Original article

Characterization, survival analysis, and expression of IGFR in tumor samples from patients diagnosed with Ewing family tumors treated at the Barretos Cancer Hospital☆

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Abstract

Objectives: Study the clinical characteristics of patients diagnosed with Ewing family tumors (EFTs) and survival analysis based on risk criteria and expression of the surface protein known as insulin-like growth factor (IGFR).

Methods: This was a retrospective cohort study based on clinical data from 77 patients diagnosed with EFTs treated by the Department of Pediatric Oncology at the Barretos Cancer Hospital in a period between 2003 and 2012. Biological samples of patients were examined for the presence of the surface receptor IGFR.

Results: The overall survival rate (OSR) of patients included in the study was 45% at five years, and EFS was 30% at five years. Metastasis at diagnosis was present in 44.2% of the sample; 88.2% of the sample was male (p < 0.001). The evaluation of the expression of IGFR in biological samples of patients was associated with the variable metastasis at diagnosis (p < 0.001). Worse prognosis was observed in patients with extrapulmonary metastasis (p = 0.009). The local treatment of neoplasia presented better prognosis in patients undergoing local surgical treatment (p < 0.001).

Conclusions: These results showed a higher incidence of metastasis at diagnosis in patients with EFTs treated at the Barretos Cancer Hospital (BCH). Extrapulmonary metastases were a negative prognostic factor in this study. Surgical treatment of the primary tumor was a factor for better prognosis. Strong expression of IGFR was more frequent in patients with metastases at diagnosis, but did not represent a prognostic factor for EFTs.

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Introduction

Ewing’s Bone Sarcoma (EBS) is the second bone malignancy frequent in this age group, its incidence being second only to osteosarcoma. Histologically, Ewing sarcoma (ES) is defined as tumor composed of small cells, round, immature and usually rich in glycogen. The tumor features genetic alterations in approximately 80% of the cases defined as the reciprocal translocation between chromosomes 11 and 22 – t(11;22) (q24;q12), resulting in the expression of the protein EWS/FLI-1. The reciprocal translocation between chromosomes 11 and 22 is also associated with the overexpression of cell surface glycoprotein CD99. Immunohistochemical markers, cytogenetics, molecular genetics, tissue culture and the detection of the same chromosomal translocation t(11;22) in the so-called primitive neuroectodermal tumor (PNET) or peripheral neuroepithelioma in Ewing’s sarcoma of soft tissues (ESST) and Askin tumor indicates that all these tumors originate from the same primordial stem cell. Thus, all these tumors are grouped in what we call Ewing Family of Tumors (EFTs). In the United States, the annual incidence of EFTs is 2.9 cases per million individuals up to 20 years of age. In the European continent, around 900 new cases are diagnosed annually. EFTs is rare in black individuals, slightly predominant in males, occurring most commonly in the second decade of life. With the introduction of chemotherapy in the treatment of ES associated with surgery and/or radiotherapy, the survival of patients with localized disease increased from 10% to 70% in five years. In spite of this gain, survival for patients with metastatic disease at diagnosis is 20% and for individuals who are refractory to treatment or relapsing disease, the survival rate does not reach 10%.

There are several prognostic factors involved in EFTs; literature correlates the prognosis to the primary tumor site, tumor volume, age at diagnosis, gender, lactate dehydrogenase (LDH) levels and the presence of metastasis. Some EFTs studies make inference to a better prognosis on extremity lesions compared to lesions that compromise the axial skeleton. Unresectable tumors located in the axial skeleton are associated with a worse prognosis. However, a recent multicenter study with 114 patients found no significant difference in prognostic value with respect to axial or extremity location of the tumor. ESSTs are associated with worse prognosis in relation to EBS. Tumor size is a predictive factor of prognosis in EFTs, tumors with size equal to or greater than 8.0 cm are associated with a worse prognosis. Neoadjuvant chemotherapy-induced tumor necrosis is a prognostic factor, and patients with necrosis rate above 90% in the resected specimen show increased survival. Patients younger than 15 years have a better prognosis compared to adolescent patients with the same age or older than 15 years and adults. American studies portray better prognosis in individuals aged less than 10 years compared with those aged 10–17 years at
diagnosis. However, review of two German clinical trials for
the treatment of ES involving patients older than 40 years at
diagnosis had a survival comparable to adolescents treated
in the same trial. Women diagnosed with EFTs have bet-
ter prognosis than men. High serum LDH levels before
treatment are associated with a worse prognosis, and this
increase can also be correlated with large primary tumors and
metastasis.

