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OVEREXPRESSION OF IGF1 PROGNOSTIC FACTOR IN CHILDHOOD OSTEOSARCOMAS

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ABSTRACT

Osteosarcoma is a highly malignant intraosseous tumour formed by tumour cells producing osteoid, bone or chondroid material (1). Considering the reserved prognosis of this neoplasia, we attempted to identify and analyze some biomarkers in order to perform a multivariate analysis and to assess the predictive power of the evolution.
In search of new and reliable biomarkers we studied IGF1 overexpression in osteosarcoma. IGF1 is an insulin-like polypeptide hormone with a vital role in normal bone development by stimulating bone growth.
We therefore decided to investigate the role of these biomarkers in osteosarcoma. For this purpose we analyzed 46 samples from 27 patients diagnosed with Osteosarcoma in the period 2017-2021 in the Children's Emergency Clinical Hospital "Sf.Maria", Iasi.

INTRODUCTION

Osteosarcoma develops from young, immature, new bone-forming bone cells. Osteosarcoma is considered to be primary when there is a layer of normal bone tissue adjacent to the tumour.

Osteosarcoma is one of the most common malignant primary bone tumours of the long bones with an increased frequency in children and adolescents. It accounts for approximately 19% of all malignant bone tumors (2). In the United States the latest epidemiological studies show a number of 400 new cases annually in people under 20 years of age (3). Identification of biomarkers and inhibitors or combinations of inhibitors active on osteosarcoma cell lines offers the possibility of early diagnosis as well as optimal surgical approach.

These tumour markers and highlighting their interrelationships may be possible targets for specific and individualised therapies to improve survival and quality of life in paediatric patients.

The study of the osteosarcoma microenvironment, in vitro or in vivo, is a way to decipher the evolutionary behaviour of the tumour. The combination of biomarker expression and response to monotherapy or combination therapy in childhood osteosarcomas can explain a number of clinical and evolutionary issues and lead to better clinical and therapeutic management of these cases. Unfortunately, overexpression of IGF-1 and its receptor (IGF-1R) has been implicated in carcinogenesis and has been indicated as a risk factor for the development of several human cancers, including OS. Elevated levels of IGF-1 and IGF-1R have been reported in OS, leading to cancer progression through transformation, proliferation, pro-metastasis and decreased susceptibility to apoptosis. (4)

MATERIAL AND METHODS

A total of 27 cases diagnosed with Osteosarcoma in the period 2017-2021 at the "Sf. Maria" Children's Emergency Clinical Hospital Iasi were analyzed.

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Forty-six 10% formalin-fixed, buffered, EDTA-decalcified, paraffin-embedded tissue samples were obtained from these cases. 5/4 sections were made and transferred to electrostatically charged slides and used for immunohistochemistry.

Immunohistochemical reactions are based on tissue antigen-antibody binding, the latter being detected by direct conjugation with tracer molecules (direct reaction) and through a chain of other binding with free or labelled antibodies (indirect reaction with two or more phases). Abcam's (ABC) Detection IHC Kit and IGF1 antibody were used. Anti IGF1 antibody7973 (ab176523) (Monoclonal, Clone - 7973, Quantity - 100 µl). Staining with this antibody was evaluated according to a score reported by AGGELIS STAVROPOULOS et al in Oncollogy letters 20; 395; 2020 (5).

Depending on the number of positive cells the score was as follows

TABEL I

IHC score positive cell

Score 0	less than 5% immunopositive cells
Score 1	5-25% immunopositive cells
Score 2	25-75% immunopositive cells
Score 3	over 75% immunopositive cells

Staining intensity was defined as 0 - negative, 1- weakly positive, 2 - moderately positive, 3 - strongly positive
The sum of cytoplasmic staining intensity and positive cells was entered into a score of 0, 1, 2+, 3 and 4++, 5 and 6+++.

Each staining process contains both positive and negative controls to confirm the correctness of the staining system, the specificity of the staining procedures for both positive and negative staining, and verification of the staining times of the procedure.

RESULTS

By analyzing the pathology reports with the passport data of each case and correlating them with the immunohistochemical study we obtained the following information

In the 27 cases studied, the male sex prevailed (16 cases, with an age at the time of diagnosis between 8-19 years old, with a maximum of cases at the age of 16 years old (5 cases).
Two cases out of 27 were identified with metastasis, one with lung metastasis and one with skin metastasis.

