Primary tumors are rare and those localized to a single location offer the potential for cure.

Primary Spine Tumors: Diagnosis and Treatment

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Background: Primary tumors are rare and those localized to a single location offer the potential for cure. To achieve this, early recognition of the primary tumor and proper workup and treatment are essential.

Methods: The authors reviewed the literature and best practices to provide recommendations on primary spine tumor treatment. Appropriate workup of primary spine tumors and treatment algorithms are also discussed.

Results: Patients suspected of a primary spine tumor should undergo fine-needle aspirate biopsy following consultation with the surgical team to ensure the biopsy tract is surgically resectable should the need arise. Once pathology is confirmed, metastatic workup should be performed to guide the level of treatment. If a localized lesion with poor radiation and chemotherapeutic response is diagnosed, then en bloc resection may be required for cure. If en bloc resection is not feasible or metastatic lesions are present, then radiation and medical oncology specialists must work in conjunction with the surgical team to determine the best treatment options.

Conclusions: Patients with suspected primary tumors of the spine should be treated in a multidisciplinary fashion from the outset. With thoughtful management, these lesions offer the opportunity for surgical cure.

Introduction

Primary vertebral tumors are rare, accounting for fewer than 5% of all neoplasms in the spinal column, making them 40 times less common than spinal metastases. These tumors are uncommon and infrequently encountered in practice. Nevertheless, because specific diagnostic and treatment modalities may impact outcome, these lesions must be included in the health care professional's differential diagnosis.

Unlike metastatic spine tumors, primary tumors localized to a single location offer the potential for true cure. However, this possibility may be eliminated by late recognition or improper workup. Because many of these lesions poorly respond to chemotherapeutic
agents and radiation therapy, missteps can have a devastating effect on outcome. Thus, this paper focuses on a systematic approach to the diagnosis and treatment of primary spine tumors.

**Workup**

**Clinical Presentation**

Most patients with primary spine tumors present incidentally or following a workup for nonspecific axial skeletal pain. As these tumors often originate in the vertebral body, symptoms are usually due to periosteal stretching with growth and localized bony destruction. Thus, unremitting pain that worsens at night or in the supine position is common. Mechanical pain due to instability may be reported. Radicular or myelopathic symptoms due to neurological element compression are rare. A new or progressive deformity, especially in younger patients, may be a presenting feature. If a tumor is suspected, or if a patient has persistent symptoms, then imaging studies should be pursued. Important clinical features and implications for treatment that distinguish primary from metastatic spine lesions are noted in Table 1.

**Diagnostic Imaging**

Imaging studies remain the most important diagnostic modality in the face of a primary spinal column lesion. In many cases, due to the nonspecific presenting features, plain radiography is initially performed. Although radiography is an excellent screening tool, it should be noted that a negative radiograph is not definitive. Computed tomography (CT) provides superior information on cortical bone and tumor calcification, while magnetic resonance imaging is excellent at delineating soft tissue, paraspinal lesions, neural encroachment, bone marrow infiltration, and epidural extension.

In some cases, radiographic imaging can provide a definitive diagnosis. However, frequently imaging narrows the differential, supplying valuable information about the involvement and proximity of the tumor to neighboring structures. Although this may not be of definitive use from a diagnostic perspective, imaging suggests in many cases that a surgical approach will be required, and, prior to more invasive testing such as biopsy, it is reasonable to involve a surgeon in the patient’s care at this point.

Depending on the differential diagnosis suggested by the imaging studies, it might be reasonable to begin the staging process, particularly in cases in which distant metastases are likely and may provide an easier biopsy target than with spinal imaging alone. In these cases, a technetium bone scan or positron emission tomography (PET) to look for metabolic activity in remote skeletal sites is a reasonable approach.

**Biopsy**

Lesional biopsy is often the most important step toward diagnosis, as well as a stumbling block of the treatment paradigm. Technical mistakes resulting in tumor spread may preclude complete resection in a potentially curable patient, thus a multidisciplinary approach that combines an experienced interventionist in direct consultation with the surgeon responsible for potential resection is appropriate to avoid errors at this stage. Consideration for biopsy must be given to lesions that do not have a diagnostic appearance and harbor malignant characteristics such as bony destruction. More benign appearing lesions, particularly those in the posterior elements in younger patients, should be watched for signs of activity. Common primary tumor types are outlined in Table 2.

