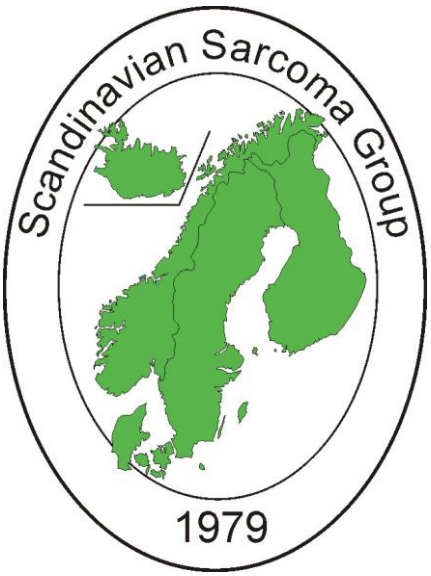
SSG Morphology and Tumorbiology Group
2012

# GUIDELINES FOR HANDLING OF SURGICAL SPECIMENS AND HISTOPATHOLOGICAL DIAGNOSIS OF EWING SARCOMA/PNET



These revised guidelines for diagnosing Ewing sarcoma and PNET are based on the previous recommendations from1998 used in the treatment protocols ISG/SSG III and ISG/SSG IV. The recommendations of diagnosis for the work-up and diagnosis of Ewing sarcoma family of tumors uses the experience from the daily practice of the pathologists in the Scandinavian Sarcoma Group, the ongoing peer-review by the Morphology group of specimens from tumors in the data base and protocols and working meeting in Turku and Lund in 2011.

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**EWING SARCOMA/PNET**
**GUIDELINES FOR DIAGNOSIS**

BIOPSY METHOD

The best material for diagnosis is obtained from fine needle aspiration (FNA) and/or surgical biopsies. Tru-cut or other coarse needle biopsies can also be used especially on the soft tissue component of the tumor, but it is often difficult to get good material from the intra-osseous part of the tumor by this method.

The combination of FNA and a true-cut biopsy is optimal.

Fresh specimens should be rapidly submitted to the Department of Pathology. First priority should be to fix tumor tissue in formalin for morphological evaluation. Fresh tumor tissue should be saved for ancillary diagnostic investigation such as genetic analysis (FISH, RT-PCR and karyotyping). Aspirates or imprints (touch preparations) from needle biopsies and surgical biopsies for cytological examinations and FISH are recommended. It is also important to save tumor tissue for banking for later research whenever possible. Be aware of which treatment protocol the clinicians are using to save required tissue for entering patients in these protocols.

## DIAGNOSIS

The diagnosis (cytologic or histological) should be based on the examination of routinely stained material (FNA or histologic sections + imprints) supplemented with ancillary diagnostic methods. Priority is to fix tumor tissue in formalin for microscopic evaluation and secondly genetic analysis. Imprints and FNAs should be stained with May-Grünwald-Giemsa (MGG), but can be supplemented with hematoxylin and eosin (H&E) or Papanicolaou (PAP.

## ANCILLARY DIAGNOSTICS

*Immunohistochemistry*

Immunohisto-cytochemistry is important especially for ruling out possible differential diagnosis. The following antibodies are useful and the selection of a panel of antibodies is based on morphological evaluation of the biopsy and clinical information;

CD99
Vimentin
Fli-1
Desmin
Myf-4
Cd45
TdT
Pax5
CD3
CD20
CD79a
CD10
CD34
MPO
NSE
Neurofilament (NF)
Chromogranin
Synaptophysoin
S-100 protein
CD56
AE1/AE3
EMA

*Histochemistry*

PAS

*Genetic analysis*

Ewing sarcoma has pathognomonic genetic aberrations. The methods to use are FISH, PCR and karyotyping. FISH and RT-PCR are recommended and are fast methods. Aspirates form fine-needle aspirations and imprints (touch preparations) and formalin-fixed paraffin-embedded tissue can be used for FISH. Gene fusions detected by RT-PCT can use fresh, frozen or formalin-fixed paraffin-embedded material. Cytogenetic analysis require unfixed, fresh tumor tissue and takes rather long time and is therefore not fast enough in the diagnostic procedure, but is a valuable method for validating the diagnosis.