The most common location was in the femur (15 cases), with a maximum in the distal femur (5 cases), followed by the tibia (4 cases).
The pathological diagnosis was as follows:

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TABLE II
Identified histopathological forms of osteosarcoma

Osteoblastic osteosarcoma	9 cases
Chondroblastic osteosarcoma	7 cases
Poorly differentiated osteosarcoma	6 cases
Telangiectatic osteosarcoma	2 cases
Pleomorphic osteosarcoma	2 cases
Well-differentiated osteosarcoma	1 case

In all cases analyzed, immunohistochemical staining for SATB2 was previously performed to certify the diagnosis of osteosarcoma, and to eliminate malignancies that may histopathologically mimic an osteosarcoma, especially undifferentiated forms (6).

In 1 case out of 27, IHC staining was negative - possible primary block processing defect.

In the case of IGF1 staining, the following aspects were identified:

1 case did not stain due to a technique defect,

All 27 cases analysed were positive for IGF1 with more than 60% positive cells in the cell population,

The more poorly differentiated the histopathological form of osteosarcoma, the more weak to moderate the staining was (in 5 cases out of 6, i.e. 83.33%). In the metastatic cases, staining for IGF1 was weak.

Staining for IGF1 was intense in 8 out of 9 (88.88%) of osteobasal osteosarcomas and in 4 out of 7 (57.14%) of chondroblastic osteosarcomas.

In pleomorphic osteosarcomas, IGF1 staining was intense in 2 cases out of 2 (100%). In telangiectatic osteosarcomas, staining was intense in 2 cases out of 2 (100%). Staining was also intense in well-differentiated osteosarcomas.

In poorly differentiated osteosarcomas, staining was intense in 4 cases out of 6 (66.66%).

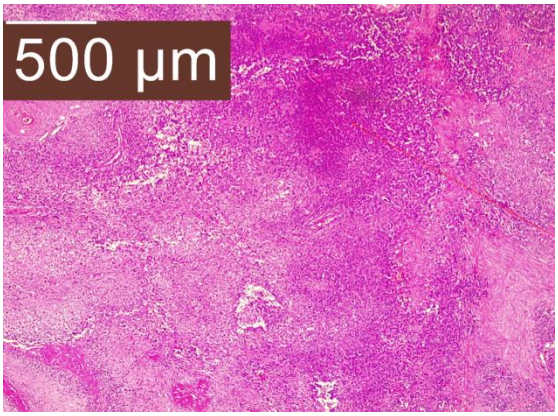

Fig 1. Hex4 chondroblastic osteosarcoma

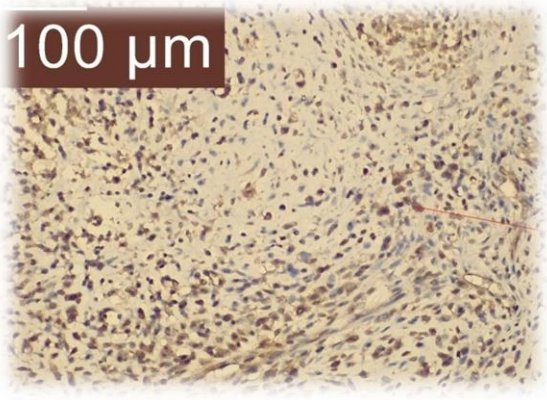

Fig. 2 Chondroblastic osteosarcoma, IGF1 weak to moderate cytoplasmic staining x10

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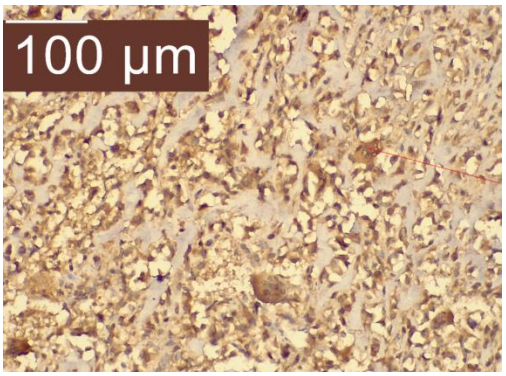

Fig.3 Osteoblastic osteosarcoma-IGF1 metastasis,
moderate to strong cytoplasmic staining x20

