Postoperative Complications in Otospongiosis: Usefulness of MR Imaging

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BACKGROUND AND PURPOSE: Sensorineural hearing loss (SNHL) is a rare complication of stapes surgery that may arise for many reasons. Usually, the pathogenesis of SNHL can be established by clinical and CT examinations. The purpose of this study was to evaluate the utility of MR imaging when CT findings are normal or not contributive.

METHODS: Eleven patients with SNHL (in some instances, associated with vertigo) after stapedectomy, in whom CT showed no well-defined cause, were examined by MR imaging.

RESULTS: MR studies established the additional findings of reparative intravestibular granuloma (n = 2), intralabyrinthine hemorrhage (n = 1), and bacterial labyrinthitis (n = 1). In five cases, MR findings were similar to CT findings. In two cases, CT and MR results were normal. Revision surgery was performed in five patients and confirmed the MR findings in each case.

CONCLUSION: If CT is not contributive as to the origin of SNHL and vertigo occurring after stapes surgery, then MR imaging may be helpful in these patients.

Sensorineural hearing loss (SNHL), often associated with vertigo, is a rare complication after stapes surgery (1). The pathogeneses are varied and depend mainly on the duration of the symptoms (1). Direct trauma to the membranous labyrinth, bulging of the prosthesis into the vestibule, postoperative reparative granuloma, oval window fistula, suppurative or nonsuppurative labyrinthitis, and endolymphatic hydrops are the primary known etiopathogenic factors (2).

Until recently, systematic surgical revision was the method of choice for identifying some of these underlying causes of SNHL. Currently, high-resolution CT (HRCT) is considered the first-line imaging technique for delineating the extent of disease, and it may improve the results of surgical intervention (3). Postoperatively, HRCT can depict the status of the reconstructed prosthetic ossicular chain, identify the position of the tip of the prosthesis, and may identify those cases that require repeat surgery. Moreover, HRCT can depict a malpositioned prosthesis, postoperative middle ear granuloma, perilymphatic fistula, and rare inner ear malformations at the origin of perioperative “gusher” syndrome.

The purpose of this study was to evaluate the contribution of MR imaging to the diagnosis of membranous labyrinthine complications occurring after stapes surgery when clinical and CT exploration fail to identify the cause of SNHL and vertigo.

Methods

This prospective study, conducted between 1994 and 1999, included 11 patients aged 30 to 76 years (mean age, 50 years). Inclusion criteria were 1) documentation of SNHL after stapes surgery and 2) persistent unknown origin of SNHL after clinical and CT examinations.

Surgical procedures included stapedectomy in eight patients and small fenestral stapedotomy in three patients. After surgery, all 11 patients experienced severe SNHL, and six patients also had profound hearing loss (in the operated ear). Vertigo was encountered in nine patients (90%) and tinnitus in two (20%). No facial palsy was encountered.

All patients underwent HRCT of the temporal bone in both axial and coronal planes with 1-mm contiguous slices and a 512 × 512 matrix. CT scans were examined to determine the position of the prosthesis; a tip protruding more than 2 mm into the vestibule defined an intravestibular bulging of the prosthesis. Other signs evaluated included mineralization of the long process of the incus and bony labyrinth as compared with the contralateral healthy ear, aeration of the oval window and the sinus tympani, ossification of the membranous labyrinth, and associated inner ear malformation (eg, vestibular aqueduct dilatation, abnormal cochlear segmentation). MR imaging was performed in all patients 1 to 7 days after CT.
exploration on a 1.0-T superconducting active shielded magnet. Three-millimeter-thick contiguous 2D spin-echo T1-weighted images (500/15/4 [TR/TE/excitations]) without and with administration of intravenous gadopentetate dimeglumine and 4-mm-thick axial 2D spin-echo T2-weighted sequences (2500/90/1) with a 0.8-mm gap were obtained in axial and coronal planes. Finally, 1-mm-thick contiguous axial 3D Fourier transformed constructive interference in steady state (20/8/1) sequences centered on the inner ear were obtained.

