

Tumor necrosis and prognosis in osteosarcoma

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Osteosarcoma of the extremities affects mainly children and young adults. Histologically, the tumors are divided into low grade osteosarcomas (grade I and II) treated by surgery only and high grade osteosarcomas (grade III and IV) treated by preoperative chemotherapy followed by chemotherapy. High grade osteosarcomas have a variable histological appearance: classical osteoblastic, chondroblastic, fibroblastic, telangiectatic and small cell osteosarcoma. Either one type or all can be present in the same tumor. The primary osteosarcoma diagnosis is based on fine needle aspiration (White et al. 1988, see also pages 48–50) and/or open biopsy. The morphologic diagnosis is preferably supported by additional examinations like cyto- or histochemistry with alkaline phosphatase. DNA-ploidy analysis, electron microscopy and/or chromosomal analysis (White et al. 1988, Åkerman et al. 1996, Walaas and Kindblom 1990).

The use of chemotherapy with high dose methotrexate in high grade osteosarcomas has improved the survival during the past decades. Additional combination of methotrexate with adriamycin and cyclophosphamide has further increased the disease-free survival. This preoperative chemotherapy has led the increased use of limb saving surgery instead of amputation (Bacci et al. 1993).

Several techniques have been previously applied to estimate the preoperative chemotherapy response. Tumor necrosis have been estimated with four or three degrees (Rosen et al. 1976, Rosen et al. 1979, Picci et al. 1985). More recently it has been suggested that patients be divided into good responders ($\geq 90\%$ necrosis) and poor responders ($< 90\%$ necrosis) (Bacci et al. 1993). Patients with a good response to preoperative chemotherapy show a higher disease-free survival rate (Bacci et al. 1993, Rosen et al. 1976, Rosen et al. 1979, Picci et al. 1985).

Examination of the surgical specimen

Gross dissection

The best results in the gross examinations are achieved with a clinical-radiological and histological correlation. The gross dissection is done together by the pathologist and orthopedic surgeon after a review

of the preoperative radiologic examinations. The tumor is dissected and macroscopic resection margins are evaluated. Macroscopic tumor growth within the bone, cortical destruction and/or tumor borders and soft tissue infiltration are reported. The uninvolved skin and soft tissue are removed. The bone containing the tumor is divided sagittally into two parts for macrophotography (Figure 1). In some cases the bone pieces can be formalin fixed until the following day and than fixed for two hours in 90 % alcohol. This fixation restores the normal colours for macrophotography but also improves the fixation procedure. Two or more 3 mm bone slices are sawn out in the sagittal plane. At least one slice is preserved for the large whole tumor section (Figures 1 and 2). The other slice is cut into smaller blocks and correlated with a drawing so each block's area can be identified, i.e. central part, cortex with tumor in growth, bone marrow cavity, resection margin etc.). The surgical margin is marked with ink. The Enneking classification of the surgical margins is used: intralesional, marginal, wide or radical (Enneking et al. 1980).

Decalcification and staining

Both large section and small tissue pieces are decalcified in Parengy's solution. The histological sections are stained by hematoxylin and eosin and van Gieson.

Histopathologic examination

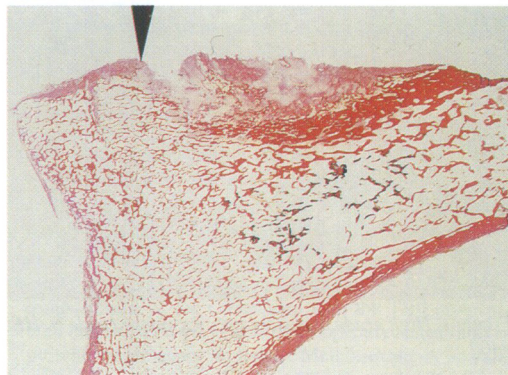
The chemotherapy effect can be graded into four (I–IV) or two grades: good or poor response. The four grade system: (Rosen et al. 1979). *Grade I*: Little or no identified effects. *Grade II*: Acellular tumor osteoid, necrotic and/or fibrotic material attributable to the effect of chemotherapy together with histologically viable tumor (10–90% tumor necrosis). *Grade III*: Predominant areas of a cellular tumor osteoid, necrotic and/or fibrotic material with only scattered foci of histologically viable tumor cells identified ($< 10\%$ viable tumor). *Grade IV*: No histologic evidence of viable tumor identified within the specimen; only mature osteoid, necrosis and/or vascularized fibrous tissue (100% necrosis) (Figure 3–7).

