A commentary by Matthew Allen, VetMB, PhD, is linked to the online version of this article at jbjs.org.

Use of the Reamer/Irrigator/Aspirator Decreases Carotid and Cranial Embolic Events in a Canine Model

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Background: Approximately 2 million patients in the United States annually undergo total joint arthroplasty with reaming and placement of intramedullary nails, resulting in extravasation of bone marrow and fat into the circulatory system and potentially causing fat embolism syndrome. Acute and chronic changes in mental status documented after these procedures may be related to embolic events. The Reamer/Irrigator/Aspirator (RIA) device has been shown to decrease intramedullary pressure during reaming. We hypothesized that the use of the RIA in a canine model would reduce the number of microemboli detected in the carotid artery and brain compared with nailing either with or without reaming.

Methods: Twenty-four large canines underwent unreamed nailing (UR), sequentially reamed nailing (SR), or RIA-reamed nailing (RIA) of bilateral femora (eight dogs per group). During reaming and nailing, the number and size of microemboli transiting the carotid artery were recorded. After euthanasia, the brain was harvested for immunostaining and measurement of microinfarction volumes.

Results: Total embolic load passing through the carotid artery was 0.049 cc (UR), 0.045 cc (SR), and 0.013 cc (RIA). The number and size of microemboli in the UR and SR groups were similar; however, the RIA group had significantly fewer larger-sized ($>200\text{-}\mu\text{m}$) emboli ($p = 0.03$). Pathologic examination of the brain confirmed particulate emboli, and histologic analyses demonstrated upregulation of stress-related proteins in all groups, with fewer emboli and less evidence of stress for RIA reaming.

Conclusions: RIA reaming decreased microemboli compared with traditional reaming and unreamed nailing, suggesting that intramedullary pressure and heat are important variables. The documented embolic events and brain stress may help to explain subtle neurobehavioral symptoms commonly seen in patients after undergoing long-bone reaming procedures.

Clinical Relevance: RIA reaming decreased cranial embolic events and may have an ameliorating effect on postoperative neurologic sequelae.

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Approximately 2 million patients in the United States annually undergo procedures involving intramedullary reaming¹. Instrumentation of the medullary canal during orthopaedic procedures has been associated with fat embolism syndrome, the clinically diagnosed triad of petechiae (skin or mucosal), neurologic impairment, and hypoxemia^{2,3}. This syndrome is commonly described in relation to long-bone fractures

and manipulation during orthopaedic surgical procedures⁴⁻⁶. Multiple studies have also examined fat emboli during total hip arthroplasty and have correlated fat emboli with cerebral emboli using transcranial Doppler⁷⁻¹⁰. Additionally, systemic embolization has been seen with pressurization of the medullary canal^{11,12}.

More recently, studies have examined cognitive deficits after orthopaedic procedures, mostly focusing on total joint arthroplasty

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and hip fracture surgery, with the rate of cognitive impairment noted to be as high as 73%¹³⁻¹⁷. Although many etiologies for these cognitive deficits have been discussed, to our knowledge, no one has yet directly demonstrated whether embolic events may be at least partially responsible. Richards et al. showed that intramedullary nailing with reaming was a risk factor for long-term cognitive impairment in trauma patients, even when controlling for other injury factors, and this finding corroborates that of another study of persistent cognitive impairment a year out from injury^{18,19}.

It is known that the acts of reaming and nailing cause fat emboli to enter the circulation. Because of the hypothetical risks of these emboli, surgeons historically attempted suction and irrigation while reaming, and this eventually led to the development of the Reamer/Irrigator/Aspirator (RIA; DePuy Synthes)²⁰. The RIA device has subsequently been shown to substantially decrease pressure and heat in the canal during reaming²¹⁻²³. In addition, examinations of systemic effects, including fat emboli in the lung, showed significant decreases when using the RIA device compared with traditional reaming²⁴⁻²⁷. However, none of these studies directly investigated the effect of the use of the RIA device on cerebral embolic events.

In the present study, we employed a canine model to determine alteration of blood-brain barrier permeability by reaming, microembolization, and the effect of the passage of gaseous and fat emboli through the cerebral microvasculature. With this model, we performed intramedullary nailing after sequential reaming, after reaming with use of the RIA device, and after no reaming. Our hypothesis was that RIA reaming would decrease the number of embolic events and demonstrate a lower permeability of the blood-brain barrier. In addition, we hypothesized that RIA reaming would cause less brain-tissue hypoxia and cell stress in the brain, theoretically decreasing the cognitive impairments that may be seen in humans after these procedures.

