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ORIGINAL RESEARCH

ONYX versus n-BCA for embolization of cranial dural arteriovenous fistulas

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ABSTRACT

Purpose To evaluate the efficacy of n-butyl-2-cyanoacrylate (Trufill n-BCA) versus ethylene vinyl alcohol copolymer (ONYX) for the embolization of cranial dural arteriovenous fistulas (DAVF).

Methods Fifty-three consecutive patients with cranial dural AVF were treated with liquid embolic agents from November, 2003 to November, 2008. These 53 patients had 56 lesions treated with arterial embolization. Patients embolized to completion underwent follow-up angiography at 3 months to assess for durable occlusion.

Results Twenty-one lesions were treated with n-BCA. Seven patients treated with n-BCA had initial angiographic occlusion of their DAVF, which were durable at 3 months. Six patients had adjunctive treatment with coils and/or polyvinyl alcohol particles, but none of these were occluded by endovascular treatment alone. Eleven patients underwent post-embolization surgery for closure of their DAVF. There was one death related to intractable status epilepticus at presentation. One patient developed a major stroke from venous sinus thrombosis after embolization. Thirty-five lesions were treated with ONYX in 34 patients. Twenty-nine patients treated with ONYX had initial angiographic occlusion of their DAVF by embolization alone. One patient had recurrence at 3 months and was re-treated out of 27 total follow-ups. Four patients underwent post-embolization surgical obliteration of their lesions. No deaths or major strokes occurred in this cohort.

Conclusion Initial angiographic occlusion ($p=0.0004$) and durable angiographic occlusion ($p=0.0018$) rates for embolization of cranial DAVF show a statistically significant higher efficacy with ONYX compared with n-BCA. Patients embolized with ONYX underwent surgery less frequently compared with those treated with n-BCA ($p=0.0015$).

Cranial dural arteriovenous fistulas (DAVF) are a protean group of lesions involving the meninges. The first cases of DAVF as a separate entity from cavernous carotid fistulas were reported in the literature as early as 1936.¹ Arterial supply to these lesions is usually via dural and falcine arteries arising from the anterior or posterior circulation, with less common contribution from parenchymal arteries. Venous shunting occurs into the dural venous sinuses or directly into cortical or spinal veins. Patients may present with subarachnoid hemorrhage, parenchymal hemorrhage, seizure, myelopathy, headache, tinnitus, or decline in mental status.^{2–3} These lesions have been categorized by Awad,⁴ Borden *et al*⁵ and Cognard *et al*⁶

according to location and the risk of intracranial hemorrhage based on patterns of venous drainage. Initial hemorrhage can range up to 65%,⁶ and patients with previous intracranial hemorrhage may have up to a 35% risk of another neurological event within 2 weeks.⁷

The first reported series of treatment cases was by Halbach and colleagues^{8–10} in the 1980s. Treatment options typically include arterial and/or venous embolization followed by surgery or radiosurgery as needed. Trufill n-butyl-2-cyanoacrylate (n-BCA; Codman, Raynham, Massachusetts, USA) mixed with ethiodol for external carotid and skull base branches has been used for arterial embolization while coil embolization can be used for venous sinus embolization. Two series of cases using ethylene vinyl alcohol copolymer (ONYX) (in dimethyl sulfoxide; DMSO) (ev3, Irvine, California, USA) for DAVF treatment by Nogueira *et al*¹¹ and by Cognard *et al*¹² have demonstrated promising results. The goal of our study is to compare embolization occlusion rates using n-BCA or ONYX in a consecutive series of patients with cranial DAVF at a single institution.

METHODS

Fifty-three consecutive patients with 56 DAVF of the skull base or calvarium embolized with n-BCA or ONYX from November 2003 to November 2008 were retrospectively reviewed. Patients having DAVF with ophthalmic artery supply and carotid cavernous fistulas were excluded from this study.

