Surgical nuances for nasoseptal flap reconstruction of cranial base defects with high-flow cerebrospinal fluid leaks after endoscopic skull base surgery

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Extended endoscopic endonasal approaches have allowed for a minimally invasive solution for removal of a variety of ventral skull base lesions, including intradural tumors. Depending on the location of the pathological entity, various types of surgical corridors are used, such as transcribriform, transplanum transtuberculum, transcellar, transclival, and transodontoid approaches. Often, a large skull base dural defect with a high-flow CSF leak is created after endoscopic skull base surgery. Successful reconstruction of the cranial base defect is paramount to separate the intracranial contents from the paranasal sinus contents and to prevent postoperative CSF leakage. The vascularized pedicled nasoseptal flap (PNSF) has become the workhorse for cranial base reconstruction after endoscopic skull base surgery, dramatically reducing the rate of postoperative CSF leakage since its implementation. In this report, the authors review the surgical technique and describe the operative nuances and lessons learned for successful multilayered PNSF reconstruction of cranial base defects with high-flow CSF leaks created after endoscopic skull base surgery. The authors specifically highlight important surgical pearls that are critical for successful PNSF reconstruction, including target-specific flap design and harvesting, pedicle preservation, preparation of bony defect and graft site to optimize flap adherence, multilayered closure technique, maximization of the reach of the flap, final flap positioning, and proper bolstering and buttressing of the PNSF to prevent flap dehiscence. Using this technique in 93 patients, the authors’ overall postoperative CSF leak rate was 3.2%. An illustrative intraoperative video demonstrating the reconstruction technique is also presented.

Key Words • skull base defect • nasoseptal flap • cerebrospinal fluid • tissue glue • rhinorrea • dural sealant • endoscopic surgery • endoscopic endonasal approach • dural defect • tissue sealant

Endoscopic endonasal skull base surgery has benefited from significant advances over the past decade. In the EEA to the skull base, a minimal access technique is used via the transnasal route to expose and remove various midline ventral skull base lesions from the frontal sinus to the craniocervical junction.15 Endoscopic endonasal approaches have many advantages over more traditional “open” transcranial approaches, including the absence of brain retraction and manipulation, better panoramic endoscopic visualization, and increased postoperative comfort and cosmesis for the patient. Furthermore, improved instrumentation and the introduction of 3D endoscopy have made this approach increasingly effective for accessing and removing ventral skull base lesions.6,31 However, the EEA was initially criticized for its high rate of postoperative CSF leakage when used to remove large intradural lesions beyond the confines of the sella, such as meningiomas, craniopharyngiomas, and clival chordomas, through large skull base dural defects. These leaks initially occurred in approximately 20%–30% of patients who required repair of large (> 2-cm) skull base defects.9,18,37 Thus, the utility of the EEA was initially limited to very small defects that could be successfully repaired using multilayered synthetic or autologous non-vascularized tissue grafts.
One of the most significant advances for the endoscopic endonasal technique was the introduction of the pedicled PNSF, also referred to as the Hadad-Bassagasteguy flap for closure of large skull base dural defects.12 This technique was first described by Oskar Hirsh in 1952 as a random vascularized rotational flap for the endonasal closure of CSF leaks.14 The PNSF is harvested from the mucoperichondrial and mucoperiosteal coverage of the nasal septum, and its vascular supply is the posterior septal branch of the sphenopalatine artery. Since the technique’s reintroduction in the literature, multiple studies have reported increasing success with closure of large skull base defects. Currently, the incidence of postoperative CSF leakage of 3.2%. Although the technique’s reintroduction in the literature, multiple studies have reported increasing success with closure of large skull base defects. Currently, the incidence of postoperative CSF leakage of 3.2%. Although the technique’s reintroduction in the literature, multiple studies have reported increasing success with closure of large skull base defects. Currently, the incidence of postoperative CSF leakage of 3.2%. Although the technique’s reintroduction in the literature, multiple studies have reported increasing success with closure of large skull base defects. Currently, the incidence of postoperative CSF leakage of 3.2%. Although the technique’s reintroduction in the literature, multiple studies have reported increasing success with closure of large skull base defects. Currently, the incidence of postoperative CSF leakage of 3.2%. Although the technique’s reintroduction in the literature, multiple studies have reported increasing success with closure of large skull base defects. Currently, the incidence of postoperative CSF leakage of 3.2%. Although the technique’s reintroduction in the literature, multiple studies have reported increasing success with closure of large skull base defects. Currently, the incidence of postoperative CSF leakage of 3.2%. Although the technique’s reintroduction in the literature, multiple studies have reported increasing success with closure of large skull base defects. Currently, the incidence of postoperative CSF leakage of 3.2%. Although the technique’s reintroduction in the literature, multiple studies have reported increasing success with closure of large skull base defects. Currently, the incidence of postoperative CSF leakage of 3.2%. Although the technique’s reintroduction in the literature, multiple studies have reported increasing success with closure of large skull base defects. Currently, the incidence of postoperative CSF leakage of 3.2%. Although the technique’s reintroduction in the literature, multiple studies have reported increasing success with closure of large skull base defects. Currently, the incidence of postoperative CSF leakage of 3.2%. Although the technique’s reintroduction in the literature, multiple studies have reported increasing success with closure of large skull base defects. Currently, the incidence of postoperative CSF leakage of 3.2%. Although the technique’s reintroduction in the literature, multiple studies have reported increasing success with closure of large skull base defects. Currently, the incidence of postoperative CSF leakage of 3.2%. Although the technique’s reintroduction in the literature, multiple studies have reported increasing success with closure of large skull base defects. Currently, the incidence of postoperative CSF leakage of 3.2%. Although the technique’s reintroduction in the literature, multiple studies have reported increasing success with closure of large skull base defects. Currently, the incidence of postoperative CSF leakage of 3.2%.