The paradigm for molecular targeted therapies has been
discussed taking IGF1 into consideration. Insulin-like growth
factor 1 (IGF-1) is a hormone that functions as the major medi-
ator of growth hormone (GH)-stimulated somatic growth, as
well as a mediator of GH-independent anabolic responses
in many cells and tissues. The IGF1-mediated molecular
pathways have recently emerged as important effectors of
neoplastic transformation in various types of cancer. For the
oncogenic transformation in ES the presence of the IGF-1R
receptor is needed. The involvement of the expression of
the IGF1 receptor has also been studied in squamous cell
carcinoma of the larynx and may be used as an indepen-
dent prognostic factor for recurrence and survival in patients
undergoing surgical resection.

Materials and methods

A cohort study was conducted with retrospective data collec-
tion which analyzed medical records of 101 patients age of
30 years and diagnosis of EFTs that were seen and treated by
the Department of Pediatric Oncology at BCH in the period
between 2003 and 2012, with follow up until 12/31/2012. The
study excluded patients with a diagnosis of EFTs initially
treated at another institution and patients treated at BCH, but
not treated by the Department of Pediatric Oncology; 24
patients met the exclusion criteria.

Sixty eight paraffin blocks containing biological sam-
ple of patients diagnosed with EFTs treated at BCH in the
period 2003–2012 were separated; the blocks were stored
in the Anatomical Pathology Department at BCH. Slides
related to the blocks were evaluated for diagnostic
confirmation.

The most representative block of each case was selected
so that the immunohistochemical staining using standard
marked slides could be carried out. Slides were incubated
with the following ready-to-use primary antibodies: Anti-IGF1
(Rabbit polyclonal to IGF1 Receptor Abcam®). Positive controls
are the ones accompanying reactions and immunohistochemical
staining pattern with cytoplasmic positivity in normal pan-
cretic tissue. Standardized evaluation for markers was based
on the “Quick score” Q=p x |20–22 where P, which corresponds
to the percentage of positive epithelial cells that are diffuse
and uniform, was assessed as follows: (0: negative; 1: <25%
of positive cells in the cytoplasm; 2: 26–50%; 3: >50%) and I
which assesses the staining intensity (1: mild; 2: moderate; 3:
intense); we get the score by multiplying the values of P x I.

The expression was considered weak positive in cases with
score from 0 to 5 (1), and strong positive on score 6 or higher
(2).

For the characterization of the clinical characteristics and
survival analysis of the sample, patient data were entered into
a database using the statistical program SPSS (Version 19.0,
Inc., Chicago, IL).

A descriptive analysis of the clinical characteristics of
patients was conducted by means of measures of central ten-
dency (mean and median), dispersion (standard deviation) for
quantitative variables. Frequencies and percentages were cal-
culated for categorical clinical variables.

Pearson’s chi-square test ($\chi^2$) was used to evaluate the asso-
ciation between two categorical independent variables.

The analysis of overall survival based on risk criteria (age at
diagnosis, gender distribution, primary tumor location, local-
ized or metastatic disease at diagnosis, site of metastasis,
treatment protocol, local treatment modality, serum LDH lev-
eels and expression of IGF1) was performed by considering
death by any cause as an event of interest, and, end of follow-
up (alive patient) or loss of follow up as reasons for study
termination. On the other hand, on the analysis of event-free
survival, we adopted, as an event of interest, the disease pro-
gression (progression and/or local or distant recurrence) or
death by any cause, and as reasons for study termination,
patients that were alive without progression of the disease,
without local or distant recurrence, or loss of follow up. Sur-
vival curves were calculated by the Kaplan–Meier method and
for the comparison of survival curves we used the Log-rank
test. Prognostic factors were evaluated according to the model
of the univariate Cox regression for OS and EFS. For purposes of
survival analysis, only patients treated in the period between
2003 and 2010 were included.

Analyses were performed with the statistical program SPSS
(version 19.0; Inc., IL). We adopted a significance level of 5% for
all cases.

Results

The average age of patients was 15 years (standard deviation
[SD] = 5.42 years) ranging from 2.34 to 28.57 years and there
was a predominance of males in 63.6% of cases. Upon evalu-
ating the ethnic group, most of the patients were white,
comprising 83.1% of our study and only 2.6% of subjects were
black (Table 1).

