Osteosarcoma: Diagnostic dilemmas in histopathology and prognostic factors

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Abstract

Osteosarcoma (OS), the commonest malignancy of osteoarticular origin, is a very aggressive neoplasm. Divergent histologic differentiation is common in OS; hence triple diagnostic approach is essential in all cases. 20% cases are atypical owing to lack of concurrence among clinicoradiologic and pathologic features necessitating resampling. Recognition of specific anatomic and histologic variants is essential in view of better outcome. Traditional prognostic factors of OS do stratify patients for short term outcome, but often fail to predict their long term outcome. Considering the negligible improvement in the patient outcome during the last 20 years, search for novel prognostic factors is in progress like ezrin vascular endothelial growth factor, chemokine receptors, dysregulation of various micro ribonucleic acid are potentially promising. Their utility needs to be validated by long term followup studies before they are incorporated in routine clinical practice.

Key words: Osteosarcoma, prognostic factors, vascular endothelial growth factor, ezrin

Introduction

Osteosarcoma (OS) is the commonest osteoarticular malignancy of nonhematopoetic origin. Conventional OS is a high grade intramedullary neoplasm, often arising in the metaphysis of long tubular bones.[1,2] The burden of the disease at large is immense considering the young age of patients, residual disability consequent to radical surgeries and long periods of rehabilitation.[3] OS is a great histologic mimicker and poses the diagnostic challenge especially in small tissue biopsies. Triple diagnostic approach, i.e., clinical, radiological and histopathological is essential in all cases. Despite initial significant improvement in patient outcome mainly owing to preoperative systemic chemotherapy (CT) and advancements in surgical techniques, up-to 40% patients still die of the tumor.[4,5] Patient outcome depends upon a number of variables. The search for novel prognostic markers continues in order to improve prediction of patient outcome and identify potential molecular therapeutic targets.

This review describes the approach toward diagnosis of OS including the dilemmas faced. It also stresses the need for triple diagnosis, especially in atypical cases. The prognostic factors, both traditional and novel, are also listed. PubMed search key words were identified in MeSH database. The key words used were Osteosarcoma, prognosis, diagnosis, differential, immunohistochemistry, biomarker. Articles were shortlisted by systematic search; preference was given to original articles, large series systematic reviews and meta-analyses. The search strategy used various combinations of MeSH terms mentioned above and prognosis with limits of species 'human'. The search was refined by applying limits such as article type in combination or serially. Approximately 100 article abstracts were short-listed after reading. For the purpose of citation, preference was given to full text articles, but not restricted to them alone. Case reports were included when misdiagnosis of certain histologic type was reported to emphasize the point.

Of the 82 references cited in manuscript, full text articles were accessible in 65; 17 abstracts were used.

Diagnostic Dilemmas of Osteosarcoma

Osteosarcoma is a mesenchymal tumor having evidence of osteoid or bone production by malignant stromal cells in some portion of the tumor. Bizarre tumor cell morphology

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Access this article online	
Quick Response Code:	Website: www.ijoonline.com
	DOI: 10.4103/0019-5413.132497

and divergent differentiation are common.[1,2,6] The histologic diagnosis of OS rests solely upon demonstration of unequivocal osteoid being laid down by malignant cells. Few specific variants of OS are peculiar in their anatomic location; others may lack obvious atypia and have subtle histologic features. Being a vivid mimicker OS may pose diagnostic challenge especially when coupled with atypical radiologic presentation, which is described in 20% of cases.[7] Peculiar sub-types of OS include surface OS, intracortical, low grade intramedullary, multicentric, secondary (e.g. postirradiation and those arising in Paget's disease). OS arising in the jaw and spine are also considered separately.[1,2]

Demonstration of osteoid is essential to diagnosis of OS; however, the amount varies widely between tumors. The diagnosis is often straight forward in the presence of abundant osteoid; although in fibroblastic areas, it may be confused with collagen. Minimal/equivocal osteoid requires additional sections, at times resampling and hence increases laboratory turnaround time. Markers of osteoblastic differentiation such as osteocalcin, osteonectin, SATB2 have been proposed to be potentially useful in such cases. Recently, Conner and Hornick have reported their results of SATB2, an osteoblastic differentiation marker, expression in osseous and soft tissue tumors.[8] They found it to be a marker of osteoblastic differentiation in both benign and malignant tumors. They recommended its use as an adjunct in settings to make the distinction between hyalinized collagen and osteoid.