MR imaging is considered a safe procedure for patients who have undergone stapedectomy; previous studies have shown that the piston of the prosthesis is not ferromagnetic, with the exception of the McGee stapedectomy piston prosthesis (4, 5). All CT and MR examinations were evaluated by two radiologists. The MR images were studied for T1 and T2 signal intensity of the anterior and posterior labyrinth, especially in the region of the oval and round windows, and for membranous labyrinthine enhancement after injection of contrast material.

Results
The study population included nine women and two men (age range, 30–76 years; mean age, 50 years). The left ear was the most frequently involved (in nine cases, versus two in the right ear). Symptom onset varied from 2 days to 20 years after stapedectomy: in three cases, onset was within 2 days; in two cases, within 2 months; in four cases, within 1 to 5 years; and in two cases, onset was more than 5 years after stapedectomy. In all patients, symptoms were represented by SNHL, which was either total (n = 7) or partial (n = 6). Vertigo was present in eight patients, tinnitus in two (Table 1). No patient had facial palsy. Three patients received medical treatment with antibiotics and steroids, resulting in partial amelioration.

CT Findings
CT findings were normal in two cases and abnormal in nine. CT showed hypodensity in the oval niche in one patient (case 1), periprosthetic hypodensity in four patients (cases 2, 5, 8, and 9), hypodensity in the vestibular aqueduct associated with enlargement of the internal auditory canal and modiolus in one patient (case 3), and periprosthetic granuloma associated with a neumora of the belly of the stapedius muscle (n = 1, case 5), intravestibular bulging of the prosthesis (n = 2, cases 7 and 11), intravestibular bulging of the prosthesis associated with periprosthetic granuloma (n = 1, case 9), and a granuloma filling the oval window niche and periprosthetic region (n = 1, case 1) (Fig 4).

MR Findings
MR studies showed postoperative hemorrhagic labyrinthitis, as determined by T1 and T2 hyperintensity of the membranous labyrinth in one patient (case 2) (Fig 1). In two patients, a reparative intralabyrinthine granuloma (or intravestibular granuloma), as determined by intermediate signal intensity on T1-weighted images and hypointensity on T2-weighted images of the membranous laby-

Discussion
Otospongiosis is a common disease affecting young persons, especially Caucasian women. It may be inherited as an autosomal dominant trait, but frequently occurs as an isolated event. The disease is bilateral in 85% of patients and often symmetrical (3). The well-established treatment of otospongiosis is stapedectomy. Stapedectomy was first introduced in 1956 by Shea, who used a poly-
TABLE 1: Clinical and imaging findings

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Clinical Findings</th>
<th>CT Findings</th>
<th>Abnormalities of the Membranous Labyrinth on T1-Weighted MR</th>
<th>Abnormalities of the Membranous Labyrinth on T2-Weighted MR</th>
<th>Contrast Enhancement</th>
<th>Bone Signal</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (Fig. 4)</td>
<td>Total SNHL, vertigo, and tinnitus</td>
<td>Hypodensity of the oval window niche</td>
<td>Hyperintensity of the oval window niche</td>
<td>Hyperintensity of the oval window niche and along the prosthesis</td>
<td>Prosthesis and oval window niche</td>
<td>Normal</td>
</tr>
<tr>
<td>2 (Fig. 1)</td>
<td>Total SNHL and vertigo</td>
<td>Hypodensity along the prosthesis</td>
<td>Hyperintensity of lymphatic and perilymphatic fluid</td>
<td>Hyperintensity of the vestibule and lateral SSC</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>3</td>
<td>Partial SNHL and vertigo</td>
<td>Intravestibular bulging prosthesis, hypodensity of the vestibular aqueduct, enlargement of the IAC</td>
<td>Normal</td>
<td>Normal</td>
<td>Endolymphatic duct and vestibule</td>
<td>Bone enhancement</td>
</tr>
<tr>
<td>4</td>
<td>Vertigo followed by total SNHL</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>5</td>
<td>Vertigo followed by partial SNHL</td>
<td>Intravestibular bulging prosthesis, periprosthetic granuloma</td>
<td>Hyperintensity of stapediaian muscle and facial nerve</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>6 (Fig. 3)</td>
<td>Vertigo followed by total SNHL</td>
<td>Filling of the middle ear, periochlear demineralization</td>
<td>Hyperintensity of middle ear, decrease in signal intensity of labyrinth</td>
<td>Normal</td>
<td>Anterior and posterior labyrinth and IAC</td>
<td>Normal</td>
</tr>
<tr>
<td>7</td>
<td>Total SNHL and vertigo</td>
<td>Intravestibular bulging prosthesis</td>
<td>Hypointensity of the vestibule</td>
<td>Normal</td>
<td>Oval window niche</td>
<td>Normal</td>
</tr>
<tr>
<td>8 (Fig. 2)</td>
<td>Total SNHL and vertigo</td>
<td>Penprosthetic hypodensity</td>
<td>Decrease in signal intensity of posterior labyrinth</td>
<td>Hyperintensity of the vestibule</td>
<td>IAC, lateral and posterior SSC, oval window niche</td>
<td>Normal</td>
</tr>
<tr>
<td>9</td>
<td>Partial SNHL and tinnitus</td>
<td>Intravestibular bulging prosthesis, periprosthetic granuloma</td>
<td>Normal</td>
<td>Hyperintensity around the prosthesis</td>
<td>Prosthesis</td>
<td>Normal</td>
</tr>
<tr>
<td>10</td>
<td>Total SNHL</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>11</td>
<td>Partial SNHL</td>
<td>Intravestibular bulging prosthesis</td>
<td>Normal</td>
<td>Normal</td>
<td>Hyperintense lesion of the labyrinth</td>
<td>Normal</td>
</tr>
</tbody>
</table>