Materials and Methods

The study was a diagnostic study to compare unreamed (UR) (n = 8), sequentially reamed (SR) (n = 8), and RIA-reamed (n = 8) nail insertion. We performed bilateral surgical procedures on the dogs' femora to replicate polytraumatized patients' injury patterns and to maximize our opportunity to detect embolic injuries in these canines. We based our sample size on past experiences with these procedures and outcomes^{11,28,29}. Enrolling eight animals per group allowed power = 0.80 to detect effect sizes as small as $d = 1.5$ (standard deviation [SD]) using a simple t test with two-sided $\alpha = 0.05$.

Surgery

Twenty-four dogs (mean weight of 31 ± 2 kg) were anesthetized with intravenous (IV) methohexital sodium (20 mg/kg) followed by IV fentanyl (200 μ g/kg) and midazolam (0.5 mg/kg). Additional fentanyl (0.8 to 1.5 μ g/kg/min) plus midazolam (1.0 μ g/kg/min) was infused intravenously as required to maintain anesthesia. Following intubation, the dogs were mechanically ventilated using 30% oxygen and 70% room air and maintained at a PaCO₂ (partial pressure of arterial carbon dioxide) level of 35 to 40 mm Hg and PaO₂ of >150 mm Hg. Normal body temperature (37.5° to 38.5°C) was maintained with a heating pad. The left femoral artery and vein were cannulated for continuous monitoring of blood pressure and heart rate, arterial blood sampling, and IV infusions. The right jugular vein was cannulated for blood sampling. After the dog was placed in the sphinx position, the cisterna magna was accessed via a midline incision on the neck. A soft, vinyl microcatheter (1.22 mm in diameter) was positioned through the fascia covering the cisterna magna into the cerebrospinal fluid (CSF)-filled space. After CSF aspiration was confirmed, the catheter was secured, and the muscle and skin were closed with sutures. The dog was then repositioned onto its back.

Reaming and Insertion of Intramedullary Nails

The intercondylar notch at the distal end of both femora was exposed by electrocautery dissection. A drill (LDX112, 600 rpm; Black & Decker) with a 2.5-mm Kirschner wire was used initially to penetrate the bone, followed by use of an 8-mm cannulated opening reamer. A 2.5-mm ball-tipped guidewire was then placed into the medullary canal, and for the UR group, was then followed by manual insertion of a cannulated nail (titanium cannulated Humeral Nail-EX, 9×300 mm; DePuy Synthes). For the SR group, flexible 8.5, 9.5, and 10.5-mm reamers were placed over the guidewire sequentially before insertion of the nail. For the RIA group, reaming was performed using an 11-mm RIA reamer irrigated with room-temperature saline solution and constant suction (>100 mm Hg) before nail insertion; this was placed after the guidewire, with no prior sequential smaller reamers used. Fluoroscopy was performed to check both reamer and nail position, to ensure reaming and nailing to the same depth in all animals. The sequence of instruments used for each group is presented in Table I.

All dogs remained anesthetized for four hours after bilateral femoral nail insertion. Following euthanasia (40 mEq KCl/kg, IV), the brain was removed and weighed, and two adjacent sections were taken for histologic analysis (see Appendix). Previous work in our laboratory has used this technique to document embolic injury in the brain^{28,29}.

Blood and CSF Sampling

A bolus infusion (femoral vein) of fluorescein sodium salt (SF) (F6377; Sigma-Aldrich) (8 mg/mL; molecular weight [MW] = 376.28) was given for twelve minutes at 0.4 mg/kg/min, as jugular venous blood (3 mL) and CSF (0.5 mL via passive drainage) samples were collected at zero, six, and twelve minutes after SF infusion. Thereafter, the infusion rate was reduced to 0.01 mg/kg/min as blood and CSF samples were collected at twenty-minute intervals for the remainder of the six-hour experiment before euthanasia. SF infusion and sample collection (blood and CSF) were initiated at least one hour prior to the orthopaedic interventions to allow the SF to reach a steady-state concentration in the blood. The SF concentration of all serum and CSF samples was determined from duplicate 100- μ L aliquots using a fluorescence microplate reader with an excitation wavelength of 485 to 530 nm (FMax; Molecular Devices).