With regard to location, the bone involvement prevailed,
affecting 58.4% of all cases. On patients with bone lesions,
28.8% affected the axial skeleton and in the injuries that com-
promised soft tissues (ST) the axial region was affected in
75.5% of patients (Table 1).

Upon evaluating the presence of metastases, we observed
that 44.2% of patients had metastatic lesions at diagnosis
(Table 1). Among patients metastatic at diagnosis 88.2% were
male (p < 0.001).

The main site of metastasis was the lung making up 52.9%
of the sample, followed by bone metastasis with 8.8% (Table 1).

The analysis of serum LDH levels on patients included in
this study showed that 38.6% of patients had values above 1.5
times the upper reference limit (400 mg/dl) (Table 1).

Immunohistochemistry was performed on 68 tumor sam-
ple from patients included in the study. The strong
expression of IGF1 was observed in 51.5% of the sample while
48.5% had weak positive expression (Table 1).

The main sites showing bone involvement were the femur
and pelvic bones with 22.2% involvement in each of these
Table 1 – Clinical characteristics and prognostic variables of interest of 77 patients diagnosed with EFTs treated at BCH, period 2003–2012.

| Variable                        | n    | (%)  |
|---------------------------------|------|------|
| **Age**                         |      |      |
| ≤15 years                       | 35   | 45.5 |
| >15 years                       | 42   | 54.5 |
| **Gender**                      |      |      |
| Male                            | 49   | (63.6) |
| Female                          | 28   | (36.4) |
| **Race**                        |      |      |
| White                           | 64   | (83.1) |
| Black                           | 2    | (2.6) |
| Brown                           | 10   | (13.0) |
| Yellow                          | 1    | (1.3) |
| **Location of the primary tumor**|    |      |
| Skeletal                        | 45   | (58.4) |
| Axial                           | 32   | (41.6) |
| Extremity                       | 13   | (16.9) |
| Soft tissue                     | 32   | (41.6) |
| Axial                           | 8    | (10.5) |
| Extremity                       | 24   | (31.5) |
| **Disease at diagnosis**        |      |      |
| Localized                       | 43   | (55.8) |
| Metastatic                      | 34   | (44.2) |
| **Metastasis site**             |      |      |
| Lung                            | 18   | (23.2) |
| Bone                            | 3    | (3.9) |
| Lung + bone                     | 8    | (10.4) |
| Central nervous system          | 3    | (3.9) |
| Other locations                 | 2    | (2.6) |
| **Treatment protocol**          |      |      |
| Euro Ewing                      | 10   | (13) |
| Brazilian                       | 46   | (60.8) |
| South American                  | 21   | (27.3) |
| **Local control**               |      |      |
| Surgery                         | 18   | (23.2) |
| Radiotherapy                    | 30   | (39.4) |
| Surgery + radiotherapy          | 15   | (19.7) |
| Without local control           | 14   | (18.3) |
| **Serum LDH level** a           |      |      |
| <600 mg/dl                      | 43   | (61.4) |
| ≥600 mg/dl                      | 27   | (38.6) |
| **Expression of IGFR** b        |      |      |
| Weak positive                   | 33   | (48.5) |
| Strong positive                 | 35   | (51.5) |

a 7 patients had no serum LDH levels at diagnosis.
b 9 patients had no biological samples in the files of the Pathology Dept.

segments. For tumors located in soft tissues, the rib cage (Askin tumors) was the main site affected totaling 43.8% of patients.

All patients received chemotherapy prior to the local treatment of the neoplasia at BCH. The local treatment of the lesion with radiotherapy and/or surgery was as follows: 22.6% of patients were submitted only to surgery, 39.4% underwent exclusive radiotherapy, 19.7% underwent surgery and radiotherapy and 18.3% patients died before the end of neoadjuvant chemotherapy (Table 1).

The treatment modality applied to the primary site of neoplasia associated with the location of the tumor showed that in lesions with axial involvement, surgical treatment was performed in 29.7% of lesions with axial involvement and 55.9% in extremity lesions (p = 0.023). Exclusive radiotherapy was indicated in 64.9% of patients with axial lesions and 52.9% of patients with tumors located in the extremities (p = 0.218). A combination of modalities such as local treatment with surgery and radiotherapy was present in 18.9% of patients with axial lesions (p = 0.069). Among patients metastatic at diagnosis 71.9% underwent local treatment with radiotherapy (p = 0.048). The IGFR expression showed an association with the variable metastasis at diagnosis (p < 0.001).