There are 4 main biopsy techniques: fine needle aspirate biopsy (FNAB), core needle biopsy, incisional biopsy, and excisional biopsy. In patients in whom results from imaging studies suggest a differential diagnosis that includes only benign lesions, excisional biopsy may be appropriate for both diagnosis and treatment. However, the likelihood of tumor disruption and local spread is high for both incisional and excisional biopsies, thus FNAB is recommended if the lesion is likely to harbor a malignant histology. Core needle biopsy allows the health care professional to obtain a column of tissue. It is a reasonable consideration if FNAB is nondiagnostic, although a higher likelihood of tumor spillage may exist and tract resection should be considered. To reduce the likelihood of tumor spread, sealing the biopsy site with bone wax or using the coaxial technique is recommended.

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**Table 1. — Distinguishing Features of Primary and Metastatic Spine Tumors**

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<thead>
<tr>
<th>Percentage</th>
<th>Primary Tumor</th>
<th>Metastatic Tumor</th>
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<tbody>
<tr>
<td>Presenting Features: Demographics</td>
<td>Younger</td>
<td>Older</td>
</tr>
<tr>
<td>Location</td>
<td>Vertebral body/posterior elements</td>
<td>Vertebral body</td>
</tr>
<tr>
<td>Time to presentation</td>
<td>Longer duration</td>
<td>Shorter duration</td>
</tr>
<tr>
<td>Treatment: Surgery</td>
<td>En bloc</td>
<td>Piecemeal</td>
</tr>
<tr>
<td>Radiation</td>
<td>Proton beam</td>
<td>Conventional</td>
</tr>
<tr>
<td>Chemotherapy</td>
<td>Unlikely</td>
<td>Common</td>
</tr>
<tr>
<td>Treatment goal</td>
<td>Cure</td>
<td>Palliation</td>
</tr>
<tr>
<td>Planned surgical morbidity</td>
<td>Low</td>
<td>High</td>
</tr>
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</table>
Among the 4 techniques described above, CT-guided FNAB is the most common procedure, yielding a tissue diagnosis in 70% to 80% of procedures.5,6 The procedure also has a low complication rate and a lower likelihood of an extralesional spread of tumor cells.5,7,8 The importance of avoiding open biopsy cannot be overemphasized. In one series of patients with chordoma, 8% of the 25 patients undergoing FNAB followed by en bloc resection had a recurrence, yet 3 of the patients who underwent open biopsy (including 2 with subsequent en bloc resections) developed local tumor recurrence.9

Although the risk of tumor cell spillage is lessened by the FNAB approach, if possible, resection of the biopsy tract is still recommended. Thus, it is beneficial for the interventionalist and spine surgeon to discuss the likely surgical trajectory prior to biopsy. Along with selecting a trajectory easily incorporated into the planned surgical incision, marking the biopsy location is also helpful. Thus, in most cases, early referral to a tertiary center capable and with the appropriate surgical expertise is beneficial prior to biopsy despite diagnostic uncertainty.

Pathological diagnosis must involve a thorough review. If necessary, FNAB can be repeated to ensure adequate tissue for diagnosis. Because the diagnosis impacts treatment planning and prognosis, a second opinion is often encouraged. Primary tumors are very rare, so sending specimens to a recognized expert to confirm the diagnosis can be useful. In cases of typical or atypical hemangiomas, it is not uncommon for the biopsy results to be interpreted as normal bone marrow.

### Metastatic Workup
Pathological diagnosis, in combination with the results of a thorough metastatic workup in malignant disease, dictates the treatment plan. Metastatic lesions at presentation alter the extent and type of therapy; for example, solitary lesions may undergo local treatment, while metastatic lesions necessitate a systemic approach to therapy. Evidence of metastasis also affects...
surgical decision-making. In patients without evidence of metastatic disease, aggressive en bloc resection of malignant lesions poorly responsive to adjuvant therapy may offer the opportunity of cure. Such a possibility is eliminated with evidence of metastatic lesions, changing the surgical plan from aggressive surgery with planned functional loss to a less aggressive debulking with preservation of function or completely forgoing resection.

