Appropriate statistical testing was conducted for 6 comparative studies (5 cohort studies and 1 case control study). However, when statistical testing was repeated for 1 of the studies that claimed to have a difference in the infection rate, no statistically significant difference was found.

**Discussion**

Surgical site infections account for 22% of all health care–related infections, resulting in an estimated $1 to $10 billion in additional medical costs annually. Postoperative infections carry serious economic and social costs for the patient, the hospital, and the community. It is estimated that the costs associated with a single postoperative spinal infection are more than $100,000. The majority of patients who sustain these infections never return to work, resulting in indirect loss of productivity to society. For these reasons, much research has been conducted to address this issue.

The rate of hospital- and community-acquired *S. aureus* infections has been increasing worldwide since its discovery in the 1960s. It has been reported as the responsible pathogen in 48.6% of surgical site infections in orthopedic surgeries, with a 56% rate of resistance to beta-lactam antibiotics. This is important because first-generation cephalosporins have been widely used as prophylaxis against surgical site infections for decades. Studies have shown these antibiotics to be safe and effective for use prior to orthopedic surgery, and they remain the most commonly used and preferred agents for prophylaxis.

Recently, the emergence of MRSA and the clear relationship between the nasal carriage of MRSA and postoperative surgical site infections has led clinicians to begin using more selective antibiotics. Meanwhile, studies in a general surgical population have shown that vancomycin prophylaxis may increase the rate of infections in patients.

### Figure 2: Forest plot showing no difference in infection rate for intravenous (IV) prophylaxis vs no IV prophylaxis. Abbreviation: CI, confidence interval.

<table>
<thead>
<tr>
<th>Study ID</th>
<th>Year</th>
<th>Exposed</th>
<th>Control</th>
<th>OR (log scale)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ritter</td>
<td>0.52015</td>
<td>0.5005</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tyranakis</td>
<td>1.129</td>
<td>1.306</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smith</td>
<td>13/2015</td>
<td>24/2221</td>
<td></td>
<td></td>
</tr>
<tr>
<td>META-ANALYSIS</td>
<td>14.53145</td>
<td>25.52257</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Figure 3: Forest plot showing decreased infection rate using powder vancomycin in spine surgery. Abbreviation: CI, confidence interval.

<table>
<thead>
<tr>
<th>Study ID</th>
<th>Year</th>
<th>Exposed</th>
<th>Control</th>
<th>OR (log scale)</th>
</tr>
</thead>
<tbody>
<tr>
<td>O’Neill</td>
<td>0.5505</td>
<td>5.5545</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Swee</td>
<td>2911</td>
<td>21821</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RefKerry</td>
<td>4966</td>
<td>16334</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Martinaki</td>
<td>13.91525</td>
<td>0.9509</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rhyys</td>
<td>0.51995</td>
<td>1.55005</td>
<td></td>
<td></td>
</tr>
<tr>
<td>META-ANALYSIS</td>
<td>20.52225</td>
<td>44.515405</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
who are not carriers.\textsuperscript{6,32} This is particularly concerning because \textit{S. aureus} resistance to glycopeptides such as vancomycin has led some authors to recommend against nonselective uses of these antibiotics for prophylaxis.\textsuperscript{33,34}

The goals of the current systematic review were to determine whether vancomycin prophylaxis, in addition to standard prophylaxis, was effective in decreasing surgical site infections in clean orthopedic surgery in local or parenteral forms; whether vancomycin is safe to administer on a prophylactic basis locally or parenterally; and the state of the literature to address the first and second goals.

This study found that local vancomycin, in the form of intrawound powder in the case of spine surgery or antibiotic cement in the case of adult reconstruction, is safe and effective according to the best available literature. Parenteral (intravenous) vancomycin does not appear to be as useful; although studies available on this modality are limited, the available data suggest that it provides no benefit over standard prophylaxis.\textsuperscript{18,20,22} It should be noted that 3 studies are available on this modality in adult reconstruction,\textsuperscript{20,22,23} but only 1 in spine surgery.\textsuperscript{18}

In terms of safety, vancomycin given in prophylactic doses appears to be safe to use in local and parenteral forms.\textsuperscript{7,8,16,20} Data from single studies suggest that the adverse effects of parenteral vancomycin were limited to infusion reactions and responded to slowed infusion rates and antihistamines.\textsuperscript{20} No adverse effects were noted for local administration, specifically kidney toxicity or red man syndrome. Serum levels of vancomycin with local administration are low to nonexistent, according to the best available data.\textsuperscript{24} The effect size of vancomycin powder is associated with a number needed to treat of 46 (ie, 46 patients would need to be treated with vancomycin powder to prevent 1 deep postoperative infection).