Intraoperative video demonstrating multilayered standard transcranial approaches.3,7,13,17,18,22–24,28,30,34,37,38 As a result, the PNSF has become the primary workhorse for closure of large skull base defects with high-flow CSF leaks that develop after endoscopic skull base surgery.29 In our experience with 93 patients requiring PNSF reconstruction of large skull base defects after an EEA, we have, to date, been able to achieve an overall rate of postoperative CSF leakage of 3.2%. Although the technique is directed by the otolaryngologist. When performing the endonasal approach to the ventral cranial base have been previously described.22,23 In general, we prefer to harvest the PNSF at the beginning of the operation and rotate it into the posterior nasopharynx or maxillary sinus during the cranial base exposure and tumor removal until later use for reconstruction. Care is taken to ensure that the vascular pedicle is protected from trauma to prevent complications. The nose and nostrils are prepared with povide-dine-iodine (Betadine) solution followed by placement of oxymetazoline hydrochloride (Afrin)—soaked pledgets. The abdomen and right thigh are also prepared for potential autologous fat and/or fascia lata graft harvesting for multilayer reconstruction prior to final placement of the PNSF.

Flap Design and Harvest of the Nasoseptal Flap

The details of how we perform the initial endoscopic endonasal approach to the ventral cranial base have been previously described.22,23 In general, we prefer to harvest the PNSF at the beginning of the operation and rotate it into the posterior nasopharynx or maxillary sinus during the cranial base exposure and tumor removal until later use for reconstruction. Care is taken to ensure that the vascular pedicle is protected from trauma to prevent compromise of the flap. We use a similar method to Hadad and colleagues22 to harvest the PNSF. We prefer to use a No. 15 blade knife rather than a fine-tipped monopolar cautery when making the incisions along the nasoseptal mucosa. We believe that using a knife blade to make the appropriate cuts ultimately increases the available surface area of the PNSF for optimal coverage by avoiding tissue retraction and shrinkage associated with electrosurgery. Furthermore, the increased vascularity at the edges of the flap may play a role in adherence to the skull base defect. Avoidance of electrosurgery may also improve donor-site re-mucosalization of the nasal septum by decreasing thermal trauma at the edge of the remaining nasoseptal tissue.