The type of metastatic workup may be dictated by pathological diagnosis because specific pathologies have specific metastatic predilections. In many cases, PET is an excellent option, although a modification may be needed as it may predominantly provide information on the chest, abdomen, and pelvis. For instance, PET can stage angiosarcoma, which is a highly aggressive lesion, provided that complete limb imaging is included. CT of the chest, abdomen, and pelvis as well as bone scans are also appropriate options. In cases of suspected plasmacytoma or multiple myeloma, a skeletal survey, bone marrow biopsy, and immunoelectrophoresis are useful.

Treatment
Once the diagnosis and the metastatic disease burden are established, attention should be turned to treatment. Patients with primary tumors require a multidisciplinary approach involving medical oncology, radiation oncology, and spine surgery specialties. Coordination of care is paramount to optimize the response for each treatment modality.

Chemotherapy/Radiation Responsivity
Primary tumors fall into 2 basic categories, ie, those responsive to radiation and chemotherapeutic agents and those unresponsive to such treatment modalities. Primary tumors of the spine that respond well to adjuvant therapies include hematopoietic lesions and certain sarcomas.

Hematopoietic Malignancies
Lymphoma, multiple myeloma, and solitary plasmacytoma are the most common malignant neoplasms of the spine.² Radiotherapy is the mainstay of treatment for these lesions, with excellent local control. Although these tumors are considered radiosensitive, recurrences following radiation may rarely occur and are especially seen in long-term survivors. Approximately 50% of patients with solitary plasmacytoma will develop multiple myeloma within 2 years.¹⁰⁻¹² Thus, systemic chemotherapy may be useful in cases with widespread disease or in the setting of plasmacytoma conversion to multiple myeloma.

Surgical intervention can be avoided in many cases. However, in the setting of neurological deficit due to spinal canal compromise or instability causing mobility-limiting pain, surgery may be considered. In these cases, CT scanning is helpful in defining the lesion. Canal compromise caused by bony intrusion, such as that caused by a retropulsed pathological fracture, is unresponsive to radiation and must be mechanically decompressed. In the case of radiation-responsive tumors, the goals of surgery are to decompress the spinal canal and restore the stability and load-bearing capacity of the spine. This is accomplished by performing vertebral augmentation. Complete resection and reconstruction is occasionally completed; however, at a minimum, a reasonable margin between the tumor and spinal cord (“separation surgery”) should be developed to optimize subsequent radiation treatment.

Moderately Responsive Tumors
Another group of tumors are those with an incomplete response to adjuvant therapy in which preoperative chemotherapy or radiation may be employed. Although it is preferable to preoperatively avoid cytotoxic therapies to reduce infection and optimize healing, a subset of patients exists in whom delaying surgical intervention might improve outcome.

The most important of this tumor group is Ewing sarcoma, which is also the most common primary spinal column tumor in children.¹³ The lesion is so responsive to chemotherapeutic and radiation options that surgical treatment is reserved for issues of stability and neurological compromise. Surgical treatment has not been shown to improve local control.¹⁵,¹⁴ Although survival gains have been made, Ewing sarcoma is an aggressive lesion, with a 5-year survival rate of less than 50% in certain subsets of patients.¹⁵,¹⁶

Sarcomas may benefit from preoperative chemotherapeutics. Osteosarcoma and, in particular, angiosarcoma are aggressive lesions that benefit from upfront chemotherapy followed by en bloc resection and postoperative radiation. Despite such aggressive therapy, prognosis remains poor.

Poorly Responsive Tumors
Chordomas and chondrosarcomas are poorly sensitive to chemotherapeutic agents and radiation. The mainstay of their treatment is en bloc surgical resection. Protocols for upfront proton-beam radiation and neoadjuvant therapies are currently being studied,¹⁷ and they may be beneficial in cases where en bloc resection is impossible or technically challenging to reduce intraoperative tumor spillage. The importance of avoiding seeding the surrounding area must be underscored, because survival is frequently affected by local recurrence rather than metastatic disease progression.³,¹⁸,¹⁹

General Surgical Strategies
The preferred surgical approach to a lesion is dictated by tumor pathology, morphology, and metastatic status. The 2 main surgical goals involve resecting the
tumor and reconstructing the load-bearing capacity of the spine. A general approach is outlined in Fig 1.