There is no US Food and Drug Administration approved liquid embolic agent for treatment of dural AVF. n-BCA was mixed with ethiodol 20–50% depending on diameter, flow and anatomical considerations of a given arterial pedicle. This was assessed by initial guide catheter and microcatheter angiography. Standard deposition or wedged microcatheter techniques were used. Microcatheters used included Ultraflow (ev3, Irvine, CA, USA), Prowler 10 and 14 (Codman, Raynham, MA, USA), and Echelon 10 (ev3, Irvine, CA, USA). Compatible microwires included Mirage (ev3, Irvine, CA, USA), Agility 10 and 14 standard (Codman, Raynham, MA, USA), and Synchro 2 standard (Stryker, Fremont, CA, USA). Glacial acetic acid was not used as our Institutional Review Board does not allow alteration of the US Food and Drug Administration evaluated material, especially in an off-label usage. For the ONYX cohort Marathon, Echelon 10 or 14 (ev3, Irvine, CA, USA) DMSO-compatible microcatheters were used. X-pedion 10, Agility 10 and 14 standard

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and Synchro 2 standard microwires were utilized. All cases started with ONYX 34 and then converted to ONYX 18. The plug and push technique was used, with plug times typically ranging from 20 to 30 min. In general, fluoroscopy times were greater for the ONYX cohort, but the time span of the study includes three generations of biplane equipment. Two of these are no longer used, and data are unavailable for radiation doses on the earliest equipment.

Arterial embolization of 56 lesions was accomplished to as complete an extent as possible followed by transvenous embolization, surgery or radiation as needed. The first 18 cases were treated with n-BCA and the last 32 cases were treated with ONYX. Two patients were embolized using n-BCA and then at least a year apart using ONYX for separate lesions. The third patient with two lesions was treated with ONYX when her second lesion progressed from a type I to a type IIa lesion. Other patients with type I or IIa lesions were treated because of symptomatic tinnitus. Six cases treated from September 2005 to February 2006 were a transition during which either agent was used. Three of these patients received n-BCA and three received ONYX. Six of the n-BCA cases also included the use of Target Detachable coils (Stryker, Fremont, CA, USA), Berenstein liquid coils (Stryker, Fremont, CA, USA), and/or Polyvinyl alcohol particles (Stryker, Fremont, CA, USA). One patient in the n-BCA cohort was treated with proton radiation therapy for residual DAVF. One of these n-BCA cases was treated with coil embolization of a sigmoid and transverse sinus following arterial embolization. Polyvinyl alcohol particles and dehydrated alcohol were used in one of the ONYX patients before surgery because of the tortuosity of the middle meningeal artery. Alcohol was used in two of the cases followed by the use of coils and ONYX on the venous side. In patients with initial angiographic occlusion of a DAVF by embolization, angiographic follow-up was at 3 months. Patients with recurrence underwent further embolization. Patients with residual arteriovenous shunts post-embolization had surgical obliteration of the fistula and underwent

cerebral angiography immediately following the surgery. Initial post-embolization results as well as 3-month angiographic follow-up is reported.

Statistical analysis was performed using a Fisher's exact two-tailed test to compare the two cohorts for initial angiographic occlusion and after 3-month follow-up angiography to compare durable occlusion rates. It was also used to compare the use of surgery in the groups. t-Test analysis was performed to evaluate the variable of age. Fisher's exact analysis was performed to evaluate variables of gender and Cognard classification. This research study was approved by the Institutional Review Board at our medical center.

RESULTS

The n-BCA embolization group shown in table 1 included 16 men and five women with an average age of 60.9 years. Nineteen patients had Cognard type III or IV lesions, and two had Cognard IIa+b lesions. Presenting symptoms in this group included seven patients with headache, one with tinnitus, six with stroke/transient ischemic attack, three with parenchymal bleed, three with subarachnoid hemorrhage and one with subdural hematoma. Seven patients had mental status changes including one with seizures. Five patients presented with venous sinus or jugular vein occlusions.

Seven patients had initial angiographic occlusion of their DAVF using n-BCA and these were stable at 3-month angiography. Eleven patients underwent surgery following embolization to achieve occlusion. This cohort underwent a total of 27 embolization sessions (1.23 per case) and 11 surgeries (0.52 per case) for an average of 1.75 procedures per case. Patient 11 underwent two embolizations of his supratentorial DAVF to occlusion and then surgical resection of a separate cerebellar pial arteriovenous malformation. Three of the incomplete embolization patients did not undergo surgery. One of these, patient 16, was lost to follow-up. Patient 17 was treated with arterial side embolization with n-BCA and coil embolization of the left transverse sinus but had