Note.—SNHL indicates sensorineural hearing loss; SSC, semicircular canal; IAC, internal auditory canal.
FIG 1. 58 year-old woman with postoperative hemorrhagic labyrinthitis 2 months after left-sided stapedectomy.

A, Coronal CT scan of left temporal bone shows a hypodensity along a well-located prosthesis (arrow). The lateral semicircular canal is normal.

B, Axial T1-weighted MR image shows abnormal signal intensity of the left lateral semicircular canal, as compared with right ear.

C, Axial T2-weighted MR image (2500/90) shows normal hyperintensity of the lateral semicircular canal (arrow).

D, Contrast-enhanced axial T1-weighted MR image (500/15) shows no change in the signal intensity of the labyrinth.

E, Contrast-enhanced coronal T1-weighted MR image shows an enhancing mass engulfing the prosthesis (arrow). Note the normal enhancement of the tympanic portion of the facial nerve (arrowhead) under the abnormal lateral semicircular canal.

F, Follow-up axial T1-weighted MR image at 1 year shows disappearance of the high signal intensity of the lateral semicircular canal.

ethylen strut placed on a vein graft in the oval window (6, 7). This procedure entails placement of a stapes prosthesis, extending from the oval window to the incus, and resection of the stapes superstructure.

Over the years, surgical techniques have continued to improve. Stapedectomy has increasingly been replaced by stapedotomy (the so-called small fenestra method), which results in better hearing in the low and middle frequencies, and prostheses have undergone improvements in shape, size, and material. Today, the shaft diameter of a prosthesis ranges from 0.3 to 0.8 mm (6), and pistons made of Teflon are the most widely used for reconstruction of the ossicular chain.

Complications of stapedectomy are infrequent (around 0.2% to 1%) (1). SNHL may appear suddenly after surgery or after a long delay (more than 5 years); vertigo is often the chief complaint in patients with profound SNHL, and may lead to revision stapedectomy (8). Many reports emphasize the need for rapid surgical revision in patients with deterioration of cochlear function after stapes surgery in order to prevent deafness (8). Complications of stapes surgery are always difficult to manage. Surgical revision may cause total hearing loss or disabling vertigo (1, 8, 9). Some authors have recommended the use of steroids, antibiotics, and vasodilators after surgical failure; for example, in a study by Mann et al (1), recovery of some hearing was found in six of 12 patients who followed this protocol and who did not undergo revision surgery.