In general, a trade-off exists between surgical morbidity and the completeness of resection. Surgical options can range from intralesional curettage/debulking to wide en bloc resection. Complex resections require larger operative corridors to appropriately visualize the tumor and neighboring structures in order to achieve negative margins. Limb or nerve root sacrifice with associated permanent morbidity may be planned in these larger procedures to optimize tumor resection.

En bloc resections involve the removal of the tumor in 1 nonviolated piece (Fig 2). En bloc resection conveys a survival advantage, but the procedure is far more technically demanding than removing a lesion piece by piece. In general, these cases are longer and more demanding than similar piecemeal intralesional resections. There may be a planned morbidity because adjacent structures may require sacrifice to remove the tumor in 1 piece. Nerve roots, major vessels, and dura are commonly resected along with the tumor mass to remove the lesion in en bloc fashion. In addition, planned tracheostomy, feeding tube placement, and ileostomy or colostomy may be necessary. The patient should be thoroughly counseled prior to surgery as to the expected perma-

Fig 1. — A general algorithm for the en bloc primary tumor via costotransversectomy is shown. Although each step is important, operative location, approach, pathology, and adjacent structures will dictate the order of the intervention. The basic surgical principles may also be applied to en bloc resections in other locations.

Fig 2. — A 54-year-old man presented with a chordoma incidentally found using fine-needle aspirate biopsy. (A) He underwent high sacrectomy, including thecal sac ligation below the S1 nerve roots with anticipated loss of bowel and bladder continence. (B) The tumor was hemisected following resection and is compared with preoperative T2-weighted magnetic resonance imaging.
nent loss of function. Thus, the decision to continue with en bloc resection must be based on a tradeoff between expected increased survival and planned surgical morbidity rates.

Biomechanical stability and spinal column reconstruction can be challenging. In complex cases, limbs, portions of the chest wall, and the pelvic ring may be resected along with the tumor. In general, the goal of reconstruction is to allow adequate load transfer while protecting the nearby spinal cord, remaining nerve roots, and other vital organs. It is worth noting that the patient may permanently rely on implanted instrumentation to maintain stability, as bony union in the face of massive reconstruction and cytotoxic adjuvant therapy is challenging to achieve. Despite the odds, long-term survivors are expected; therefore, fusion should be attempted. In the presence of radiation and other therapies, anterior load-bearing constructs are more likely to achieve fusion than posterior constructs, and it may be worth revising the surgical plan to encompass this type of reconstruction. In general, en bloc resection of large spinal tumors and their subsequent reconstruction are among the most challenging spinal procedures.

**Oncological Staging**

By incorporating information about pathology, general morphology, and metastatic status of a lesion, generalizations about growth and behavior can be made in order to dictate the surgical approach. The Enneking classification originally designed to stage limb lesions has been ported to primary spine tumors and provides an excellent overview (Table 3).

Benign tumors are divided into 3 categories. S1 tumors are latent, asymptomatic, have a prominent capsule, and are often observed. An example of an S1 tumor is a schwannoma. S2 lesions are active with slow growth, mild symptoms, and a thin capsule or pseudocapsule of reactive tissue. Osteoid osteomas and smaller osteoblastomas fall into this category and can be treated with intralesional curettage unless marginal en bloc resection is achievable. S3 lesions are aggressive, demonstrate rapid growth, and often have a hypervascular pseudocapsule. Aggressive osteoblastomas are the hallmark of this type, and they may be treated with marginal en bloc resection. A “marginal margin” implies that the tumor pseudocapsule has not been violated; however, additional tissue is not included in the surgical specimen. This fact is important because spinal column tumors may reach the thecal sac, and a marginal margin can provide adequate treatment without neurological sacrifice (Fig 3).

All malignant tumors require a wide en bloc resection. Although these lesions can be further categorized by location (whether confined to the vertebral body or within the paraspinal tissues) and whether islands of tumor are within the pseudocapsule or exist beyond the recognized pseudocapsule (low vs high grade), the pseudocapsule itself — unlike a benign tumor — cannot be considered a safe margin. Originally described by Roy-Camille for long-bone tumors, wide en bloc resection has been adapted to the spine. Due to the proximity of spinal cord and other vital structures to the axial spine, this procedure may not be feasible; however, limb amputation is recommended if necessary. Adjuvant therapy is generally recommended, particularly in cases of high-grade malignant lesions.