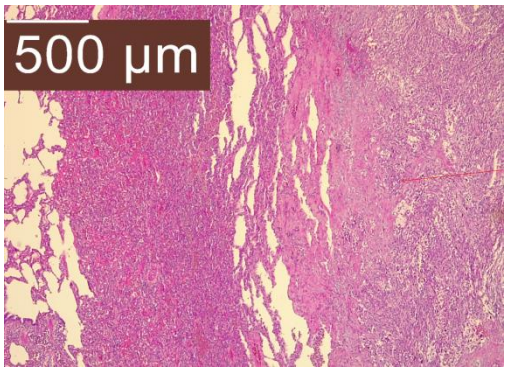

Fig.4 Osteoblastic osteosarcoma, lung
He x10 staining

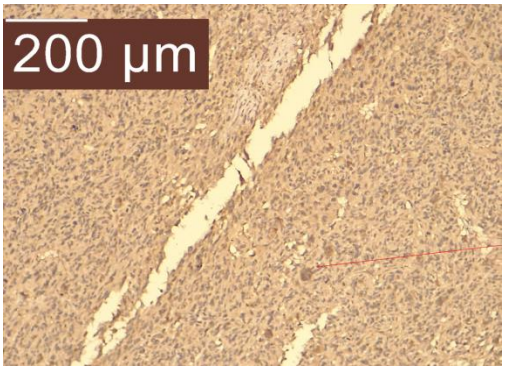

Fig.5 Osteoblastic osteosarcoma, IGF1
moderate to weak cytoplasmic staining x10

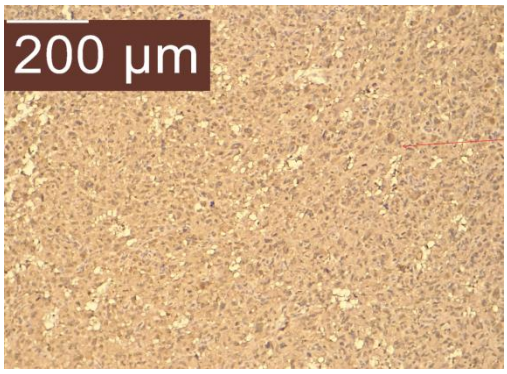

Fig.6 Osteoblastic osteosarcoma, IGF1
moderate to strong cytoplasmic staining x10

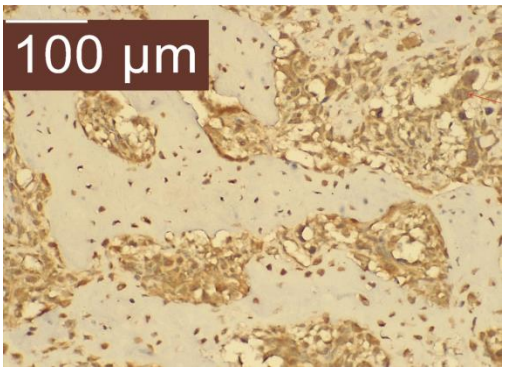

Fig. 7 Chondroblastic osteosarcoma, IGF1
strong cytoplasmic and nuclear staining x10

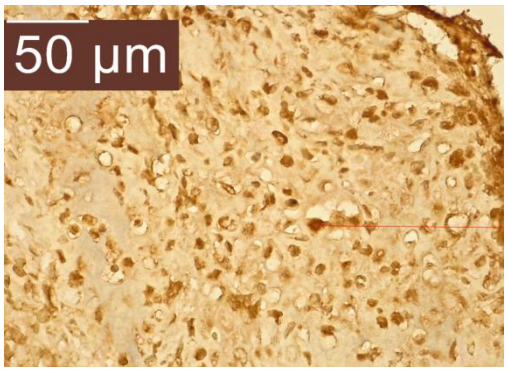

Fig. 8 Chondroblastic osteosarcoma, IGF1 staining
strong predominantly nuclear stainingx40

DISCUSSIONS

Osteosarcoma is the most common primary bone tumour in children and adolescents. In the USA the incidence of this tumour is 3.1/1million places, with a peak incidence of 15 cases/million places/year in the age group 10-19 years, with a frequency of 1.4 higher in males.

The increased rate of metastasis at the time of diagnosis explains the importance of early diagnosis and the

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identification of antibodies capable of achieving a prognostic or therapeutic evolution.

Although with current treatment protocols the 5-year survival rate can reach 50-70%, there remains a large group of patients with recurrent and/or refractory metastatic disease without therapeutic options.

The solution in these cases would be to identify factors that can help prognostic predictability among patients with Osteosarcoma, possibly leading to personalized treatment.

- From the total number of cases of osteosarcomas admitted and investigated at the Children's Clinical Hospital Sf Maria Iasi, during the period, 46 cases were included in our study and received surgical treatment and histopathological diagnosis.

- The prevalence of osteosarcomas in the age group 8-19 years was evident with a peak incidence at 16 years (5 out of 27-=18.51%). At the same time the diagnosis of osteosarcoma was more common in males (with 16 cases out of 27=59%) with a B/F ratio=1.4 which is close to the literature data.

- The most common location was in the femur - 15 cases out of 27 (55%), with a higher frequency in the distal femur (7 out of 15 cases - 46%), followed by the tibia - 4 cases out of 27 (14.81%).