Osteosarcoma is capable of divergent differentiation. It often shows chondroid and fibrous areas besides the pathognomic osteoid deposition by malignant tumor cells.[1,2,6] A tumor is designated as specific subtype only when the given differentiation exceeds 50% of tumor area.[1,2] Extensive chondroid differentiation might be confused with chondrosarcoma (CS). CS does not show osteoid deposition. They typically arise from flat bones of the trunk and proximal appendicular skeleton and present almost two decades later than OS. OS arising in the axial skeleton is uncommon and likely to be secondary.[1,2] In a retrospective comparative analysis of 10 cases, each of histologically confirmed CS and chondroblastic OS, Yen *et al*. compared number of features on radiology and magnetic resonance imaging (MRI). They found chondroblastic OS to have metaphyseal tumor location ($P = 0.039$) and aggressive periosteal reaction ($P = 0.008$) and presentation at significantly younger age (mean age: 24.7 years vs. 56.7 years, $P < 0.001$). Some of their chondroblastic OS did have radiologic and MRI findings typical of CS and posed diagnostic challenge.[9] Cases are also on record wherein initial diagnosis of CS rendered on cytology or biopsy have been revised to OS on resection specimens.[10] Unni suggests that in an adolescent patient, chondroid tumors should be considered as chondroblastic OS unless proven otherwise.[11] As management of CS and OS are very different, there is a need of markers to distinguish them on limited tissue specimens. Immunohistochemistry (IHC) for galectin-1 has been reported to be discriminatory. Gomez-Brouchet *et al*. performed galectin-1 staining on 165 bone tumors and demonstrated significantly higher intensity and proportion of positivity than in OS including chondroblastic OS in comparison to CS.[12] High grade fibrous areas of OS may be mistaken for fibrosarcoma, malignant fibrous histiocytoma and sometimes fibro-osseous lesions. The diagnosis of OS hinges on demonstration of osteoid, which may require diligent search.[2] According to Dahlin differentiation between fibroblastic OS, fibrosarcoma and malignant fibrous histicytoma is arbitrary.[13] Low grade fibroblastic areas of OS may look deceptively akin to fibrous dysplasia. Periosteal reaction and destructive lesional margins are alerting radiologic features in such situations.[1]

A typical OS may be accompanied by areas rich in giant cells (giant cell rich OS), large blood filled spaces (telangiectatic OS) or small cells with minimal osteoid production (small cell OS).[1,2,6] These tumors are likely to be misdiagnosed as giant cell tumor (GCT) of bone, aneurysmal bone cyst and Ewing's sarcoma (EWS) respectively. Numerous giant cells obscuring malignant OS cells are a rare event. Such cases may be mistaken as GCT of bone. Demonstration of atypical tumor cells is the key to correct diagnosis.[14] Careful viewing of X-ray for location, periosteal reaction and physis plate is beneficial. OS is metaphyseal centered tumor of immature skeleton often with raised periosteum. GCT arises in the epiphysis of mature skeleton; periosteal reaction occurs in the setting of complication (biopsy/fracture).[2,6] Telangiectatic OS has overall low cellularity and sparse osteoid production. Interrupted periosteal reaction and detection of bone matrix are helpful in establishing the correct diagnosis of telangiectatic OS.[2,6,15] Minimal osteoid in small cell OS makes its radiology atypical. Distinction from EWS, mesenchymal CS and lymphoma is essential. OS do not respond much to radiotherapy which is the standard treatment modality in EWS.[6] Diagnosis of OS would require demonstration of osteoid, however minimal. Positive immunostaining for galectin-1 is expected in small cell OS.[16] EWS would be decorated by CD99 and harbor translocation of EWS gene on chromosome 22. Immunostaining for leukocyte common antigen and S-100 would exclude lymphoma and mesenchymal CS respectively.[2,6,17]