Postoperative SNHL may manifest at different time intervals after surgery, depending on the underlying cause. SNHL that arises in the immediate postoperative period is most often caused by the insertion of a prosthesis that is too long, an intralabyrinthine hemorrhage, a perilymphatic fistula, or perioperative acoustic trauma. SNHL that occurs within a 2- to 5-month period after surgery is often the result of a middle ear granuloma, which may involve the vestibule (1). After several months, intravestibular granuloma, perilymphatic fistula, or prosthesis migration may be the cause of SNHL and vertigo.

Many complications of stapes surgery can be identified by CT examination, especially prosthesis dislocation and graft retraction (3). Damage to the tympanic membrane and postoperative otitis are frequent (3). Cholesteatoma formation has been reported as a poststapedectomy complication (3). Abnormal protrusion of the prosthesis into the vestibule, postoperative granuloma, suppurative labyrinthitis, and perilymphatic fistula responsible for SNHL may be identified or suggested on CT scans (1, 3). Intravestibular bulging of the prosthesis is easy to identify on CT studies; it is defined
by the tip of the prosthesis protruding more than 2 mm into the vestibule (3). Postoperative granuloma may be suggested by the persistence of soft tissue in the vicinity of the oval window 1 month after surgery (3). Suppurative labyrinthitis (10) may be suspected at a late stage if CT scans show labyrinthine bone demineralization with effacement of the intracartilaginous tissue and pseudoenlargement of the labyrinth. Perilymphatic fistula may be suspected when a fluid-filled middle ear is associated with the presence of air bubbles along the tip of the prosthesis (11).

MR imaging may be helpful for understanding the mechanism of SNHL and may reveal complementary findings to those on CT scans. Serous or serofibrinoid labyrinthitis is relatively common in the immediate postoperative period, and may cause transient SNHL, as a result of intralabyrinthine bleeding (2). CT is not contributive to this diagnosis. MR examination may be helpful by illustrating high signal intensity of the labyrinth on non-contrast T1-weighted images, related to a posttraumatic hemorrhagic lesion. Postsurgical labyrinthine bleeding is a local phenomenon involving only the vestibule and one or more semicircular canals. This occurrence underscores the value of a dedicated MR examination in these patients. Hearing loss may occur when bleeding involves the posterior membranous labyrinth, probably because of a transient biochemical modification of the endolymphatic fluid (12). When such complication occurs, the surgeon may have to remove the prosthesis, allowing decompression of the membranous labyrinth. This diagnosis has to be made within the first months, as spontaneous T1 hyperintensity signal disappears rapidly. Calcifications can be found at a late stage of a hemorrhagic process, allowing a retrospective diagnosis of hemorrhage (12). In our experience with serofibrinoid labyrinthitis, based on follow-up findings in only a few patients, no calcifications were evident. Moreover, abnormalities of the labyrinthine fluid are transient,
Fig. 4. 44-year-old woman referred for SNHL and vertigo 6 weeks after stapedectomy with a granuloma located around the prosthesis and in the oval window niche.

A, Axial CT scan of the left temporal bone shows well-located prosthesis (arrow) and partial filling of the posterior part of the oval window niche (arrowhead).

B, Noncontrast axial T1-weighted MR image (500/15) shows normal signal intensity of the labyrinthine fluid, except for slightly high intensity around the oval window (arrow).

C, Contrast-enhanced axial T1-weighted MR image (500/15) shows mild enhancement of the oval window (arrow). Tympanic portion of the facial nerve is seen laterally (arrowhead).

D, Axial T2-weighted MR image shows normal high signal intensity of the labyrinthine fluid. A discrete intravestibular bulging of the prosthesis is clearly depicted (arrow).