Quantification of Microemboli

Prior to bone reaming, an emboli detection and classification ultrasonic transducer (EDAC QUANTIFIER; Luna Innovations) was positioned at two sites: 1 mm proximal to the right common carotid artery and 1 mm proximal to the left jugular vein. The EDAC QUANTIFIER provided real-time monitoring of embolic activity at each site during the bone reaming procedures³⁰⁻³². The counting was done in an automated manner; there was no source for detection bias.

Histology

Coronal sections of the brain were collected for histologic assessment of fat microemboli. Cross-sectional blocks of brain approximately 1 cm thick were embedded in celloidin (plastic). Once hardened, these blocks were mounted on a wooden support and sectioned serially on a sledge microtome at a thickness of 50 μ m. Approximately twenty sections were cut from one block for use in our evaluation. Each section in the series was numbered sequentially. A second block (slab) was reserved.

Immunostaining

The first five of the twenty sections were stained for alkaline phosphatase (AP) according to a previously reported method^{33,34}. Details of the sectioning and staining can be found in the Appendix. Immunohistochemistry was performed with an antibody to HIF-1 α (hypoxia-inducible factor 1-alpha) (1:250; NB100-131; Novus Biologicals); HSC70/HSP73 (1B5) (heat shock protein 70) (1:4000; ADI-SPA-815-F; Enzo Life Sciences); and AP histochemistry. AP staining was used to visualize and quantify the length of arteries, arterioles, and capillaries. Previous work in our laboratory has used this technique to document embolic injury to the brain²⁹.

Stereology

Unbiased stereology is a recognized process by which spatial and histologic properties of a three-dimensional object may be estimated using systematic random sampling.

TABLE I Treatment Algorithm for the Unreamed (UR), Sequentially Reamed (SR), and Reamer/Irrigator/Aspirator (RIA) Groups

	Steps, in Sequence					
	2.5-mm Kirschner Wire	8-mm Cannulated Opening Reamer	Ball-Tipped Guidewire	Sequential Reamers (8.5, 9.5, 10.5-mm)	RIA (11 mm)	Humeral Nail (9 mm)
UR	Yes	Yes	Yes	—	—	Yes
SR	Yes	Yes	Yes	Yes	—	Yes
RIA	Yes	Yes	Yes	—	Yes	Yes

In this case, the number of labeled cells or the length of vessels per volume containing either HIF-1 α or HSP were quantified. Additional details regarding the stereology can be found in the Appendix. The labeled cells in HSP or HIF-1 α sections were counted using the Optical Fractionator probe (MBF Bioscience)^{35,36}. The length of labeled vessels per volume was measured in HIF-1 α sections using a hemispherical, 45- μ m-dissector-height, Spaceballs probe (MBF Bioscience)³⁷. Sampling was optimized to produce Gundersen error coefficients of <0.10^{38,39}. Three coronal sections were evaluated for each antibody per animal. A single observer, blinded to the treatment groups, assessed the data using Stereo Investigator software (MBF Bioscience). The precision of the stereologic measurements was assessed by calculation of the coefficient of error (CE). Analysis parameters (e.g., the number of grid sections counted and the size and spacing of the grid) were established during preliminary procedures such that a CE of <0.10 was confirmed for each sampled area.

Statistical Analysis

All analyses were conducted using SAS software, version 9.2 (SAS Institute). To examine differences among the groups (RIA, SR, and UR), we used several one-way analysis of variance (ANOVA) models for the microemboli and histology scores. When a significant group effect was observed, Holm-Sidak post-hoc tests were conducted to examine pairwise group differences. Several sensitivity analyses were conducted that considered nonparametric approaches when outliers could have influenced the results. Because of the similarity of results, only the primary analyses are presented. To examine the total number of emboli observed by group and the size of emboli, generalized estimating equations were used with group and emboli size as fixed factors with a normal distribution and log link for the outcome. Where appropriate, all testing was two-tailed, with significance considered to be $p < 0.05$.

Results

Blood and CSF Sampling

Blood SF levels from serum increased with the bolus, as expected, and then declined, to reach a steady state within three hours of infusion. This was the case in all groups (RIA, SR, and UR), and there were no significant differences among the groups. Levels from CSF are shown in Figure 1; the percent change was calculated as the change from the last CSF sample taken prior to femoral reaming to the time indicated. There were significant changes within the groups over time following reaming and nail insertion. Specifically, the UR group continued to have an elevated mean percent change compared with baseline, and the RIA and SR groups had decreased percent changes compared with baseline. The RIA group, in particular, had both the lowest percent change as well as the fewest outliers, as evidenced by the similar mean and median seen in the figure.