Low grade central OS is a rare type of locally aggressive OS with limited potential for metastases.[2,18] It shows fibrous areas with minimal atypia, small bony trabeculae with or without chondroid areas. Radiologically, it may resemble fibrous dysplasia by virtue of deceptive circumscription and lack typical features of OS. Low grade central OS

with predominant bony element and minimal fibrous element may be mistaken either for osteoblastoma or peri-lesional bony trabeculae. Careful search for minimal atypia, abnormal mitoses, attention to lesional radiologic borders and periosteal reaction are helpful in preventing mis-diagnosis.[2,6] The distinction has clinical relevance. Unless the tumor is de-differentiated, its outcome is distinctly better; 5 years survival being more than 80%.[6,19] Unlike conventional high grade OS which have complex cytogenetic aberrations, low grade OS (both central and parosteal) specifically harbor amplification of chromosome 12q13-15 region including murine double minute type 2 (MDM2) and cyclin-dependent kinase 4 (CDK4) leading to respective protein over-expression. Staining for MDM2 and CDK4 is hence useful in difficult cases. Positive results are seen in low grade central OS, fibrous dysplasia is typically negative.[19]

Osteosarcoma with epicenter outside bone cortex are termed surface OS.[2,6] Different types of surface OS are parosteal OS, periosteal OS and high grade surface OS. This definition precludes medullary involvement, which is typically primary tumor site in conventional OS. These tumors arise a decade later and barring high grade surface OS have a better prognosis than conventional OS. Parosteal OS, the commonest surface OS are sclerotic lesions attached to the underlying bone. They are histologically similar to low grade central OS and share the same genetic alteration, i.e., amplification of MDM2 and CDK4 genes.[20] Sometimes they may be mistaken to be osteochondroma; lack of marrow fat, subtle anaplasia and radiologic correlation allow correct diagnosis.[21] Parosteal OS do not require CT and have distinctly better 5 year survival (>90%) with surgery alone. De-differentiation worsens prognosis and is known to occur in almost 25% cases.[6] Periosteal OS are located more toward diaphysis with fusiform periosteal involvement unlike parosteal OS which outgrow their attachment in metaphyseal region.[6,22] Periosteal OS shows grade 2 cartilaginous matrix with small areas of osteoid deposition. It is less aggressive than conventional OS, but worse in comparison to parosteal OS. The largest series until date has reported 84% survival at 10 years.[22] Wide excision is sufficient, preoperative CT does not improve survival. High grade surface OS may arise de novo or else be de-differentiated parosteal OS. Their outcome is no better than conventional high grade OS arising from medulla.[2,6]

Practical Considerations

Tumor samples for tissue diagnosis of OS may be obtained by any one of the following ways - fine-needle aspiration (FNA), needle biopsy or open surgical biopsy. FNA is minimally invasive and cost-effective; however it is also likely to be least sensitive. Dodd *et al*. reviewed FNA results of 40 patients of OS accumulated over 8 years in their institution. They found the technique to have high accuracy (95%) with moderate sensitivity (65%). Inconclusive diagnoses were more likely in cases of OS variants.[23] Koscick *et al*. compared diagnostic performance of FNA and needle biopsy in a series of 144 cases of which 17 were primary bone tumors (excluding EWS). The diagnostic concurrence rate for osseous tumors was 59% only when compared to 73% for the entire series.[24] Other authors have reported diagnostic accuracy of needle biopsies with failure rates as low as 9.3%.[25] Obtaining needle biopsies under radiologic guidance has advantage of avoiding cystic and necrotic areas.[26] The diagnostic yield of open biopsies may be as high as 98%.[27] Hence, an open biopsy should be performed in case of conflict between radiologic and preliminary pathologic diagnoses.

Prognostic Factors of Conventional High Grade Osteosarcoma

The outcome of OS patients excluding the specific histologic/anatomic types mentioned above depends upon a number of variables. These prognostic factors may be classified as traditional and novel. The need for novel prognostic factors stems from a better understanding of molecular pathways of carcinogenesis in recent times and potential therapeutic targeting.

Traditional Prognostic Factors

Age at presentation

Young adults between 25 and 30 years of age fare best.[7] Patients older than 40 years have significantly poorer survival statistics than young adults even when secondary OS are excluded.[28-31] In a multiinstitutional study of 86 patients of OS older than 40 years of age, Iwata *et al*. found that these patients were more likely to have truncal location and metastatic disease at initial presentation, factors associated with worse outcome.[28] Elderly patients, >60 years fare worst.[32,33] Shaylor *et al*. followed 26 patients of OS secondary to Paget's diseases of bone. They noted rapid decline in survival from 53% at the end of 1st year to 25% by 2 years' time; significantly no patient survived until 5 years.[33] The negative influence of increasing age on final outcome has been attributed to reduced ability to tolerate CT and often refusal for radical surgery.[29] Survival statistics in children were earlier inferior to young adults with increased risk of recurrence.[4,7,34] This is no longer the case.