In addition, according to cost data from the authors’ institution, this intervention would cost approximately $2.50 per patient. As such, it would cost approximately $115 to prevent 1 deep spine infection (oral communication, pharmacy personnel, The Children’s Hospital of Philadelphia, November 2011). Because the outcome is expensive and life chang-

\begin{table}[h]
\centering
\caption{Antibiotic Regimens}
\begin{tabular}{|l|l|l|l|}
\hline
\textbf{Study} & \textbf{Preoperative} & \textbf{Intraoperative} & \textbf{Postoperative} \\
\hline
Rahman et al\textsuperscript{5} & Not defined & 500 mg to 1 g vancomycin powder & Not defined \\
\hline
Assor\textsuperscript{15} & 500 mg IV cephazolin & 1-2 g vancomycin powder mixed with HAC & 500 mg IV cephazolin every 12 h for 2 d \\
\hline
Buttaro et al\textsuperscript{16} & 1 g IV cephazolin & Bone graft with 1 g vancomycin powder & 1 g IV cephazolin every 8 h for 24 h (3 doses total) \\
\hline
Chiu & Lin\textsuperscript{17} & 500 mg IV cephazolin and 80 mg IV gentamicin & 1 g vancomycin powder in 40 g of cement & 500 mg IV cephazolin every 6 h and 80 mg IV gentamicin every 12 h for 36 h; followed by 500 mg oral cephazolin every 6 h for 7 d \\
\hline
Klekamp et al\textsuperscript{18} & Not described & Not described & Not described \\
\hline
O’Neill et al\textsuperscript{19} & 1 g IV cephazolin & 1 g vancomycin powder & 1 g IV cephazolin every 8 h for 24 h (3 doses total) \\
\hline
Ritter et al\textsuperscript{20} & 1 g IV vancomycin + 80 mg gentamicin & None given & None given \\
\hline
Sweet et al\textsuperscript{21} & 1 g IV cephazolin & 1 g vancomycin powder mixed with cement and 1 g vancomycin powder sprinkled in wound & 1 g IV cephazolin every 8 h for 24 h (3 doses total) \\
\hline
Tyllianakis et al\textsuperscript{22} & Group A, 1.5 g IV cefuroxime; group B, 500 mg IV fusidic acid; group C, 1 g IV vancomycin & None given & Group A, 750 mg IV cefuroxime at 8 and 16 h; group B, 500 mg IV fusidic acid at 8 and 16 h; group C, 1 g vancomycin at 12 and 24 h \\
\hline
Smith et al\textsuperscript{23} & Not defined & Not defined & Not defined \\
\hline
Molinari et al\textsuperscript{24} & Not defined & 1 g vancomycin powder & Not defined \\
\hline
Pahys et al\textsuperscript{25} & Not defined & 500 mg vancomycin powder & Not defined \\
\hline
\end{tabular}
\end{table}

\textit{Abbreviation: IV, intravenous.}
ing and the intervention is relatively benign and inexpensive, the authors feel that this constitutes a clinically effective and cost-effective treatment. In addition, the abstract that was most favorable toward intravenous vancomycin for joint replacement patients had an effect size that would be associated with a number needed to treat of 161, approximately one-quarter as efficacious as local treatment. In the best case, given data from the authors’ institution, this would represent $9581 per infection saved (oral communication, pharmacy personnel, The Children’s Hospital of Philadelphia, November 2011). If the full systematic review is to be believed, no benefit over standard treatment would exist.

Limitations of this systematic review include a low level of evidence and scientific rigor inherent in the included studies. None of the studies were well-designed, randomized, placebo-controlled, double-blind trials. The member studies had issues with selection bias and observer bias outlined in the quality analysis. In addition, publication bias may have existed, as indicated by our Egger’s intercept for all study quality by increasing the peer review rigor to which the studies were subjected. It is unclear whether this is specifically vancomycin providing the increased protection or whether local antibiotics provide a locally inhospitable environment for bacteria. Gentamicin was used by Borkhuu et al in neuromuscular spinal fusions with an apparent difference in deep or superficial infections, the added expense, and the lack of an apparent difference in deep or superficial infection rates, the authors do not recommend the use of parenteral vancomycin as prophylaxis for clean orthopedic surgery. It may be of benefit in situations where the patient is allergic to cefazolin or is a carrier of MRSA, although few data exist in the orthopedic literature to support this. However, local vancomycin appears to be safe and effective to use as additional antibiotic prophylaxis when large prosthetic or fixation devices are used. The authors give the use of vancomycin powder a U.S. Preventive Services Task Force level B recommendation: the benefits outweigh the risks, and the data are consistent and compelling. In addition, pilot data from an informal survey performed of major spine centers suggests that this method is becoming the standard of care in the spine community (oral communication, J.P. Dormans, MD, The Children’s Hospital of Philadelphia, November 2011). Further study is warranted on this particular modality.

REFERENCES