The most common fusion genes in Ewing sarcoma are *EWSR1-FLI1* and *EWSR1-ERG*. The other translocations listen in the table are very rare.

| **Genetic findings in "Ewing sarcoma family of tumors" *** | | |
|---|---|---|
| **Translocation** | **Fusion genes** | **Prevalence** |
| t(11;22)(q24;q12) | *EWSR1-FLI1* | 80-95% |
| t(21;22)(q22;q12) | *EWSR1-ERG* | 5-15% |
| t(7;22)(p22;q12) | *EWSR1-ETV1* | <1% |
| t(17;22)(q12;q12) | *EWSR1-E1AF* | <1% |
| t(2;22)(q33;q12) | *EWSR1-FEV* | <1% |
| inv(22) | *EWSR1-ZSG* | <1% |

* Not complete list

THE FINAL DIAGNOSIS

The final diagnosis should be based on examination of routinely stained material (FNA and histology), characteristic immunohistological findings (vimentin and CD99 positivity) and genetic results. As the tumor may be partly necrotic and an ancillary method may fail, it is important to save material as indicated above.

In the diagnostic procedure of bone sarcoma it is always important to correlate the results of the radiological examinations and clinical findings with the morphology in multidisciplinary teams.

In small round cell tumors it is important to rule out differential diagnosis as

- Malignant lymphoma
- Neuroblastoma
- Rhabdomyosarcoma
- Synovial sarcoma
- Wilms tumor
- Small cell carcinoma

**MACROSCOPIC INVESTIGATION OF THE SURGICAL SPECIMEN**

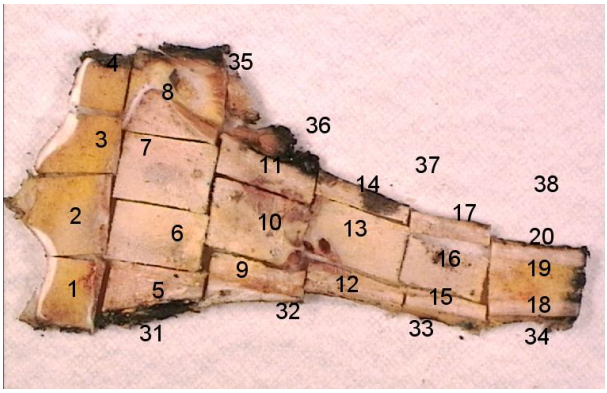
Ideally the surgeon and the pathologist should examine the specimen together regarding orientation of the specimen and resection margins. Photo documentation is recommended. The tumor can be localized in bone or soft tissue.

Slice the bone along the longitudinal axis through the middle of the marrow cavity. Wash the surface with water to remove dust from the sawing. The specimen and the tumor should be measured (in three dimensions) as well as the distance from the tumor to the resection planes. The type of tissue in the resections margins should be recorded. Suspected areas with necrosis and viable tumor are noted

Most patients have received preoperative chemotherapy and therefore the extent of necrosis should be evaluated. One slice containing the largest diameter of the tumor should be cut in numbered blocks and marked on the image.

Important areas to be sampled:

- Subperiostal reactive new bone areas
- The medullary canal
- The soft tissue extension of the tumor should be sampled because this is often the only location with viable tumor tissue




**PICCI GRADING SYSTEM FOR HISTOLOGICAL CHEMOTHERAPY RESPONSE**
The Picci system is based on microscopic evaluation of the amount of remaining viable tumor after the chemotherapy and is a three grade system. Depending on the treatment protocol the postoperative treatment will be guided by the histological treatment response.