The histological definition of osteosarcoma is based on the presence of malignant cells that produce osteoid.The more aggressive the tumor is, corresponding to a high degree of malignancy, the lower the osteoid production may be, resulting in chondroblastic or fibroblastic variants.

For our study 27 cases were identified that had paraffin blocks containing sufficient tissue material and haematoxylin-eosin stained diagnostic slides.

In our study the usual histopathological diagnosis was as follows:

- Osteosarcoma osteoblastic 9 cases (33.33%)
- Chondroblastic osteosarcoma-7 cases (25,92%)
- Poorly differentiated osteosarcoma-6 cases (22,22%)
- Pleomorphic osteosarcoma-2 cases (7.40%)
- Telangiectatic osteosarcoma-2 cases (7.40%)
- Well differentiated osteosarcoma-1 case (3,70%)

It is thus observed that in our study group the most frequent form was osteoblastic osteosarcoma (7).

IGF1is a protein with a vital role in normal bone development. IGF1 also stimulates bone growth.

Angiogenesis is a private process during osteosarcoma development. Although the role of IGF1 in activating angiogenesis is not fully known, in 2016 Geo and Cheng demonstrated that IGF1 increased VEGF expression in promoting angiogenesis in osteosarcoma cells(8).

This opens up the opportunity for therapeutic use of angiogenesis inhibitors.

Activation of endothelial cells by angiogenic factors leads to the production of proteolytic lesions that degrade the extracellular matrix. Degradation of the underlying membrane allows endothelial cells to proliferate and migrate to the surrounding tissue to form new vessels. (9)

These new vessels provide cancer cells with oxygen and nutrition and play an important role in cancer cell survival and metastasis.

Thus angiogenic therapies could be an interesting approach in osteosarcoma therapy.

IGF1 has also been studied in various cancers such as, breast cancer, clear cell renal cell carcinoma, prostate cancer, lung.

Besides its contribution in stimulating angiogenesis, there are studies showing its role in the cellular mitogenic

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process and whether its expression could be correlated with therapy.

Alecksic (10) et all report its nuclear localization in clear cell carcinoma, pre-invasive breast lesions and non-tumoral tissues associated with a high proliferation index.

A correlation between unclear IGF expression, poor prognosis of clear cell carcinoma and rhabdomyosarcoma has been demonstrated.

Its underexpression appears to be correlated with reduced ability of cancer cells to form colonies.

Using immunohistochemistry Mukama et all (11) demonstrate that exclusively nuclear expression of IGFIR compared to nucleo-cytoplasmic or cytoplasmic expression correlated with a better prognosis in patients with unresectable or metastatic osteosarcoma.

In contrast exclusive nuclear IGFIR expression in colorectal cancer patients was correlated with poorer prognosis.

Nuclear expression in breast cancer correlated with an increased risk of recurrence.

Immunohistochemical study of IGF1 expression revealed the following:

1. No exclusive nuclear expression, (associated with a favorable prognosis)

2. In the cases studied we had cytoplasmic and nuclear expression respectively.

3. 1 case did not stain by technique defect

4. All the 26 cases studied were positive for IGF1 with more than 60% positive cells in the cell population (denoting a poor prognosis).

5. The more poorly differentiated the histopathological form of osteosarcoma, the more weak to moderate the staining (in 5 cases out of 6, i.e. 83.33%).

6. In cases of metastasis staining for IGF1 was weak (in contrast to the literature which suggests that underexpression is associated with a low risk of metastasis).

7. IGF1 staining was intense in 8 out of 9 cases (88.88%) of osteobasal osteosarcomas and in 4 out of 7 cases (57.14%) of chondroblastic osteosarcomas (which is associated with a poor prognosis).

8. In pleomorphic osteosarcomas staining for IGF1 was intense in 2 cases out of 2 (100%).

9. In telangiectatic osteosarcomas, staining was intense in 2 cases out of 2 (100%).

10. Staining was also intense in cases of well-differentiated osteosarcoma

11. In poorly differentiated osteosarcomas, staining was intense in 4 cases out of 6 (66.66%).

CONCLUSIONS

1. IGF1 was overexpressed in all cases of osteosarcoma certified positive for SATB2- this correlates with a poor prognosis.In this context, the question arises as to the therapeutic use of angiogenesis inhibitors in osteosarcoma.
2. We had no case of exclusive nuclear overexpression of IGF1 (associated with a favorable prognosis in the literature).
3. No correlation was identified between histopathological form and IGF1 overexpression, nor between degree of differentiation and overexpression.

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