In the two grade chemotherapy response evaluation (Bacci et al. 1993), the good response means tumor

Figure 1. Chondroblastic osteosarcoma of the proximal tibia.



A. Macrophotography.



B. Large section. van Gieson.

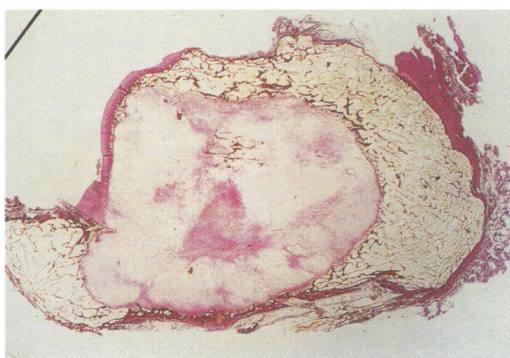


Figure 2. Osteosarcoma of the calcaneus, large section. Response grade III. van Gieson, $\times 2$.

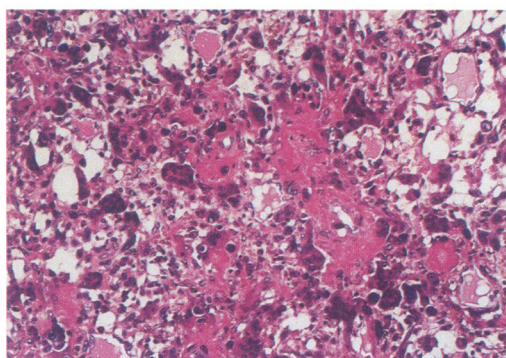


Figure 3. High grade osteosarcoma. Response grade I. HE, $\times 400$.

necrosis $\geq 90\%$. The poor response is defined as $< 90\%$ of tumor necrosis.

Comparing both systems, grade I and II correspond to a poor response ($< 90\%$ tumor necrosis); by contrast, the good response $\geq 90\%$ necrosis is equal to grade III and IV.

Comments on the microscopic evaluation

There is usually no problem to diagnose grades I and IV (no or complete response). However, it can be difficult to decide whether grade II and III is present (90% response or less), which has clinical importance, since the therapy schedule is changed in poor responders (grade I and II). In grade III, *only scattered* small areas of viable tumors are allowed (Figure 6). The interpretation of single atypical cells (Figure 7) can be difficult, too. It is now accepted that they represent remaining tumor cells (response, grade III).

The best response is to be expected in the central parts of the tumor. The remaining viable tumour is

usually found in the cortex, subcortex, zones close to the physis and articular cartilage and invaded soft tissue. The remaining tumor tissue distribution is related to the tumor vascularization (Picci et al. 1985). Therefore, the sampling site for histological evaluation is important as the result may vary depending upon the site.

Discussion

The introduction of aggressive chemotherapy in high grade osteosarcoma within the last two decades has improved the metastases free survival to 60–70% compared to 10–15% after local treatment only (Bacci et al. 1993).

Chemotherapy has increased the use of limb saving surgery. The choice of drugs and doses have been constantly modified. The prognosis prediction in good histological responders was reported by Rosen et al. (1979) and the results have been later confirmed by others (Bacci et al. 1993).

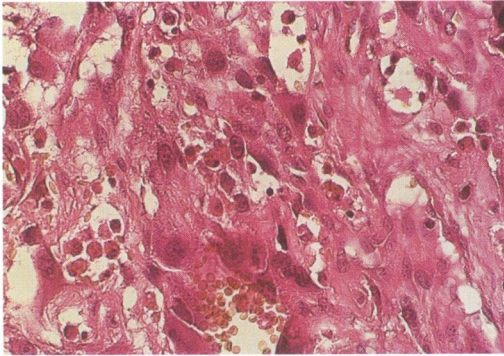


Figure 4. High grade osteosarcoma. Response grade II. HE, $\times 400$.