*Grade I*
The chemotherapy response when the surgical specimen contains at least one "macroscopic nodule of viable tumor. A macroscopic nodule is defined as an individual nodule that is larger than one 10X objective magnification field or scattered nodules' that individually are smaller than one 10X field but the total summation of the areas these nodules exceeds one 10X field (Fig. 1)
*Grade II*
The chemotherapy response when the surgical specimen contains one or several nodules of **viable** tumor; the total summation of the areas of all nodules are less than one 10X filed (Fig. 2)
*Grade III*
The chemotherapy response when **no viable** nodules of tumor are identified in the surgical specimen (Fig. 3).

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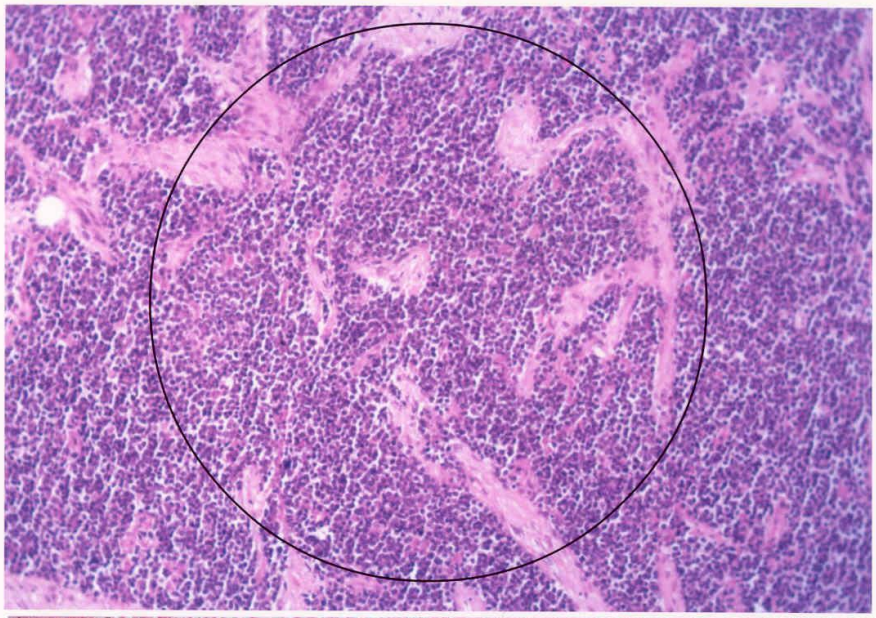

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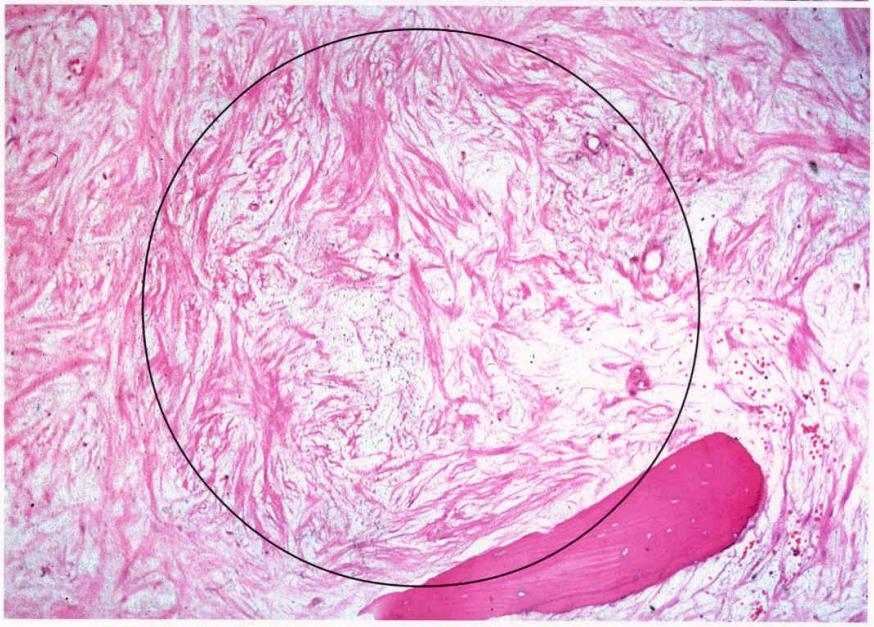

