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PROGNOSIS OF EWING SARCOMA BY QUANTITATIVE HISTOPATHOLOGICAL METHODS

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ABSTRACT

Ewing's sarcoma is an increased incidence tumor, with radiological polymorph appearance that mimics a multitude of bone diseases. It has an incompletely elucidated origin, monotone microscopic image, but high aggressiveness. Exceptional sensitivity to radiotherapy requires precise diagnosis as early as possible. These peculiarities suggested the evaluation of stereological microscopic structure and its proliferative activity. For this purpose we had studied anatomopathologicaly 42 bone tumors from children with ages between 3-18 years from Iasi hospitals. Quantitative measurements were performed using the interactive digital program PRODIT 5.2. The quantified stereological parameters objectifies the microscopic appearance of Ewing's sarcoma, but can not be used to assess the prognosis and the tumor progression. Quantification of the proliferative activity can be used to assess the prognosis only in case of sure diagnosis, the values above 2/10 hpf indicating increased risk.

INTRODUCTION

Ewing sarcoma was described for the first time in 1921 by James Ewing under the name, diffuse bone endothelioma", a very aggressive form, but very sensitive to radiotherapy (1).

Regarding its origin, there are two theories which are discussed: one considers that ES to be born from embryologic cells in the neural crest (2, 3) and the other considers to take origin from the pluripotent cells named mesenchymal stem cells (4, 5).

The microscopic appearance is usually monotonous with small uniform cells having oval or round nuclei, alternating with fibrillary, reticular appearance, but there are also many atypical forms (6, 7, 3).

The high incidence (75% of all cases) of Ewing sarcoma encountered in children, described in speciality literature (5) as well evidenced by personal followed cases, along with polymorph radiological appearance, that mimics a multitude of bone diseases, but also its unknown origin (8) , had suggested the evaluation of stereological microscopic structure and its proliferative activity.

METHODS AND MATERIALS

We had studied anatomo-pathologicaly 42 bone tumors from children with ages between 3-18 years, from Iasi hospitals, which we followed both clinically and surgically. Tumoral fragments were obtained from biopsies or from surgical resected samples. The histopathological exam was done on paraffin slides of 5 micrometers, died by usual H&E diagnostic techniques.

On stereological exam we used digital overlapping of standard grid over the achieved video image. The Weibel parallel grid was adapted at studied tissue in order to fulfil optimal quantification conditions.

After establishing the reference structures (tumoral cells, blood vessels and haemorrhages, fibroconnective stroma, bone or metaplastic cartilage) was studied using 40x no camera lens on a test area of 0.139628 mm^2 which corresponds to 480 dots on the Weibel parallel grid test, having a distance of d = 18.33 micrometers.

It was quantified:

- *Mitotic activity index* (MAI) which represents the total number of mitotic figures counted on these fields;
- *Mitotic rate* which appreciate the percentile value of mitoses on quantified fields;
- *Mitotic density* which represents the number of mitosis per mm^2.

RESULTS AND DISCUSSIONS

Microscopic appearance of classic Ewing sarcoma evidentiate the presence of undifferentiated cells with increased density or cellular monotony with small, round cells, with little, clear cytoplasm and invisible membrane (fig. 1), located in lobules separated by fibrous bands or by necrotic areas (fig. 2). The nuclei are usually round (fig 3) with irregular surface (indentations), sometimes small nucleoli being visible.

The islands of tumoral cells invades the bones lamellas from the near tumoral nucleus (Fig. 4).

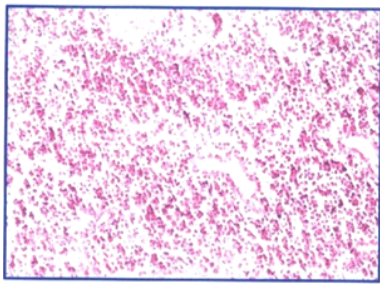

Fig. 1. Ewing sarcoma H&E stain, x200. Proliferation of round cells, decreased cytoplasm and prominent nucleus.

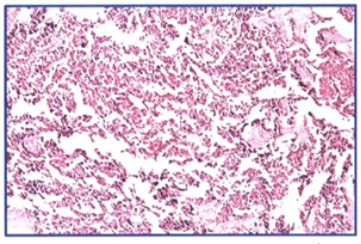

Fig. 2. Ewing sarcoma H&E stain, x200. Proliferation of tumoral cells alternate with small necrosis areas.

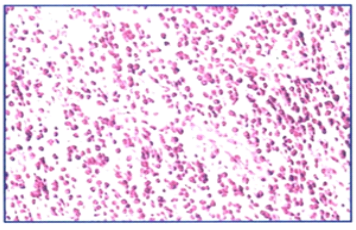

Fig. 3. Ewing sarcoma, H&E stain, x400. Round or oval cells, with reduced cytoplasm and big nucleus.

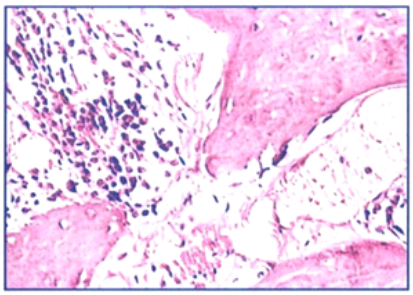

Fig. 4. Ewing sarcoma, H&E stain, x200. Tumoral cells which invades bony lamella.