Gender

The prognostic impact of gender on final outcome is not fully established. Male gender has been reported to be associated with poor response to CT, more likelihood of local recurrence and death (up to 4 times) and higher case fatality ratio.[30,35-38] Others have failed to document favorable prognostic association of female gender.[4,7,34,39]

Serum alkaline phosphatase (AP) enzyme level

Serum AP values should be interpreted in context of age; higher values are physiologic in children and adolescents. Bacci *et al*. found elevated serum levels to correlate with significantly shorter event free survival (EFS).[4] It is noteworthy, that serum AP level may not be elevated at presentation in up to 50% patients. This is likely to happen in anaplastic tumors with little osteoid deposit.[2] Rise in serum AP value in postoperative period may precede clinical local recurrence and/or metastasis by months.[1,4,35]

Radiologic findings

Up to 20% of OS present with atypical radiologic features. Although, it may contribute to delay in diagnosis, it has not been found to have any influence on final patient outcome.[7,39] Fracture secondary to typical OS is an uncommon event, described in <5% cases.[6,7] Telangiectatic OS may have fracture in up to 25% cases.[2] By increasing unexpected chance of micro-metastasis, fracture is theoretically an adverse event. However, it has not been found to be an indicator of either recurrence or overall survival.[4,40,41]

Tumor site

Tumors arising in truncal location fare worse in comparison to those located in limbs.[7,30,31,37,40] Bielack *et al*. have reported significant difference in 10 years actuarial survival between groups (axial 29.2% vs. limb 61.7%, $P < 0.0001$).[30] OS of femur fare significantly worse when compared to those located distally in tibia.[7,30,42] Spinal location accounts for <5% of all OS. Sacrum followed by lumbar and thoracic segments is commonest sites of affliction.[43] Almost 2/3rd patients of spinal tumors die within 2 years of diagnosis.[44,45]

Gnathic OS constitute <10% OS.[1,2] These tumors present later in life, 4-5th decade.[46,47] Maxilla is more likely to be involved than the mandible. They are usually considered a separate category in view of their low histologic grade, less frequent metastases and better prognosis. Female gender and chondroblastic predominance may be associated with worse prognosis.[46] Nongnathic craniofacial OS often arise in the background of head and neck irradiation or Paget's disease in elderly individuals.[6]

Tumor burden

Bulky tumors have worse outcomes than smaller ones.[4,30,36,37,48,49] Primary tumor burden may be expressed either as tumor size or volume as determined on preoperative radiologic investigation.[50,51] Petrilli *et al*. found the risk of death to be 3.4 times higher when tumor diameter exceeds 15 cm.[36] Gobel's criteria are followed widely to calculate tumor volume.[51] However, collapse and/or displacement in the setting of pathologic fracture may lead to inaccurate tumor measurements. Tumors with volume exceeding 150-200 mL have significantly less likelihood of limb salvage, poor response to CT and increased risk of recurrence.[4,30,37] Up to 20% OS patients have evidence of metastases at their initial presentation. The risk of mortality is significantly more in those with metastases at initial presentation.[30,31,38,41,52,53] They are also likely to have shorter EFS than their counterparts with limited disease.[28,41] Some authors do not consider stage at presentation as a prognostic factor, rather a consequence of other poor prognostic factors.[7]

Histology

The impact of histology on patient outcome is modest.[48] Fibroblastic differentiation has been considered better prognostic histology. It has been reported to be a significant predictor of CT related necrosis and associated with lower risk of death (bordering statistical significance) than other histologic sub-types.[4,7] Worse outcome in chondroid predominant tumors has been noted by some authors.[29,38] Telangiectatic OS no longer denotes inferior prognosis. It is especially sensitive to CT given its high cell turn over and increased vascularity.[15]

Response to preoperative chemotherapy

Systemic CT is warranted in almost all cases of OS. It is an important therapeutic strategy to reduce tumor bulk, make surgical procedure easier, better the chance of limb salvage, improve opportunity to obtain tumor free margin, increase the number eligible patients for conservative treatment and targeting of micrometastases.[4,5,30,37,42,48] It is a consistently strong prognostic factor besides stage at presentation. The response may be assessed either radiologically immediately preceding surgery and/or by histologic examination of the resected specimen. Radiologic methods have the advantage of being noninvasive, guiding the type of surgery, modification and/or addition of postoperative CT. However hematomas, fibrosis could be mistaken for residual tumor itself.[42]