Patients with metastasis on presentation are candidates for palliative surgery and subsequent adjuvant therapy. The main goal of en bloc resection is to avoid

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<th>Table 3. — Basic Surgical Staging Considerations Based on Modified Enneking Classification</th>
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<tr>
<td><strong>Staging</strong></td>
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<tr>
<td><strong>Benign</strong></td>
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<tr>
<td>S1 (latent): No growth</td>
</tr>
<tr>
<td>S2 (active): Slow growth</td>
</tr>
<tr>
<td>S3 (aggressive): Rapid growth</td>
</tr>
<tr>
<td><strong>Malignant</strong></td>
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<tr>
<td>Low grade (I): IA (confined to vertebra) IB (paravertebral extension)</td>
</tr>
<tr>
<td>High grade (II): IIA (confined to vertebra) IIB (paravertebral extension)</td>
</tr>
<tr>
<td>High grade with metastasis (III)</td>
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Data from references 2, 24, and 26.
local and distant seeding by violating the tumor. Thus, if a patient has metastases on presentation, en bloc resection is irrelevant, and the patient should be directed from a high morbidity procedure and instead toward adjuvant therapies, palliative debulking, and spinal stabilization.

Consideration for en bloc resection can also be provided to patients without evidence of metastases but in whom the tumor capsule was violated (eg, cases of previous resection or open biopsy) or in the presence of local recurrence. Although not ideal, it may be possible that local seeding has occurred and en bloc resection will lessen the likelihood of distant metastases. Due to local seeding, adjuvant therapy is usually recommended.

**Surgical Staging**

Once the preferred method of resection has been determined, patients must be surgically staged to determine the technical feasibility of the procedure. Invasiveness into nearby unresectable structures is the primary reason some tumors, particularly in the case of sarcomas, are unresectable in en bloc fashion and may rely on debulking with adjuvant therapy. In all other cases, other systems have been proposed, but the determination must be whether a surgical corridor exists in order to deliver the tumor in 1 piece without disrupting vital structures. Oftentimes, the limiting factor for determining the tumor trajectory is the spinal cord, which is encircled by a bony wall composed of vertebral body, pedicles, and lamina. To remove a tumor specimen en bloc, the ring must be broken wide enough to pass around the spinal cord. Thus, if the tumor completely encircles the spinal cord, a marginal en bloc resection is not possible without violating the tumor. The break in the ring also determines the surgical corridor for tumor removal; it must be removed opposite the break in the ring. Thus, immobile vital structures beyond the spinal column may preclude removal. Nearby structures, such as the great vessels and heart, may limit the resectability of a tumor or increase the difficulty of the procedure.

Generally, location on the spinal axis predicts the technical difficulty associated with en bloc resection.

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**Fig 3.** — (A) A 34-year-old woman developed sudden back and radicular leg pain. A giant cell tumor with a pathological fracture was diagnosed via fine-needle aspirate biopsy. (B-D) Because these tumors have a high propensity for recurrence following intralesional resection, posterior and subsequent anterior en bloc resections and reconstruction were undertaken. The tumor between the great vessels was removed.
and reconstruction; surgery is more challenging from the sacrum to clivus. Distal sacrectomies may be accomplished using a posterior-only approach. Mid to upper sacrectomies may involve the posterior-only or the anterior and posterior approach to aid dissection and utilize rectus vascularized flaps to aid in closure. Total sacrectomies, in which S1 is removed, require instrumented reconstruction. In the lumbar spine, the great vessels, renal arteries, ureters, and digestive structures must be considered, as well as nerves involved in lower extremity function. In the thoracic spine, mediastinal structures preclude certain surgical trajectories, and chest wall reconstruction may be indicated. The subaxial cervical spine may be challenging because upper extremity and diaphragmatic innervation, the vertebral arteries, trachea, and esophagus are in close proximity. However, high cervical spine and clival lesions are challenging because transoral and transmandibular approaches may be required. In these cases, cranial nerves and vascular structures make resection difficult. Fig 4 outlines the overall surgical strategy.

**Strategies for Capsular Violation**

Patients commonly present following partial resection or open biopsy, which is often a diagnostic procedure (Fig 5). Although capsular violation precludes true en bloc resection as the margins are already contaminated, using the en bloc techniques to eliminate tumor spillage is the preferred approach in these situations. If it is possible to widen the margin or include a portion of the surgical tract in the specimen, then there may be a reduced likelihood of local and distant recurrence; however, no data exist on this patient population, so the approach is inferred but commonly agreed upon.