We found OS of 45% at 5 years (Fig. 1) and EFS of 30% at 5 years (Fig. 2).
Table 2 – Overall survival probability and cumulative event-free survival according to clinical characteristics and prognostic variables of interest for patients diagnosed with EFTs treated at BCH, 2003–2010.

| Variable                      | Overall survival | Cumulative event-free survival |
|-------------------------------|------------------|--------------------------------|
|                               | 2-years | 5-years | p Value | 2-years | 5-years | p Value |
| Overall survival              | 61%     | 45%     | 0.202   | 59%     | 30%     | 0.417   |
| Age group                     |         |         |         |         |         |         |
| ≤15 years                     | 72%     | 51%     |         | 69%     | 30%     |         |
| >15 years                     | 47%     | 40%     |         | 48%     | 32%     |         |
| Gender                        |         |         |         |         |         |         |
| Male                          | 62%     | 37%     | 0.555   | 67%     | 30%     | 0.587   |
| Female                        | 58%     | 58%     |         | 50%     | 32%     |         |
| Location                      |         |         |         |         |         |         |
| Skeletal                      | 58%     | 35%     | 0.457   | 59%     | 18%     | 0.243   |
| Soft tissue                   | 64%     | 53%     |         | 60%     | 40%     |         |
| Location                      |         |         |         |         |         |         |
| Axial                         | 59%     | 32%     | 0.256   | 59%     | 18%     | 0.502   |
| Extremity                     | 63%     | 58%     |         | 60%     | 40%     |         |
| Metastasis site               |         |         |         |         |         |         |
| Without metastasis            | 69%     | 53%     | 0.293   | 63%     | 48%     | 0.009   |
| Lung                          | 88%     | 45%     |         | 75%     | 33%     |         |
| Extrapulmonary                | 47%     | 40%     |         | 41%     | 10%     |         |
| Local treatment               |         |         | <0.001  |         |         | 0.008   |
| No treatment                  | 20%     | 20%     |         | 20%     | 20%     |         |
| Radiotherapy                  | 63%     | 53%     |         | 58%     | 44%     |         |
| Surgery                       | 75%     | 64%     |         | 58%     | 48%     |         |
| Surgery + radiotherapy        | 67%     | 33%     |         | 85%     | 0%      |         |
| Treatment protocol            |         |         | 0.840   |         |         | 0.647   |
| Euro Ewing                    | 78%     | 44%     |         | 67%     | 33%     |         |
| Brazilian                     | 57%     | 47%     |         | 58%     | 30%     |         |
| Serum LDH level               |         |         | 0.710   |         |         | 0.747   |
| <600 mg/dl                    | 66%     | 46%     |         | 64%     | 15%     |         |
| ≥600 mg/dl                    | 55%     | 46%     |         | 61%     | 34%     |         |
| Expression of IGFR            |         |         | 0.502   |         |         | 0.628   |
| Weak positive                 | 70%     | 43%     |         | 60%     | 15%     |         |
| Strong positive               | 65%     | 42%     |         | 61%     | 34%     |         |

We did not find statistically significant differences when evaluating OS and EFS for the variables: age group, gender, location, treatment protocol, LDH levels and expression of IGFR (Table 2).

There was statistically significant difference for both OS and EFS for the local treatment of tumor and OS rates for patients undergoing surgery of 64% at 5 years ($p < 0.001$) and 5-year EFS of 48% ($p = 0.008$) (Table 2). The simple regression model for local control with surgery was statistically significant (HR = 0.11 [0.03–0.41], $p = 0.001$) and OS (HR = 0.19 [0.06–0.61], $p = 0.005$).

We found a worse prognosis in EFS for patients with extrapulmonary metastases, showing a 5-year survival of 10% ($p = 0.009$) (Table 2). Analyses of the results by the simple regression model showed statistical significance for EFS of patients with extrapulmonary metastases compared to patients with lung metastasis (HR = 3.13 [1.33–7.37], $p = 0.009$).