The first incision is made at the junction between the floor of the nose and the nasal septum starting from a posterior to anterior direction. In cases in which a larger...
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PNSF is desired (as in cases of transcribriform defects from orbit to orbit), this incision is taken more laterally along the floor of the nose to increase the width of the flap. The second incision is made from the most inferior aspect of the sphenoid opening and advanced superiorly and anteriorly until the desired length is reached. This incision can be taken as far anteriorly as the septocolumellar junction if a longer flap is needed (as in transcribriform defects extending to the posterior table of the frontal sinus). During this second incision, it is important to preserve the olfactory mucosa located posterolaterally to prevent postoperative anosmia. The third incision involves making a vertical cut connecting the most anterior aspect of the previous 2 incisions. It is important not to perform this incision before the other 2 incisions as to prevent pooling of blood posteriorly that can obstruct visualization of the field. At this juncture, the nasoseptal flap is elevated with a Cottle elevator in a submucoperichondrial and submucoperiosteal plane from an anterior to posterior direction until the choana is reached. Lastly, a relaxing incision is made along the arc of the choana to increase the surgical freedom (that is, the range of rotation) of the flap. By taking this incision as far lateral along the choanal arch, one can significantly increase the flap’s mobilization and reach.

It is important to design the PNSF tailored to the location and the anticipated size of the skull base defect that will be created to access the particular tumor. We term this “target-specific flap design.” For transcribriform approaches for olfactory groove meningiomas or sinonasal tumors requiring large cribriform resections, we prefer to harvest large flaps that extend as far anteriorly as the septocolumellar junction. These transcribriform defects are also wider, extending from one medial orbital wall to the other. Therefore, the width of the flap is enlarged by making the inferior incision more laterally along the mucoperiosteum of the nasal floor (hard palate). To ensure adequate coverage of the skull base defect, it is better to overestimate the defect size and harvest a larger flap than to have a smaller flap with suboptimal coverage.

Preparing the Defect

After tumor removal and subsequent meticulous hemostasis is achieved, reconstruction of the cranial base defect begins with preparing the bony defect site (recipient) for the PNSF (donor). It is important to strip the bony ventral skull base and sphenoid sinus of any secretory mucosa to allow flap adherence to the bone. We typically denude approximately 1 cm of mucosa around the bony defect, so as to avoid any trapped mucosa between the layers of reconstruction, which can result in postoperative intracranial mucocoele formation. Placing the flap over secretory mucosa also risks flap dehiscence from the skull base. For successful flap adherence of the PNSF to the skull base, there must be adequate contact between the mucoperichondrial/mucoperiosteal surface of the flap and the denuded bone. Additionally, in transplanum and transsellar approaches the entire sphenoid sinus is denuded to prevent sphenoid sinus mucocoele formation. This also includes denuding the mucosa of the lateral recesses of the sphenoid sinus.

Multilayer Reconstruction of Skull Base Defect

Meticulous multilayer reconstruction of the skull base dural defect is critical for preventing postoperative CSF leakage. The skull base dural defect is initially converted from a high-flow CSF leak state to a low-flow CSF leak state by placing a piece of autologous fascia lata to cover the defect (Fig. 1A and B). It is important to harvest a piece of fascia lata that is larger than the dimensions of the dural defect. We use precut pieces of Gelfoam of different sizes as templates to approximate the dimensions of the dural defect.

For transcribriform and transsellar defects, we prefer to use an inlay technique so that the edges of the fascia are tucked underneath the dural edges. For larger transcribriform defects, we place an additional layer of acellular dermal allograft as a combined inlay/overlay layer. Nonetheless, we prefer to use an overlay technique for transplanum transsellar defects because the optic canals and optic sheaths are often exposed in this procedure, especially for treatment of tuberculum sellae meningiomas. Placing the graft on the outside as opposed to the inside will decrease the chance of graft compression of the exposed optic nerves (Fig. 1A and B). For transsellar defects, we also prefer to use an overlay technique because we usually perform wide dural openings that prohibit any graft to be tucked underneath as an inlay. For larger sellar defects, as in pituitary macroadenomas, we occasionally insert a piece of autologous fat in the sellar defect before placement of the fascia lata overlay graft. A monolayer of Surgicel is then placed over the fascia graft to hold it in position to prevent graft migration prior to placement of the PNSF (Fig. 1C and D). At this juncture, there should be almost no evidence of CSF egress from the dural repair before proceeding to the next layer.

Fig. 1. Intraoperative endoscopic photographs. A: Ventral skull base dural defect (dotted line) seen after an endoscopic transplanum transtuberculum approach to remove a suprasellar craniopharyngioma. B: The optic chiasm (OC) is visualized within the defect. An autologous fascia lata graft is placed as an overlay over the skull base dural defect to convert a high-flow CSF leak to a low-flow leak state. C and D: An overlay graft is preferred in transplanum approaches to an inlay graft because of the potential risk of graft-induced compression against the optic nerves and chiasm. A single layer of Surgicel is placed over the fascia lata graft to hold it in position and to prevent graft migration.
step of nasoseptal flap placement. If needed, a second piece of fascia lata followed by another layer of Surgicel can be placed to stop any further CSF egress.