Table 1 DAVF patients treated with n-BCA

Case	Age/sex	Location	Cognard	Embolization occl	Surg occl	Complication
1	50–59/M	R Tr-Sig	IV	–	+	
2	70–79/M	L Tr-Sig	IV	–†	+	Tr vis. P.O.
3	60–69/M	R Trans	IV	–	+	
4	70–79/M	L Sph-Par	IV	–	+	
5	80–89/F	L Sig	IV	–	–	Death, stroke
6	60–69/F	L Tent	III	+	–	
7	50–59/F	L Trans	IV	+	–	PE
8	70–79/M	L Tent	V	–	+	
9	70–79/F	R Inf Petr	IV	+	–	Dissection
10	40–49/M	R Tr-Sig	IV	+	–	
11	60–69/M	R Sag	IV	+	–	Dissection
12	60–69/M	L Trans	IV	+	–	
13	70–79/F	R Sph-Par	IV	–	+	
14	40–49/M	R Trans	IIa+b	–	+	SThr, stroke, Sz
15	30–39/M	Falx-BVR	IV	–	+	
16	50–59/M	Sag	III	–	–	ACA perf x 2
17	80–89/M	L Trans	IV	–*	–	
18	40–49/M	F Mag vv	IV	+	–	
19	50–59/M	L Trans	IV	–	+	
20	40–49/M	L Trans	IV	–	+	PE
21	40–49/M	R Trans	IV	–	+	

*n-BCA arterial and coils venous sinus.

†New fistula at edge of craniotomy site at 2 years.

ACA perf x 2, anterior cerebral artery perforation x 2; BVR, basal vein of Rosenthal; DAVF, dural arteriovenous fistula; F Mag, foramen magnum; n-BCA, n-butyl-2-cyanoacrylate; PE, pulmonary embolus; Sig, sigmoid sinus; Sph-Par, sphenoparietal sinus; SThr, sinus thrombosis; Sz, seizure; Tent, tentorium; Tr or Trans, transverse sinus; Tr vis. P.O., transient visual change post-op.

Table 2 Adverse events in DAVF embolization and surgery cohorts

Liquid embolic agent	Death	Major neurological	Minor neurological	Systemic	Technical
n-BCA	1	2	1	2	4
ONYX	0	3	0	3	4

DAVF, dural arteriovenous fistula; n-BCA, n-butyl-2-cyanoacrylate; ONYX, ethylene vinyl alcohol copolymer.

residual fistula. Because of a history of previous surgery and head and neck radiation he underwent further treatment with proton radiotherapy and has elected not to have follow-up angiography. Patient 5 died during admission (see below).

Adverse events (table 2) included a death in patient 5. She presented with a left temporal lobe hemorrhage and intractable status epilepticus, which was the cause of death. She was found to have occlusion of the left sigmoid sinus suggesting a hypercoagulable state from her gastrointestinal malignancy. There was 95% embolization of this DAVE. Another major neurological adverse event occurred in patient 14, who had seizures and strokes after embolization due to progressive venous sinus thrombosis identified on admission. No migration of n-BCA into

the venous sinuses was identified, but 5 cc ethanol had been administered adjunctively during right occipital artery embolization. Patient 16 had an anterior cranial fossa DAVF and sustained anterior cerebral artery perforations during two separate procedures, which were occluded with n-BCA embolization without clinical sequelae. Intention to treat with ONYX was originally planned for the patient; however, n-BCA was used to occlude the arterial branch perforations. Patient 2 had transient hemianopsia following surgery. Patient 9 had an asymptomatic internal carotid dissection treated with anticoagulation and patient 11 had an asymptomatic left vertebral artery dissection probably from a follow-up diagnostic angiogram. Patients 7 and 20 had pulmonary emboli, one following embolization and the other following embolization and surgery.