Histologic assessment is the most accurate method to determine response to preoperative CT. Tumor mapping is performed to assess percentage area occupied by necrosis and viable malignant cells. Huvos criteria (>90% necrosis-good response) is most commonly followed.[54-57] Poor response is a strong predictor of local recurrence, metastases and overall survival.[28,30,31,37,41,50,55] The preferential sites where viable malignant cells are most likely to persist include soft tissues, cortex, sub-cortex, ligaments and areas

in contact with cartilage.[57] Response to preoperative CT correlates significantly with tumor volume, probably with histologic type but not with anatomic location.[4,6]

Combinational prognostic indices

In view of rather limited predictive power of individual prognostic factors, many authors have attempted to improve prognostication by constructing combinational prognostic model. Using combination of age, anatomic location, symptom duration and histology, Bentzen *et al*. were able to stratify their patients (n = 184) into three different survival groups.[7] The group with best outcome (estimated 10 years survival 79%) had all favorable factors (age 25-35 years, distal extremity, symptoms >6 months and fibroblastic histology). Poor outcome (estimated 10 years survival 8%) was predicted when all adverse factors (elderly age, trunk/proximal appendicular location, short symptom interval and nonfibroblastic histology) occurred together. Typical cases (having admixed prognostic factors) had intermediate outcome (estimated 10 years survival 29%). Petrilli *et al*. stratified Brazilian patients of OS into different death risk categories based upon multivariate analysis of various prognostic factors. The relative risk of death of a male patient with >15 cm OS was more than 2.0 times when compared to a female patient with smaller tumour.[36]

Outcome after development of relapse

Introduction of preoperative CT in OS has resulted in strikingly fewer metastases and longer EFS. Despite preoperative CT, more than one third of conventional OS eventually relapse. Lungs, often with bilateral involvement are the commonest site of metastases followed by bones. The reported median time of relapse and number of metastatic lesions are 13-15 months and three respectively.[58,59] The final outcome after development of metastases depends upon number of lesions, time interval, success of metastatectomy surgery and adequacy of salvage CT. The chances of achieving complete second remission are higher with solitary metastatic lesion, disease free interval more than 24 months and accomplishment of complete metastatectomy.[58,59] Patients with local recurrence with/without metastasis tend to fare worse than those with metastasis alone.[4,50]

Incorporation of preoperative CT in management of OS (in 1980s) was a landmark event. It dramatically improved 5 years survival figures from 15% to 20% to above 60%. However, further advances in treatment of OS have been modest at best.[5,49] Pulmonary metastases remain the most common cause of death. Better understanding of tumor biology and metastasis is essential to improve both EFS and overall survival.[3,49,60,61] Experimental models allow valuable insight into carcinogenesis and identify novel prognostic and therapeutic targets.

Emerging prognostic factors (novel)

Ezrin

Ezrin is a cytoskeleton linker protein with diverse functions. It is involved in cell to cell interaction, cell to matrix adhesion and signal transduction.[62] Dysregulation of ezrin has been implicated in metastasis of various cancers including OS. Salas *et al*. compared ezrin expression by IHC in primary tumors (37) to metastases (13). They found the protein expression to be higher in metastases than the corresponding primary tumours.[63] Two meta-analyses on prognostic significance of ezrin expression in OS were published recently.[62,64] Li *et al*. and Wang *et al*. found only 5 and 7 original studies to be eligible for evaluation from 54 to 23 published works respectively. Majority of analyzed studies had used 1% cut-off criteria on IHC. Both meta-analyses concluded ezrin expression in OS tumor cells to be an independent factor predictive of death at 2 years, but not of EFS. Its association with AP levels and histologic response to CT were found to be insignificant. In an animal model, Pignochino *et al*. studied effect of Sorafenib, a multikinase inhibitor drug on OS cell proliferation and potential for lung metastases. They found the treatment to dramatically reduce tumor volume and its ability for lung metastases.[65]

P53

P53 is a protein product of a tumor suppressor gene. Patients carrying germline mutations of p53 are at significantly higher risk of developing OS than general population.[66] Fu *et al*. analyzed results of 609 sporadic cases of OS pooled from 15 studies in relation to p53 protein expression. They found significantly reduced overall survival (odds ratio = 0.29, $P < 0.001$) and disease free survival (odds ratio = 0.06, $P < 0.001$) of patients with up-regulated p53 expression in comparison to those with low or undetectable expression. They concluded that p53 was an effective biomarker of survival in patients of OS and suggested more studies with large sample size to identify the effect of p53 expression in OS.[67]