Quantification of Microemboli

Figure 2 shows the total embolic load as seen in each group measured by ultrasound (EDAC). These events were only detected during the reaming and nailing portions of each procedure. Total embolic load quantifies the sum total of all emboli during the time from the beginning of the drilling procedure to

Fig. 1

Fig. 1 Percent change in cerebrospinal fluid (CSF) fluorescein sodium salt (SF) after reaming (R) for the Reamer/Irrigator/Aspirator (RIA), unreamed (UR), and sequentially reamed (SR) groups. **Fig. 2** Total embolic load, defined as the sum of all emboli from the beginning to the completion of the procedure. Differences in embolic load were not significant due to wide variance and the small sample size of each group. However, there appeared to be fewer outliers for Reamer/Irrigator/Aspirator (RIA) reaming. UR = unreamed, and SR = sequentially reamed.

Fig. 2

Fig. 3

Fig. 3 Size and number of microemboli; note the logarithm scale on this figure. The Reamer/Irrigator/Aspirator (RIA) group had significantly fewer larger-sized (>200- μ m) emboli. UR = unreamed, and SR = sequentially reamed. **Fig. 4** An embolus (arrow) within an alkaline phosphatase-stained cerebral capillary ($\times 20$).

Fig. 4

the end of nail insertion. For each group, this time averaged thirteen minutes (SD of three minutes for RIA, five minutes for SR, and eleven minutes for UR). The RIA group had, on average, a lower embolic load (0.013 cc) than did the SR group (0.045 cc) and the UR group (0.049 cc); however, these differences were not significant because of the wide variance and small sample size of each group.

The size and number of emboli were also evaluated with the EDAC, and the results of this analysis are shown in Figure 3

(note the use of a logarithm scale on the y axis). The number and the size of the microemboli in the UR and SR groups were similar, but the RIA group had significantly fewer larger-sized (>200- μ m) emboli (mean, 140; range, twelve to 1586) compared with the UR group (mean, 739; range, thirty-six to 4042) and the SR group (mean, 251; range, twenty-one to 8345) ($p = 0.03$). There was also a lower overall number of emboli in the RIA group compared with the UR and SR groups, but this did not reach significance.

Fig. 5

Fig. 5 Heat-shock-protein (HSP)-labeled cells per brain volume. Mean differences were significant. RIA = Reamer/Irrigator/Aspirator, UR = unreamed, and SR = sequentially reamed. **Fig. 6** Hypoxia-inducible-factor (HIF)-labeled cells. Mean differences were significant. RIA = Reamer/Irrigator/Aspirator, UR = unreamed, and SR = sequentially reamed.

Fig. 6

Histology

On coronal brain sections, particulate emboli were seen on direct pathologic examination in all groups (Fig. 4).

HSP-labeled cells were evaluated and found to be significantly different when comparing RIA (mean, 464.0 cells/brain volume; SD, 195.6), SR (mean, 759.0 cells/brain volume; SD, 246.3), and UR (mean, 466.1 cells/brain volume; SD, 271.4) ($p = 0.033$, ANOVA). Using the Holm-Sidak method, pairwise multiple comparison showed that the SR and UR groups differed significantly (mean difference, 292.8; 95% confidence interval [CI], 14.9 to 570.7; $p = 0.023$), but other pairs did not differ significantly. There was a greater number of HSP-labeled cells per volume in the SR group compared with both the RIA and UR groups, which were similar (Fig. 5).

Histologic analysis of HIF-labeled cells showed a similar pattern. There was a significant difference when comparing RIA (mean, 2666.0 cells/brain volume; SD, 793.3), SR (mean, 4081.9 cells/brain volume; SD, 889.3), and UR (mean, 2554.6 cells/brain volume; SD, 1093.2) ($p = 0.006$, ANOVA). Using the Holm-Sidak method, pairwise multiple comparison showed that SR and UR differed significantly (mean difference, 1527.3; 95% CI, 458.7 to 2959.9; $p = 0.023$). Additionally, RIA and SR differed significantly (mean difference, 1415.9; 95% CI, 512.2 to 2319.6; $p = 0.006$) (Fig. 6).

Stereologic assessment showed similar HIF-labeled vessel lengths in all of the groups for a given brain volume.