The ONYX embolization group shown in table 3 included 20 men and 14 women with an average age of 56.1 years. Twenty-three lesions were Cognard III and IV lesions, four were Cognard IIa+b lesions, four were Cognard IIb lesions, two were Cognard IIa lesions and three were Cognard I lesions. Presenting symptoms in this group included 16 patients with headache, 14 with tinnitus, four with stroke/transient ischemic attack, 10 with

Table 3 DAVF patients treated with ONYX

Case	Age/sex	Location	Cognard	Embolization occlusion	Surgical occlusion	Complication
1	50–59/M	R Tent-BVR	IV	—*	+	Asystole
2	70–79/M	R Tent-BVR	IV	—	+	R CN V and VII
3	50–59/M	Sag	IV	—	+	
4	30–39/M	L Tr-Sig	IIa	+	—	
5	40–49/M	L Trans	IV	+	—	
6	60–69/F	Torcular	IV	+	—	
7	60–69/M	L Sig	IIa+b	+	—	
8	40–49/M	R Tr-Sig	IV	+	—	
9	70–79/M	Sag	III	+	—	LECA Diss
10	50–59/F	Sag	IV	+	—	Sz Dx angio
11	50–59/F	L Sig	IV	+	—	LECA clot
12	40–49/M	L Tr-Sig	III	+	—	
13	50–59/M	L Trans	IV	+	—	
14	70–79/M	L Tmp	III	—†	+	
15	60–69/F	L Trans	IV	+	—	RVA Diss
16	70–79/F	R Sig	IV	+	—	
17	50–59/F	L Tr-Sig	IV	+	—	
18	50–59/F	L Tr-Sig	IV	+	—	
19	40–49/F	L Trans	III	+	—	
20	30–39/M	R Tr-Sig	IIb	+	—	Occ Art perf
21	40–49/M	R Tr-Sig	III	+	—	
22	50–59/F	R Tr-Sig	IIa+b	+	—	
23	40–49/F	L Tr-Sig	IIb	—	—	AICA perf × 2
24	60–69/F	R Tr-Sig	IIb	—	—	
25	50–59/F	L Tr-Sig	I	+	—	
26	50–59/F	R Sig	I	+	—	
27	60–69/M	L Tr-Sig	IIa+b	+	—	
28	60–69/M	Torcular	IV	+	—	
29	50–59/M	R Inf Petr	III	+	—	R CN VII
30	50–59/F	R Tr-Sig	IIb	+	—	
31	50–59/M	L Tr-Sig	III	+	—	
32	70–79/M	L Tr-Sig	IV	+	—	
33	50–59/M	R Tr-Sig	I	+	—	
34	50–59/M	L Tr-Sig	III	+	—	
35	70–79/F	R Trans	IIa	+	—	

*ONYX and PVA.

†ONYX, PVA and alcohol.

AICA perf, anterior inferior cerebellar artery perforation; BVR, basal vein of Rosenthal; DAVF, dural arteriovenous fistula; ECA, external carotid artery; ECA diss, external carotid dissection; Inf Petr, inferior petrosal sinus; Occ art perf, occipital artery perforation; ONYX, ethylene vinyl alcohol copolymer; R CN V, VII; right cranial nerve V and VII; R CN VII, right cranial nerve VII; Sig, sigmoid sinus; Sag, sagittal sinus; Sz Dx angio, seizure during diagnostic angiogram; Tmp, temporal lobe cortical veins; Tr or Trans, transverse sinus.

parenchymal bleed, and three with subarachnoid hemorrhage. Thirteen patients presented with mental status changes including two with seizures. Patient 4 with a type IIa lesion had severe tinnitus and congestive heart failure, which improved after embolization. Seven patients presented with venous sinus or jugular vein occlusions.

Thirty-five DAVF were treated with ONYX embolization, with 29 initial angiographic occlusions and four surgical closures. A total of 54 embolizations (1.54 per case) and four surgeries (0.12 per case) were done for an average of 1.66 procedures per case. Patient 6 had two recurrences and underwent two further embolizations using ONYX to achieve a durable occlusion. Patient 1 received adjunctive PVA embolization before surgery. Patient 14 showed progression on 3-month angiography and was treated with alcohol sclerosis and PVA particle embolization before surgery. The use of ONYX resulted in the durable angiographic occlusion of 27/35 cases as assessed by 3-month angiograms after the final embolization (two patients lost to follow-up).