TABLE 2: Diagnosis and evolution of sensorineural hearing loss

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Revision Surgery</th>
<th>Interval between MR Imaging and Surgery</th>
<th>Evolution of SNHL</th>
<th>Final Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Removal of the prosthesis</td>
<td>3 mo</td>
<td>Disappearance of vertigo, persistence of SNHL</td>
<td>Granuloma located around the prosthesis and in the oval window niche</td>
</tr>
<tr>
<td>2</td>
<td>Removal of a granuloma</td>
<td>3 mo</td>
<td>No amelioration</td>
<td>Labyrinthic hemorrhage, periprosthetic granuloma</td>
</tr>
<tr>
<td>3</td>
<td>None</td>
<td>21 yr</td>
<td>Persistence of symptoms (SNHL and vertigo)</td>
<td>Reparative intravestibular granuloma, luxation of prosthesis, enlargement of IAC</td>
</tr>
<tr>
<td>4</td>
<td>None</td>
<td>5 yr</td>
<td>Persistence of symptoms (SNHL and vertigo)</td>
<td>Unknown</td>
</tr>
<tr>
<td>5</td>
<td>None</td>
<td>5 yr</td>
<td>Persistence of symptoms (SNHL and vertigo)</td>
<td>Intravestibular bulging of the prosthesis, periprosthetic granuloma, neuroma of the stapedian muscle</td>
</tr>
<tr>
<td>6</td>
<td>Treatment of bacterial labyrinthitis</td>
<td>2 mo</td>
<td>Disappearance of infectious syndrome</td>
<td>Postoperative bacterial labyrinthitis</td>
</tr>
<tr>
<td>7</td>
<td>None</td>
<td>5 yr</td>
<td>Persistence of symptoms (SNHL and vertigo)</td>
<td>Intravestibular bulging of the prosthesis with granuloma of the oval window niche</td>
</tr>
<tr>
<td>8</td>
<td>Removal of a granuloma filling the middle ear and membranous labyrinth</td>
<td>3 mo</td>
<td>Regression of vertigo</td>
<td>Reparative intravestibular granuloma</td>
</tr>
<tr>
<td>9</td>
<td>None</td>
<td>Unknown</td>
<td>Persistence of symptoms (SNHL and tinnitus)</td>
<td>Intravestibular bulging of the prosthesis with periprosthetic granuloma</td>
</tr>
<tr>
<td>10</td>
<td>None</td>
<td>5 mo</td>
<td>Partial then total SNHL</td>
<td>Unknown</td>
</tr>
<tr>
<td>11</td>
<td>Removal of the prosthesis</td>
<td>20 yr</td>
<td>Persistence of partial SNHL</td>
<td>Intravestibular bulging of the prosthesis</td>
</tr>
</tbody>
</table>

Note.—SNHL indicates sensorineural hearing loss; IAC, internal auditory canal.
which is why if diagnosis of a postoperative sero-
fibrinoid labyrinthitis is suspected, MR imaging
should be performed in the weeks immediately af-
ner the surgical procedure (12). The pathologic val-
ue of T1 hyperintensity in the immediate postsur-
gical phase has to be established by a larger cohort
of patients.

Suppurative labyrinthitis is a rare but severe
complication of stapes surgery. It may occur within
days after surgery or even after a long delay (13,
14). The routes of infection from the middle ear to
the labyrinth are probably through the space be-
tween the stapes footplate and the rim of the oval
window, a subluxated footplate facilitating the
spread of a middle ear infection in the membranous
labyrinth. At a late stage of the disease, CT may
show demineralization of the labyrinthine bone and
effacement of the enchondral bone surrounding the
footplate, with pseudoenlargement of the membra-
nous labyrinth (10). Although labyrinthine bone de-
mineralization is infrequent in bacterial labyrinthi-
tis, it is often observed in otosyphilis. In iatrogenic
tympanogenic labyrinthitis, the inflammatory pro-
cess seems to be more aggressive than in other cas-
es of bacterial labyrinthitis, perhaps because the
bacteria have been introduced directly into the ear
during surgery. MR imaging allows an early diag-
nosis, as it can show abnormalities of the membra-
nous labyrinth, manifesting primarily as areas of T2
hypointensity associated with T1 enhancement af-
after contrast administration. MR images may depict
extension of the suppurative process into the inter-
nal auditory meatus along the acousticofacial bun-
dles, which can promote intracranial complications,
such as meningitis, sigmoid sinus thrombosis, and
temporal lobe abscess. Consequently, in patients re-
ferred for meningitis and who have a history of
stapes surgery, MR imaging should be performed to
assess the inner ear as the possible origin of the
infectious process.