**Rotation and Positioning of the PNSF**

The vascularized PNSF is then brought up from the nasopharynx or maxillary sinus and rotated toward the ventral skull base repair (Fig. 2A). It is essential to maintain proper orientation of the flap so that the mucoperichondrial/mucoperiosteal surface of the PNSF is in direct contact with the ventral skull base defect. It is also important to ensure that the vascular pedicle is not twisted upon itself but maintains a uniform directionality. To ensure that the “reach” of the PNSF is maximally optimized, we aggressively remove the rostrum and floor of the sphenoid bone during the skull base exposure so that the flap is not hinged on this ledge, which tends to shorten the reach of the flap. The reach of the flap can also be lengthened by extending the incision at the proximal base of the flap near the vascular pedicle along the superior arc of the choana as mentioned above.

Using the 2-surgeon technique, the PNSF is carefully positioned over the skull base repair. Cottonoid paddies are used to apply gentle pressure on the flap from a proximal to distal fashion to rid any trapped air bubbles beneath the PNSF (Fig. 2B and C). This step ensures a good seal against the skull base so as to avoid the risk of flap dehiscence. Another single layer of Surgicel is placed around the edges of the PNSF to hold it in position and prevent flap migration (Fig. 2D). In our initial experience, we applied a dural sealant (fibrin glue or DuraSeal) over the PNSF repair at this juncture. However, in a recent retrospective review, we found that the addition of a dural sealant did not significantly decrease the rate of postoperative CSF leakage. Therefore, we have stopped using dural sealants when performing PNSF reconstruction in the aforementioned fashion, which has also decreased the surgical costs. We also believe that adding a layer of dural sealant prevents maximal buttressing force of the Gelfoam and Merocel packing against the PNSF repair. Additionally, we believe that the dural sealant has the potential to drip underneath the free edges of the PNSF and lift the flap away from the bone during the sealant expansion, thus risking flap dehiscence.

**Bolstering and Buttressing the PNSF**

Proper buttressing of the PNSF is also critical to preventing postoperative flap dehiscence. The PNSF repair is then bolstered with several pieces of 2.5 × 2.5–cm Gentamicin-soaked Gelfoam pledgets (Fig. 3A and B). An initial layer is placed directly on the PNSF repair and then compressed with cottonoid paddies with gentle suction. This step is followed by the placement of a second layer of Gelfoam. An inflatable Merocel nasal tampon lathered with Bacitracin ointment is then placed into the nasal cavity to buttress the repair. The packing expands the dead space in the nasal cavity after it becomes hydrated with Gentamicin irrigation (Fig. 3C and D). This provides buttressing support up against the PNSF repair to optimize flap adherence to the bone.

Although some have used a Foley catheter balloon to buttress the PNSF repair, we prefer to use the inflatable Merocel nasal tampon. In our opinion, the Merocel tampon expands in such a way to provide a tighter packing of the nasal cavity dead space while applying uniform pressure to the skull base repair. Because the Foley balloon is spherical in shape, the balloon has less direct contact on the PNSF repair than does the Merocel pack that expands to fill the dead space. The Merocel pack, in general, is less invasive and more comfortable for the patient while avoiding the risk of premature inadvertent removal by the patient or nursing staff.

![Fig. 2. Intraoperative endoscopic photographs. A: Rotation of the PNSF from the posterior nasopharynx toward the ventral skull base defect. B and C: Cottonoid paddies are used to apply gentle pressure against the PNSF to get rid of any trapped air bubbles beneath the nasoseptal flap (NSF), as this can potentially result in flap dehiscence. D: After final positioning of the nasoseptal flap, a single layer of Surgicel is placed over the edges of the flap against the skull base to prevent flap migration and promote flap adherence. VP = vascular pedicle.](Image 63x162 to 303x341)

![Fig. 3. Intraoperative endoscopic photographs. A and B: Repair of the PNSF is bolstered by placing Gentamicin-soaked Gelfoam pledgets (G) against the flap. C and D: An expandable Merocel nasal tampon (M) is used to buttress the PNSF repair. As the Merocel expands after hydration with Gentamicin irrigation, it fills the nasal cavity dead space to create a tamponade against the skull base repair. MT = middle turbinate; NS = nasal septum.](Image 327x141 to 567x320)
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Postoperative Management