Major neurological adverse events (table 2) include patient 2 with facial nerve palsy following embolization of the petrous branch of the right middle meningeal artery. This patient also had a trigeminal nerve injury as well as a bone flap infection following surgery. Patient 29 had a facial nerve palsy following embolization of the posterior auricular branch of his right external carotid artery. An asymptomatic event occurred in patient 1 with a posterior fossa lesion. We postulate that this could be related to DMSO administration into the left posterior inferior cerebellar artery supplying the DAVF in proximity to the floor of the fourth ventricle. The procedure was terminated and the patient was clinically asymptomatic. Patient 11 developed a clot in the external carotid artery during microcatheter navigation in the second embolization but was asymptomatic (see case B). Other technical complications included a microcatheter perforation with ONYX in the occipital artery in patient 20 and two microcatheter/wire perforations in the anterior inferior cerebellar artery in patient 23, an external carotid artery dissection in patient 9 and a right vertebral artery dissection in patient 15, none of which had clinical consequences. Patient 10 had a seizure after a follow-up angiogram. MRI documented no new abnormality.

A summary of study results is shown in table 4. There is a statistically significant difference in the initial angiographic occlusion rates between ONYX and n-BCA ($p=0.0004$). There is also a statistically significant difference in embolization durable occlusion rates evaluated by 3-month angiogram including procedures done for recurrence ($p=0.0018$). The patients treated with ONYX embolization did not undergo surgical obliteration procedures as often ($p=0.0015$). t-Test analysis shows no significant difference in the cohorts by age ($p=0.1723$). Fisher's exact analysis shows no difference in the cohorts by the variables of gender ($p=0.2490$). Fisher's exact analysis shows a statistical difference in the proportion of Cognard type IV lesions in each cohort ($p=0.0049$). However, test of uniformity was done, and

controlling for the type of lesion, DAVF closure rates are better using ONYX compared with n-BCA across lesion types. None of the patients treated with adjunctive embolic materials in the n-BCA group (six) or the ONYX group (three) were closed by embolization techniques alone. Adverse event rates comparing death plus major neurological events n-BCA (3/21) and ONYX (3/35) do not show statistical significance ($p=0.6613$).

DISCUSSION

This single center retrospective study of consecutive patients shows that the initial angiographic occlusion rate for cranial DAVF is statistically higher with ONYX compared with n-BCA ($p=0.0004$). Durable occlusion rates assessed by 3-month follow-up angiography continue to show a statistical difference ($p=0.0018$) in favor of ONYX. There are low recurrence rates for patients with initial angiographic occlusion n-BCA (0/7) and ONYX (1/26). The ONYX cohort underwent surgery less often than the n-BCA cohort ($p=0.0015$). Death and major neurological complication rates are not statistically different in the cohorts.

The goal of DAVF treatment is to prevent high flow of blood into the cortical veins. Ideally, one would seal off the fistula from the arterial feeders to occlude the proximal vein(s) completely in a controlled manner. Occluding the venous outflow limits the possibility of developing new collaterals or recanalizing previously occluded arterial supply. Lesions may derive blood supply from the external carotid arteries, the internal carotid arteries or vertebral arteries via dural and falcine branches having variable origin. Parenchymal and pial branches of the anterior and posterior circulations can also be recruited. The lowest risk branches to embolize are specific external carotid artery branches, which do not supply cranial nerves. Two patients in our series developed facial nerve palsies. One of these occurred following embolization of the petrous branch of the right middle meningeal artery. The other occurred following embolization of the right posterior auricular artery. Both of these lesions had primary supply to their respective DAVF through these branches, but the surgical approach was known to carry a risk to the facial nerves.¹³

In the past, some lesions have been difficult to close completely from the transarterial approach. Transvenous endovascular methods were developed using embolic coils for lesions involving the transverse and sigmoid sinuses. This approach can be challenging if the segment contains normal venous drainage of brain parenchyma or is isolated from previous surgery/radiation, stenosis or thrombosis. Overall, we encountered 12 of 53 patients with venous sinus or jugular vein occlusions at presentation. In some cases retrograde catheterization of cortical veins has been done for coil placement. These techniques risk cortical vein rupture or incomplete cure as a result of dural leaflets remaining open. Venous sinus occlusion can be accomplished with n-BCA. This is based on closure of inflow and/or outflow rather than cast of the sinus. There is a potential risk of incomplete closure or fragmentation and migration of n-BCA across the fistula to embolize cortical veins.