Reparative granuloma is one of the most fre-
quent complications of stapes surgery, and be-
comes manifest between the sixth and 15th day af-
ner surgery (12). By 1 month after surgery, no
postoperative soft tissue debris should persist in the
vicinity of the oval window (3). When such ab-
normality is discerned on CT scans, granuloma for-
mation should be suspected (1). Intravestibular
spread of reparative granuloma occurs in 2% of
cases. Surgical removal of the granuloma and the
prosthesis, and replacement with a different graft
and prosthesis, is often successful in preventing
permanent SNHL. MR examination may reveal a
focal decrease of the normal T2 labyrinthine fluid
signal and enhancement after contrast medium ad-
ministration, especially when a lesion of the same
signal fills the oval window niche. Therefore, MR
imaging should be performed when CT shows a
periprosthetic granuloma in a patient with SNHL.

Inner ear malformations may on occasion simu-
late otospongiosis clinically. As no imaging studies
are usually required before stapes surgery, diag-
nosis of such inner ear malformations (endolym-
phatic sac dilatation, semicircular canal dysplasia,
or incomplete segmentation of the cochlea) may
manifest with a CSF gusher in the perioperative
period, or if postoperative SNHL or disabling ver-
tigo occurs. A CSF gusher is defined by fluid out-
flow through the oval window related to an abnor-
amal congenital communication between the
subarachnoid and perilymphatic spaces (12). Iden-
tification of congenital abnormalities of the inner
ear that can give rise to a gusher is easily accom-
plished on CT studies. However, MR imaging may
also be contributive by showing an endolymphatic
sac dilatation or an abnormal segmentation of the
cochlea.

In some cases, MR imaging does not allow pre-
cise determination of the cause of postoperative
complications, although it may contribute sugges-
tive findings. For instance, perilymphatic fistula,
deﬁned by the abnormal presence of endolabyrinth-
ic ﬂuid within the middle ear, occurs as early as the
first week after stapes surgery and as late as 6
years after surgery (12, 15) and persists until an
endosteal membrane forms at the oval window.
Perilymphatic fistula occurs more frequently after
stapedectomy than stapedotomy (15), manifesting
as a persistent perilymphatic gusher. One recent study has shown that the
presence of a pneumolabyrinth can sometimes ex-
plain postoperative vertigo and is a good prognostic
sign, as, most often, a pneumolabyrinth regresses
with the vertigo within a few days (unpublished
data; Veillon F, Richm G, Roedicht N, Leve Lue C,
Tongio J, Merli D; Congress of Radiological
French Society, Paris France, 1999). In some cases
of perilymphatic fistula arising early in the post-
operative period, gusher syndrome has to be con-
sidered in the differential diagnosis. Indeed, a CSF
gusher may simulate otospongiosis clinically and
may manifest as a persistent perilymphatic fistula
after surgery. In the first 2 months after surgery,
perilymphatic fistula and granuloma may have the
same appearance on CT studies. MR imaging can
distinguish perilymphatic fistula from postoperative
debris, as the CSF is hyperintense on T2-weighted
images of the labyrinth.

Intravestibular bulging of the prosthesis may oc-
cur within days after surgery. It is defined on CT
scans by the tip of the prosthesis protruding more
than 2 mm into the vestibule (3). Sometimes, the
tip of the prosthesis may be evident as a hypointen-
sity on gradient-echo MR sequences; however,
a magnetic susceptibility artifact may cause inaccurate localization of the tip and create a false-positive MR finding of intravestibular bulging. Thus, CT remains the standard of reference for diagnosis of this complication. In our study, five patients had intravestibular bulging of the prosthesis on both MR and CT studies.

**Conclusion**

CT is currently the standard of reference for depicting pathologic processes necessitating surgical revision in patients with SNHL after surgery for otospongiosis. CT scans can show a prosthesis bulging into the vestibule, a postoperative granuloma, findings suggestive of perilymphatic fistula, or occult pericochlear abnormalities. When CT findings are normal or fail to explain the clinical signs, MR imaging may be contributory. Indeed, MR examination may reveal an intralabyrinthine hemorrhage, spread of infections or inflammatory labyrinthitis, or intravestibular spread of a reparative granuloma.

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We thank Daniel Rocher for skillful technical assistance in preparing the illustrations, Patrice Tran Ba Huy for supplying one of the cases (patient 6), and Walter Grauer for help in clarifying the manuscript.

**References**