STEREOLOGY

The monotonous histopathological picture with small uniform cells have permitted the identification of reference structures in small number:

- tumoral cells,
- blood vessels and haemorrhages,
- fibroconnective stroma,
- metaplastic bone or cartilage.

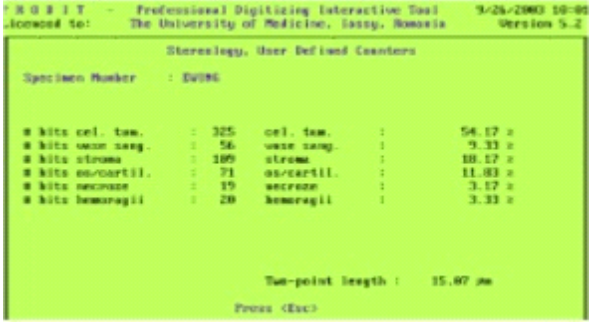

Fig.5. Stereological report of quantifications in Ewing osteosarcoma.

The quantifications performed showed:

- the existence of the largest percentage volume of tumor cells (54.17), which asserts the compact, solid aspect seen in the qualitative examination of the tissue fragments,
- the blood vessels, although very numerous, occupy a modest percentage volume (9.33), and due to the dilations in some areas,
- the formation of metaplastic bone tissue, rarely cartilaginous, occupying a percentage volume of 11.83, has insular disposition,
- the haemorrhages (3.33) and the necroses (3.17) are more abundant at the periphery of the tumor,
- the fibroconnective stroma is poorly represented (18.17), it isolating islands of small, round cells.

Tumor grading	Tumoral cells	Atipia	Mitoses	Sarcomatous vessels	Osteoid matrix	Fibrous stroma
2	6.66	3.22	3.63	8.87	22.59	55.03
2-3	11.31	4.55	3.89	19.98	28.22	32.06
3	14.77	6.63	4.15	25.57	33.39	16.55
4	17.49	7.85	6.87	26.84	33.77	7.18

Fig. 6. Stereological report of quantifications in clasic osteosarcoma.

If we compare the stereological data obtained in ES with those obtained previously (9) in different degrees of classical osteosarcoma (fig. 6), we note very few similarities: the percentage volume of tumor cells in ES is much higher than in any of the classic osteosarcomas; the percentage volume of the fibroconnective stroma in the ES is similar to that in grade 3 osteosarcoma. We can conclude that, although both tumors are osteosarcomas, the prognostic evaluation criteria are different, as well as their evolution.

PROLIFERATIVE ACTIVITY

The proliferative activity of this type of bone tumor is rather low, as demonstrated by the quantifications performed:

- *mitotic density* (mitosis /mm2) is 2.5/mm2.
- *mitotic activity index* was evaluated according to the statistical ratio of quantification to 3/10 hpf.

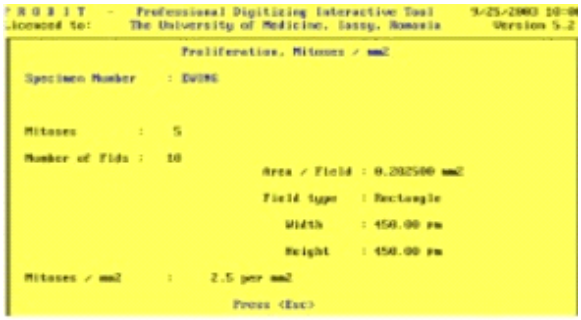

Fig. 7. Quantification report of the proliferative activity (mitosis / mm2)in Ewing sarcoma.

The mitotic density in our cases discretely exceeds the risk limit (2/10 hpf) recommended in the literature (10).

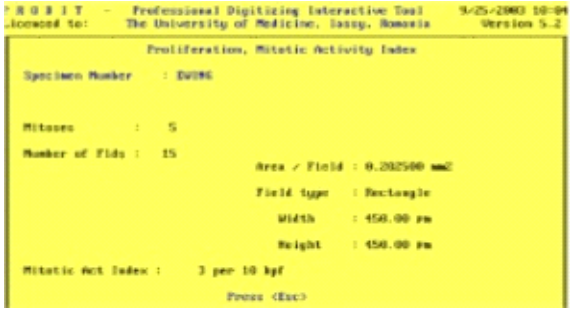

Fig. 8. Quantification report of the proliferative activity (mitotic activity index - MAI) in Ewing sarcoma.

Tumor grading	*Mitoses/mm2*	*Mitotic activity index*
2	9.6	7
2-3	16.6	12
3	20.7	17
4	25/3	19

Fig. 9. Quantification report of the proliferative activity in classic osteosarcoma.

By comparing the results of quantification of proliferative activity in ES with those of classical osteosarcomas (fig. 9) we noticed that there are no similarities (9), the values being much higher in the latter cases. In each of the two types of studied osteosarcomas, particular limits of the quantitative parameters that allow a prognostic assessment can be established.

CONCLUSIONS

1. The quantified stereological parameters objectifies the microscopic appearance of Ewing's sarcoma, but can not be used to assess the prognosis and the tumor progression, in view of varied aspects and the lack of an unit of opinion on grading.
2. Compared with previously studied osteosarcomas, the proliferative activity in Ewing sarcoma is reduced.
3. Quantification of the proliferative activity can be used to assess the prognosis only in case of sure diagnosis of ES, the values above 2/10 hpf indicating increased risk.

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