The patient is maintained on postoperative antibiotics with a third-generation cephalosporin or a penicillin-based antibiotic with β-lactamase for about 10–12 days after surgery until the Merocel packing is removed by nasal endoscopy. The patient receives stool softeners and is instructed to avoid Valsalva maneuvers, nose blowing, or activities that can raise intracranial pressure. Additionally, we do not routinely use postoperative lumbar drainage to avoid complications associated with intracranial hypotension and tension pneumocephalus because the patient is already in a CSF hypovolemic state at the end of the surgery.5 We have observed that by not placing an indwelling lumbar drain, patients are able to ambulate earlier and minimize their potential risks for thromboembolic events. Furthermore, patients are discharged from the hospital earlier, thereby promoting shorter hospital stays. In our experience of using the aforementioned reconstruction technique in 93 patients, we have observed a postoperative CSF leak rate of 3.2%. These leaks were all successfully repaired by repositioning and rebolstering the same PNSF on top of a multilayer reconstruction.

Management of Postoperative CSF Leaks

Of 93 patients who underwent multilayered PNSF reconstruction of high-flow CSF leaks after endoscopic skull base surgery, 3 suffered a postoperative CSF leak. All 3 patients underwent endoscopic endonasal reexploration and revision of the skull base repair using the same viable PNSF. There were no further leaks after the revision repair. Interestingly, all 3 patients had undergone a transplanum transtuberculum approach to a suprasellar tumor. The skull base defect created by this approach is generally large (approximately 5 cm²) and communicates freely with an opened suprasellar arachnoid cistern and, in some cases, a fenestrated floor of the third ventricle. Thus, some have suggested that transplanum defects have a higher risk of postoperative CSF leakage than other locations such as transcribriform or transsellar.9,37

Each failure was critically analyzed for the potential cause of the leak. In 1 patient delayed CSF rhinorrhea developed 4 weeks after surgery, about 1 week after a routine outpatient follow-up debridement performed via nasal endoscopy. We postulate that disturbance of the repair may have occurred during the debridement. The patient underwent endoscopic reexploration, and the nasoseptal flap was noted to be partially displaced. The defect was successfully repaired with an acellular dermal allograft followed by repositioning the same PNSF. The second patient was noted to have high CSF egress during the dural opening part of the surgery. Benign intracranial hypertension was suspected, and a postoperative lumbar drain was therefore placed postoperatively for 5 days. Two days after removing the lumbar drain, the patient presented with a CSF leak. A lumboperitoneal shunt was inserted, and a revision of the PNSF repair was undertaken using the same flap. The third patient developed delayed intraventricular tension pneumocephalus 1 week after surgery. An emergency ventricular catheter was placed to aspirate air, and endoscopic reexploration was performed thereafter. A ball-valve leak was noted in the repair, and successful revision of the repair was performed using the same fascia lata graft and PNSF.

Discussion

Endoscopic endonasal approaches to access ventral skull base pathologies have become increasingly effective over the last decade (Fig. 4). In properly selected cases, this approach provides various advantages over more conventional open transcranial methods to access and remove lesions of the midline ventral skull base, sellar region, and parasellar region, including avoidance of a transcervical or transcranial incision, craniotomy or transfacial osteotomies, and risks associated with brain retraction and manipulation.11,15,16,18–20,22,23,25,26,33 Furthermore, this approach offers panoramic visualization of ventral skull base structures from the frontal sinuses to the craniocervical junction with the aid of angled endoscopes.1,22,23

Initially, the EEs were restricted to the removal of small lesions through smaller skull base defects (< 1 cm). These smaller defects were usually closed using a combination of nonvascularized free tissue and synthetic grafts, applied in a variety of methods. The success rate for closing small lesions with this method was approximately 95%, which showed EEs to be an effective method when the operation required creating a small skull base defect.12 However, one of the major criticisms