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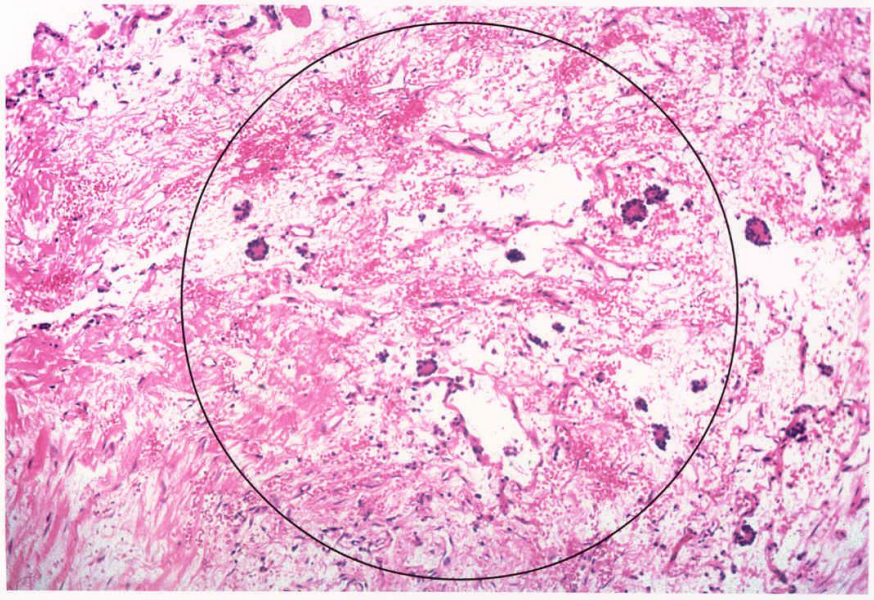

## References

1. Picci P, Rougraff BT, Bacci G, Neff JR, Sangiorgi L, Cazzola A, Baldini N, Ferrari S, Mercuri M, Ruggieri P, et al. Prognostic significance of histopathologic response to chemotherapy in nonmetastaticEwing's sarcoma of the extremities. J Clin Oncol. 1993;11(9):1763-9

2. Li S, Siegal GP Small cell tumors of bone. Adv Anat Pathol. 2010;17(1):1-11. Review

3. Maheshwari AV, Cheng EY. Ewing sarcoma family of tumors. J Am Acad Orthop Surg. 2010;18(2):94-107. Review.

4. Toomey EC et al. Recent advances in the molecular pathogenesis of Ewing's sarcoma. *Oncogene* 2010, 29:4504-4516

5. Gamberi G, Cocchi S, Benini S, Magagnoli G, Morandi L, Kreshak J, Gambarotti M, Picci P, Zanella L, Alberghini M Molecular diagnosis in Ewing family tumors: the Rizzoli experience--222 consecutive cases in four years.J Mol Diagn. 2011;13(3):313-24.

6. Khoury. JD. Ewing sarcoma family of tumors: a model for the new era of integrated laboratorydiagnostics. *Expert Rev. Mol. Diagn*. 2008,8(1): 97-104.

7. Sankar S, Lessnick SL. Promiscuous partnerships in Ewing's sarcoma. Cancer Genet. 2011 ;204(7):351-65. Review

8. Devoe K, Weidner N. Immunohistochemistry of small round-cell tumors.Semin Diagn Pathol. 2000;17(3):216-24. Review

9. Paulussen M, Bielack S, Jürgens H, Casali PG; ESMO Guidelines Working Group. Ewing's sarcoma of the bone: ESMO clinical recommendations for diagnosis, treatment and follow-up. Ann Oncol. 2009;20 Suppl 4:140-2. Review. N.