Vascular endothelial growth factor

Vascular endothelial growth factor is a key regulator of angiogenesis, an event essential to all cancers and metastases. Kaya *et al*. investigated the impact of VEGF expression as determined by IHC on patient outcome. Of the 27 cases, 15 developed pulmonary metastases. The distribution of cases was 14/17 and 1/10 of VEGF positive and negative cases respectively.[68] Recent meta-analyses on prognostic significance of VEGF expression in OS have also shown VEGF expression to have adverse prognostic impact on both disease free and overall survival at 5 years.[69,70] Clinical trials exploring endostatin as a candidate for anti angiogenic therapy have yielded encouraging results. Xu *et al*. compared outcome of 54 OS patients receiving

intravenous endostatain in combination with four cycles of CT to 62 cases who received CT alone. The treated group had increased EFS and decreased occurrence of metastases; however overall survival was not affected. They concluded that anti angiogenic therapy using endostatin had potential to prevent progression of metastases.[71]

Chemokines and chemokine receptors

Chemokines are small peptides, initially identified as molecules mediating communication between various types of leukocytes through G-protein coupled receptors. Chemokine receptor, CXCR4 has been implicated in determining metastatic destination of tumor cells.[72] Laverdiere *et al*. estimated messenger ribonucleic acid (RNA) expression levels of CXCR4 and CCR7 in 47 OS patients and sake its correlation with patient outcome. CXCR4 and CCR7 were expressed in 63% and 43% cases respectively. Levels of both showed correlated inversely with overall survival ($P < 0.0001$ and $P < 0.03$ respectively) and metastasis free survival ($P = 0.002$ and $P = 0.007$ respectively). In addition, CXCR4 expression correlated with advanced stage at presentation and poor EFS ($P < 0.001$).[73] Kim *et al*. tested CTCE-9908, a small CXCR4 antagonist in two murine OS metastases models. The test group had 50% reduction in number of gross metastatic nodules and a marked reduction in micro-metastatic disease compared to controls.[74]

Micro ribonucleic acid (miRNA)

These are small fragments of noncoding RNA, which act at posttranscriptional level and hence regulate numerous important cell functions.[75] Depending upon their function, their effects may be classified as either oncogenic or tumor suppressive (increased or decreased respectively in malignancy).[76] Up-to 22 miRNAs have been found to be up-regulated in OS cell lines and human tissues and almost equal number have been found to down-regulated. Some miRNAs have also been implicated in drug resistance. The testimony to their involvement in OS lies in development of several experimental therapeutic strategies aimed to block oncogenic miRNA or use mimics of tumor suppressive miRNA.[77]

Other emerging novel prognostic markers in osteosarcoma

Genome wide expression studies and proteomics have identified differential expression of several genes and proteins in OS. Up-regulated proteins include growth factors, their receptors, enzymes and others involved in various cellular functions.[3,60,61] Few examples of such identified markers are up-regulation of enzyme matrix metalloproteinase 2, cycloxygenase 2 enzyme expression, insulin like growth factor receptor type 1 and RANK-L expression, all associated with inferior outcome.[3,60,61,78-80] Research is also ongoing to determine markers of poor response to CT, an indirect measure of outcome. Polymorphism of endothelin-1 and peroxiredoxin2 expression has been found to be associated with increased risk of chemo-resistance to OS.[81,82]

To conclude, OS is a great mimicker capable of posing diagnostic challenge to all, i.e., surgeons, radiologists and pathologists alike. Any case which fails to show concurrence between triple diagnostic approaches should be viewed with suspicion. Prognosis of conventional high grade OS remains grim, especially in those who present with metastases. Molecular techniques permit detailed analysis of cellular pathways at play in OS carcinogenesis and offer scope of identifying novel prognostic markers and therapeutic targets. Of the many new markers currently under investigation, few have shown promising results and likely to be clinically useful in future.

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How to cite this article: Wadhwa N. Osteosarcoma: Diagnostic dilemmas in histopathology and prognostic factors. Indian J Orthop 2014;48:247-54.

Source of Support: Nil, **Conflict of Interest:** None.