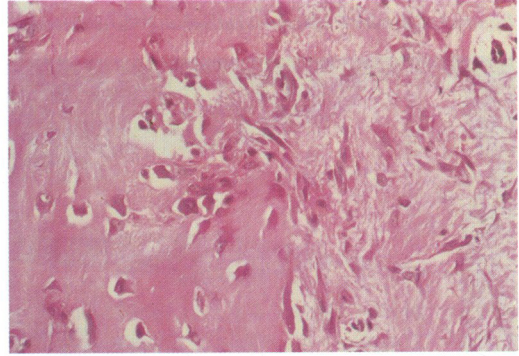


Figure 5. Osteosarcoma (central portion of Figure 2). Response grade III with scattered viable cells, mature bone and fibrosis. HE, $\times 380$.

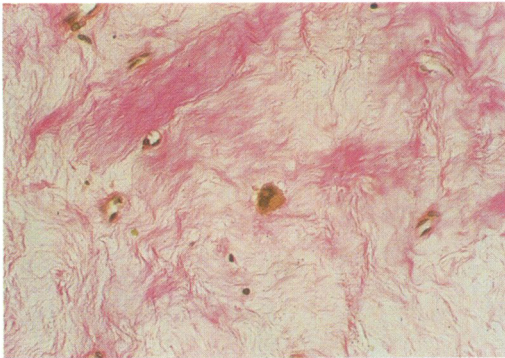


Figure 6. The same tumor as Figure 2. Response grade III with extensive fibrosis with remaining single atypical tumor cell. van Gieson, $\times 380$.

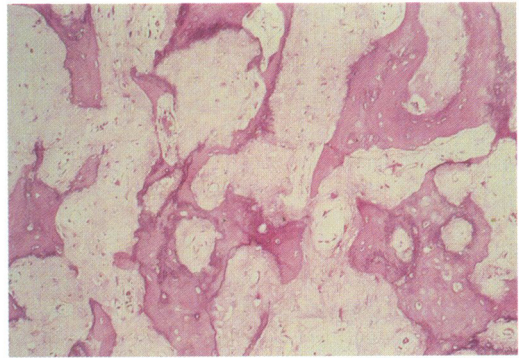


Figure 7. Osteosarcoma. Response grade IV with mature osteoid and bone marrow fibrosis. HE, $\times 120$.

The surgical margins have decreased by using an aggressive chemotherapy, and wide and possibly even marginal surgery with good chemotherapy response may only carry a low risk for local recurrence (Bacci et al. 1993).

The histologic response of chemotherapy in high grade osteosarcoma is not related to sex or age of the patients, tumor site, size or grade. However, chondroblastic tumors show significantly lower percentage of good responders (Bacci et al. 1993).

The correlation between the clinical-radiologic and morphologic response is often good, but not constant. However, the good radiological response correlates better with the good histologic response. By contrast, the "poor radiologic response" can hide all rates of necrosis.

Angiography with reduced tumor vascularity has a high prediction value (Chuang et al. 1992, Jaffe et al. 1993). Magnetic resonance imaging has been used to evaluate tumor response in osteosarcoma through estimation of tumor volume and in the amount of both

intra- and extramedullary edema (Holscher et al. 1992).

Several authors have tried to standardize the classification of necrosis grade in osteosarcoma (Rosen et al. 1976, Rosen et al. 1979, Picci et al. 1985). However, there is no absolute objective method for the evaluation of single viable cells. The procedure is highly dependent on the experience, diligence and effort of the pathologist estimating non-viable and viable tumor areas. Thus, with an adequate number of slides, a good correlation between morphology, therapy response and metastasis-free survival can be achieved. This correlation is of clinical importance, regardless which classification was used.

In poor responders, there is a possibility of switching to other chemotherapeutic agents with adjuvant treatment (Saeter et al. 1991).

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