![Fig. 4. Preoperative sagittal (A) and coronal (B) T1-weighted post–Gd enhanced MR images demonstrating a large suprasellar retrochiasmatic craniopharyngioma. The tumor was removed completely via an endoscopic transplanum transtuberculum approach. Immediate postoperative sagittal (C) and coronal (D) T1-weighted post–Gd enhanced MR images showing no evidence of residual tumor. The PNSF is well visualized with bright enhancement along the ventral skull base (arrow). Modified with permission from Liu JK, Eloy JA: Endoscopic endonasal transplanum transtuberculum approach for resection of retrochiasmatic craniopharyngioma. J Neurosurg 32 (Suppl):E2, 2012.](image-url)
of EEs during their infancy was the relatively high rate of postoperative CSF leaks when the approach was used for removal of larger lesions through a large (>2-cm) defect when using the same multilayered nonvascularized free graft reconstruction technique. This type of closure began with a subdural inlay graft of collagen matrix to eliminate intradural dead space and was followed by an extradural inlay graft of acellular dermis to provide closure of the cranial defect. If an epidural graft was insufficient or a more complete closure was desired, the application of grafts over the defect on the nasal side was often performed, with careful denudation of the surrounding mucosa to prevent mucocele formation. Finally, the grafts would be bolstered by a combination of absorbable packing, gelatin sponge squares, and possibly synthetic glue. The basic premise established by the closure of traumatic, idiopathic, and smaller skull base defects was expanded upon by using different materials for closure, namely endogenous nonvascularized tissue grafts, including autologous fat grafts, fascia lata grafts, temporalis fascia grafts, and occasionally autologous bone grafts. These grafts would be used at various points in the closure, either as subdural inlay grafts or as overlay grafts for the defect. However, even with optimal closure, endoscopic reconstruction of larger dural defects resulted in an unacceptably high CSF leak rate of approximately 20%–30%. Furthermore, the use of vascularized flaps for the repair of dural defects in transcranial approaches, including pericranial, galeal, and temporoparietal flaps, were not feasible for minimally invasive endonasal approaches. Not only did externally accessing these cranial flaps contradict the minimal access nature of EEs, but it could drastically increase the risk for postoperative morbidity and infection.

The high postoperative CSF leak rate associated with EEs for large skull base lesions limited this approach to only small lesions, leaving larger lesions for traditional transcranial approaches. However, the recent advent of pedicled endonasal mucosal flaps for repair of large skull base defects considerably expanded the role of EEs in accessing and removing these larger lesions. Initially, the intranasal flaps were taken from random locations, with varied success. Of these, the PNSF has been the most widely studied and has gained the most popularity for closure of large skull base defects created after EEs. This flap consists of a neurovascular pedicled flap from the mucoperiosteum and mucoperichondrium of the nasal septum, which is supplied by the posterior septal branch of the sphenopalatine artery. The greatest advantage of this flap is that it can be harvested endoscopically before creating the skull base defect, thereby eliminating the need for external flap harvesting. Furthermore, this flap provides the same advantages as other vascularized flaps, namely faster healing and a lower incidence of graft migration. Another major advantage to this flap is that it provides a very large surface area (approximately 25 cm²) for the coverage of large defects created from EEs. The PNSF is effective in covering defects greater than 6 cm in the anterior cranial fossa, particularly the transcribriform defects that extend from the posterior table of the frontal sinuses to the planum sphenoidale in the sagittal plane and between both orbits in the coronal plane. However, maximally extensive defects from multiple-corridor EEs, such as those that extend from the sella turcica to the posterior table of the frontal sinus, may require bilateral PNSFs or other forms of repair.

Conclusions
The PNSF used in conjunction with a multilayered reconstruction is an effective method for repair of high-flow CSF leaks from large skull base defects created after endoscopic skull base surgery. The key aspects to successful reconstruction include meticulous multilayered reconstruction performed by 2 surgeons (the 3- to 4-hand technique), careful preparation of the skull base defect and graft site, accurate flap design and preservation of the vascular pedicle with avoidance of trauma to the flap, and optimal positioning and buttressing of the PNSF repair. Careful consideration of all these steps in the PNSF reconstruction is critical to minimizing postoperative CSF leakage.

Disclosure
The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

Author contributions to the study and manuscript preparation include the following. Conception and design: Liu, Eloy. Acquisition of data: all authors. Analysis and interpretation of data: all authors. Drafting of the article: Liu, Schmidt, Choudhry. Critically revising the article: Liu, Schmidt, Eloy. Reviewed submitted version of manuscript: Liu, Schmidt. Approved the final version of the manuscript on behalf of all authors: Liu. Study supervision: Liu, Eloy.

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