Discussion

Our RIA reaming group had significantly fewer microembolic events, specifically, fewer larger-sized emboli, as well as a lower total embolic load compared with the other two groups (although the differences in total embolic load were not significant because of the small sample size). The lower change of fluorescein levels was also indicative of a less permeable blood-brain barrier in the RIA group. Our histologic analysis specifically showed that HIF and HSP were similar between the UR and RIA groups compared with a significant increase in both for the SR group, again demonstrating less cell stress and hypoxia that could potentially cause clinical symptoms. The similarity between UR and RIA implies that reaming with an RIA device eliminates the added brain-cell stress from reaming, while still getting the benefit of reaming intraoperatively.

With cerebral events that can potentially lead to substantial permanent impairment in an individual patient, it is important to have a technique that decreases the number of catastrophic outliers (in this case, large emboli or very large changes in blood-brain barrier permeability or HIF/HSP). In all cases, we found that RIA reaming had fewer outliers compared with the other techniques, a finding that suggests that such a reaming technique could help to prevent such important consequences.

Multiple reports have discussed an intracardiac shunt, specifically, a patent foramen ovale (PFO), as a pathway for emboli to go from the venous circulatory system. Emboli through such a right-to-left cardiac shunt may end up in the brain as opposed to the lungs, where side effects are more commonly seen⁴⁰⁻⁴³. However, only 25% to 40% of human subjects have a PFO⁴⁴⁻⁴⁷. Because of the

paradox that almost every patient seems susceptible to embolic events, many authors have discussed a possible transpulmonary pathway for emboli. Those papers hypothesized that, from transcapillary leakage, pressure reversal from arterial to venous systems, or capillary damage, emboli may enter the oxygenated arterial circulation, leading to showers of cerebral and renal emboli^{7,8,48-51}. In a postmortem study of patients with orthopaedic injuries, Nikolić et al. concluded that a PFO was not required for systemic embolization and that arteriovenous shunts and other circulatory pathways in the lung were more important routes for embolic phenomena than anatomic cardiac anomalies⁵². Intrapulmonary arteriovenous shunts were previously described in canines in an exercise-induced state, but more recently, using both human and dog models, intrapulmonary arteriovenous shunts have been demonstrated even at rest⁵³⁻⁵⁵. In our experimental design, we did not evaluate the dogs for an intracardiac shunt or PFO; however, it would be highly unlikely that 100% of the animals had a PFO. In contrast, 100% of the animals did have cerebral emboli, which increases the likelihood that at least some of these emboli transferred to the cerebral circulation by an intrapulmonary pathway.

The histologic stains that we chose evaluated HSP and HIF. Heat shock protein 70 has been shown to indicate cell stress from a variety of sources⁵⁶; the protein itself also influences surrounding neurons and affects their tolerance to hypoxia⁵⁷. HIF-1 α is a factor that responds to hypoxia and increases angiogenesis, among other roles. These two factors have widely been used as markers of brain damage due to hypoxia. In our evaluations, the RIA animals were equivalent, on average, to the UR group, but had fewer outliers than either the SR or UR groups, suggesting the potential of leading to fewer catastrophic outcomes in an awake-patient population. However, our model was potentially limited, in part, because we could not sample the entire brain, which might have led to detection bias regarding embolic events. In addition, as stated above, we did not evaluate these animals for PFO; however, a low prevalence of PFO has been found in canines⁵⁵. Finally, we did not follow embolic events to other areas in the body, specifically pulmonary, which could have helped to further elucidate the clinical importance of these events in terms of quantity.

Because this was a terminal canine model, it is difficult to assess symptoms of cerebral dysfunction that may be seen in a human, and the authors of previous studies have noted that many of these events are subclinical^{18,19,58,59}. However, in a polytraumatized patient or a patient with pulmonary or cerebral injury from a trauma in addition to a long-bone fracture, decreasing emboli could be potentially life-saving. In addition, with the recent focus on changes in mini-mental status scores and cognitive impairment in patients after intramedullary canal manipulation, perhaps these clinical findings have always been evident and are only now being recognized¹³⁻¹⁹.

In conclusion, cerebral embolic events are common with nail insertion; however, the quantity of these events was reduced using the RIA system. In addition, damage to the blood-brain barrier and cell damage in the brain were less with use of the RIA device compared with traditional reaming. We believe that use of the RIA can have a substantial ameliorating effect on adverse cerebral events, including fat embolism syndrome, and can potentially

decrease patients' lifelong risk for permanent sequelae of neurologic damage.

Appendix

eA Additional details of the immunostaining and stereology are available with the online version of this article as a data supplement at jbjs.org. ■

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