Another frequent scenario involves unintended capsular violation during the initial en bloc resection in a previously unviolated tumor. If possible,
oversewing the tear in the tumor capsule can preserve the structural integrity of the tumor, because further manipulation is often necessary during removal. Regardless of whether or not the tear is reparable, the area can be coated in a fibrin sealant to prevent spillage. Oftentimes the soft internal structure of the tumor will extrude through the tear. In such a case, thoroughly removing the extruded component and inspecting the nearby area are both imperative.

In some cases, tumor morphology may require modified en bloc resection in which tumor violation is planned to protect nearby neural and vascular structures (“planned transgression”). For instance, if the tumor wraps around the spinal cord and no window wide enough exists for the spinal cord to slip through when the mass is removed, either the spinal cord or the mass must be incised. Although such a scenario is neurologically devastating, planned paraplegia with en bloc resection and spinal cord sacrifice in the setting of aggressive sarcoma is arguably an option; commonly, however, it is the tumor that is incised. In these cases, the same techniques apply. Oftentimes it is possible to achieve a useful exposure window by removing the posterior elements, violating the tumor as it extends through the pedicles. In such a scenario, carefully protecting surrounding structures and promptly coating the remaining pedicle with bone wax is the appropriate option. Because a patient in this case has contaminated tumor margins, any instruments in contact with the tumor must be considered contaminated and, thus, be permanently removed from the field.

Clearly communicating tumor violation to radiation and medical oncologists is important. Depending on tumor pathology, close imaging follow-up may be the standard therapy following en bloc resection; however, upfront adjuvant therapy may be desirable in cases of contaminated margins.

Postoperative Adjuvant Therapy
Following optimal surgical debulking, patients should be considered for adjuvant chemotherapy and radiation. If a patient has undergone successful en bloc resection (with marginal margins in a benign lesion or clean wide margins in a low-grade malignant lesion), then the patient should be observed for signs of distant metastases or local recurrence. Adjuvant therapy should be timed to allow adequate wound healing and to decrease the risk of infection.

Local control is achieved with radiation. Primary tumors are often considered for proton beam radiotherapy (PBR) alone or in combination with intensity-modulated radiotherapy (IMRT). The advantage of PBR is the steep Bragg peak, allowing high-dose radiation delivery near critical structures. Although IMRT is conformal, it does not have the same steep drop in dose, thus it effectively delivers a through-and-through dose of radiation. However, PBR is geographically limited and expensive, so combination PBR/IMRT has been employed to achieve a similar effect.28

Chemotherapeutics also play an important role, particularly in sarcomas and hematologic malignancies. In other cases, such as giant cell tumors, experimental chemotherapeutics have been attempted with reasonable effect in patients with inoperable tumors or recurrences.

Ongoing Monitoring
Depending on pathology, patients should be followed at variable intervals. Although many benign tumors have a very low incidence of recurrence, certain lesions such as giant cell tumors have an
80% local recurrence rate with intralesional resection. Malignant lesions require ongoing periodic monitoring for local recurrence and periodic restaging for metastatic lesions. Due to the multispecialty team involved in patient care, the coordination of monitoring is valuable.

**Recurrence/Late Metastasis**

If a patient has local recurrence or distant metastasis, then further treatment and possible restaging are both warranted. In the case of distant metastasis, the mainstay of treatment is adjuvant radiation and chemotherapy, although accessible solitary lesions may be amenable to resection. Local recurrence presents a different challenge. Provided no distant lesions are present during the metastatic workup, pursuing treatment as though it was a disrupted primary lesion might be appropriate. Although such a patient is likely to have a higher risk of repeat local recurrence than a patient undergoing first-time en bloc resection, initiating treatment may prevent late distant metastasis. However, it is worth noting that, in certain tumor types, survival may be related to local recurrence more than distant metastases. Due to the rarity of these lesions, little data exist on the efficacy of en bloc resection for recurrence.

**Conclusions**

Although primary spine tumors are rare, they must remain high on the differential diagnosis, because early diagnostic and treatment decisions may have far-reaching implications for additional treatment options and survival rates. However, appropriate diagnostic tests, thoughtful biopsy techniques, and challenging surgery may provide a cure.

**References**