Several strategies have been reported in the literature for the treatment of DAVF including transarterial and transvenous methods followed by surgery or radiation as needed. Occlusion rates for embolization of lesions involving the tentorium, straight sinus, transverse sinus and sigmoid sinus range from 29.8% to 100% using various methods.^{8–12 14–17} The highest initial occlusion rate using arterial embolization with n-BCA was achieved by Nelson *et al*¹⁵ with 23 of 23 lesions completely occluded by the wedged microcatheter technique. This treatment consists of closing segments of a lesion with n-BCA or PVA particles to decrease competing inflow followed by a wedged microcatheter

Table 4 Results of DAVF treatment n-BCA versus ONYX

Liquid embolic agent	No of DAVF	Initial angio occlusion	Rec	Durable angio occlusion	Surgical occlusion post-INR	Inc
n-BCA	21	7	0	7	11	3
ONYX	35	29	1	27 (+2 pending f/u angio)	4	2

Angio, angiogram; DAVF, dural arteriovenous fistula; Inc, incomplete; n-BCA, n-butyl-2-cyanoacrylate; ONYX, ethylene vinyl alcohol copolymer; post-INR, post embolization; Rec, recurrence.

injection of n-BCA to seal the dural vascular connections. It is not always possible to achieve a microcatheter position that allows this type of deposition especially in large lesions. Also, with complex lesions requiring multiple sessions of embolization, small inaccessible branches may remain, necessitating adjunctive therapy. A third point about this method is that PVA does not always result in permanent occlusion of arterial supply.

Nelson *et al*¹⁵ also reported that during the time of the study 11 out of 11 transverse and sigmoid sinus lesions were successfully treated by transvenous coil embolization, indicating that the n-BCA technique was not robust enough to treat all types of DAVF. The series by Guedin *et al*¹⁶ had a closure rate of 35 out of 45 by arterial embolization using n-BCA, including two of three patients treated adjunctively with transvenous coils (the third was treated by transvenous coil embolization only). Seven of the ONYX cases in our series employed a strategy of transarterial venous sinus occlusion, with sparing of the vein of Labbe. All were successful and showed durable occlusions at 3 months.

Cognard *et al*¹² published a series of 30 patients with DAVF treated using ONYX, with 24 completely occluded in that cohort. However, they state in their series that venous sinus coil embolization was employed for lesions centered around the transverse and sigmoid sinuses. In our study we used ONYX to treat a wide range of DAVF including the seven cases mentioned above. These seven cases were treated by transarterial venous sinus occlusion using ONYX rather than transvenous coil obliteration of the sinus. We had one case of transvenous sinus coil embolization in the n-BCA group and three cases in the ONYX group following transarterial embolization. Despite the adjunctive treatment, angiographic occlusions were not achieved. Furthermore, DAVF can present with venous sinus or jugular thrombosis or occlusion in up to 39% of cases,¹⁸ and these may not lend themselves to standard transvenous coil embolization.

ONYX has possible advantages over n-BCA described in the literature.^{19–21} ONYX can be delivered more slowly than n-BCA. Embolization from the artery through to the dural vein can potentially be better controlled with ONYX. ONYX is cohesive and non-thrombogenic allowing for better packing of a lesion and making parenchymal venous occlusion less likely. Slower delivery also allows the interventionalist to control and optimize the amount of artery-to-artery embolization. ONYX can accomplish closure of supply from potentially dangerous anastomoses (eg, neuromeningeal trunk/ascending pharyngeal artery) via injection from lower risk pedicles. One can perform arteriography in the pauses of 30–120 s to evaluate residual shunts in dural leaflets²² and the normal vessels as the lesion is occluded.

Finally, there are separate learning curves to the use of either liquid embolic agent. The n-BCA cases were treated by one senior and one junior staff, while the ONYX cases were treated by three senior and two junior staff. Five of the six incomplete ONYX embolization cases were done before we refined our technique of delivery. Cases were usually done with multiple staff present, so the level of staff experience was not significantly different in the cohorts.

CONCLUSION

This single center retrospective study shows a statistically significant improvement in initial and durable occlusion rates for embolization using ONYX compared to n-BCA in patients with cranial DAVF. This was accomplished with a similar low risk of complications. Our results suggest that ONYX embolization can be considered as the initial treatment modality of cranial DAVF.

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Contributors All of the authors contributed patient care for this cohort. They provided data analysis, review and editing comments during the research and manuscript writing phases.

Competing interests None.

Ethics approval Ethics approval was provided by Massachusetts General Hospital Institutional Review Board.

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Data sharing statement Data for our study are available to other researchers if they choose to gain access by Massachusetts General Hospital Institutional Review Board approved methods.

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