Categorizing metastatic patients into two subgroups, lung metastases and extrapulmonary metastasis, EFS was 10% at 5 years for patients with extrapulmonary metastasis ($p = 0.015$) (Table 3). The univariate Cox regression showed

Table 3 – Overall survival probability and cumulative event-free survival according to the variable location of metastases in patients diagnosed with metastatic EFTs treated at BCH, in the period 2003–2010.

| Variable     | Overall survival | Event-free survival |
|--------------|------------------|---------------------|
|              | 2-years | 5-years | p Value | 2-years | 5-years | p Value |
| Metastasis   |         |         |         |         |         |         |
| Lung         | 88%     | 45%     | 0.164   | 75%     | 33%     | 0.015   |
| Extrapulmonary | 47%   | 40%     |         | 41%     | 10%     |         |
better prognosis for patients with lung metastases compared to patients with extrapulmonary metastasis (HR = 2.82 [1.18–6.76], p = 0.009).

Discussion

The average age of patients enrolled in our study was 15 years (standard deviation [SD] = 5.42 years). The literature emphasizes a higher incidence in the second decade of life, with the majority of patients affected being young people under 20 years.6,24 Our sample consisted of 63.6% of males and 36.4% females, and literature describes the incidence of EFTs slightly higher in males. Retrospective study based on the database belonging to the National Cancer Institute’s Surveillance, involving 725 cases in the period between 1989 and 2007 obtained a ratio of incidence between males and females of 3:2.25 Another study with a sample of 300 patients in the period between 1980 and 2005 shows a frequency of 58% in males.5 Only 2.6% of black subjects participated in our study. Our study is consistent with the literature since EFTs is described as being rare in black people.5 A study involving 220 patients found 93.2% of white patients and 6.8% of other races.27

EFTs may be present in skeletal or soft tissues. Of the patients included in the study, 58.4% had disease with skeletal location and 41.6% located in soft parts, while some studies present a higher incidence in soft tissues. A multicenter study involving 114 patients in different cancer treatment centers in Turkey demonstrates a rate of 53.5% for tumors located in soft tissues.11 In contrast, a study conducted at St. Jude Children’s Research Hospital found 87.9% of patients with involvement of the skeletal neoplasia.28 Besides the skeletal and soft tissue location, the location of EFTs can be further subdivided into axial and extremity; in our study, 48.0% of patients had neoplasia with axial location while 52.0% were skeletal location. Among those located in soft tissues, 75.5% were axial, while in skeletal 71.1% affected the extremity. The main sites presenting skeletal involvement were femur and bones of the pelvis (22.2% for each anatomical region) and in tumors having soft tissue involvement it was the rib cage (43.8%). The literature infers that the main sites showing EFTs skeletal involvement are bones of the pelvis, femur, tibia and humerus.28 In EFTs with soft tissue involvement, the most frequent location is the thoracic wall.11 In contrast, some cases show involvement in the soft tissues, more commonly on extremities.3,30,31 The evaluation of localized or metastatic disease at diagnosis showed that 44.2% of patients had metastatic disease, with the pulmonary site affected in 46.8% followed by the skeletal site with 28.6%. This finding may suggest and be explained by the delayed diagnosis in some of these patients because of the difficulty of access to specialized centers, since according to literature about 25–30% of patients diagnosed with EFTs presented metastases at diagnosis.32,33 Other studies show a rate of metastases at diagnosis of 13% in patients with primary tumor location in the soft tissues.34 The preferred site of metastasis described in literature is consistent with the findings in this study, where lung is the main site (50% of cases), followed by bone involvement (25%) and/or bone marrow (20%).33 In our study, lung was the most common site of metastasis with 52.9%. We observed that 88.2% of patients metastatic at diagnosis were male, with an association between the variables gender and metastasis at diagnosis (p < 0.001).

Among the patients included in the study, 18.3% died before local treatment. Of patients undergoing local treatment, surgery was carried out in 22.6% of the cases, exclusive radiotherapy in 39.4% and the combination of surgery with radiotherapy in 19.7%. The complete surgical removal is considered the modality of choice for local treatment, showing a lower rate of local recurrence compared with isolated radiotherapy for the treatment of the primary tumor.10 The low rate of isolated surgery and/or combined with radiotherapy seen in our study may be explained once we got a rate of 44.2% for metastases at diagnosis. Metastasis is considered the worst prognostic factor for patients with EFTs.32 In metastatic patients with unresectable metastases, surgical treatment, which can often involve loss of body segments, is losing its place to local treatment with radiotherapy. Among patients metastatic at diagnosis 71.9% underwent radiotherapy as treatment of the primary site of the tumor, displaying an association between the variables metastatic disease and radiotherapy (p = 0.048). Upon evaluating the local treatment modality associated to the primary site of the tumor, we observed that in lesions of axial involvement the surgical treatment was performed in 29.7% and in the lesions of extremity in 59.5%, showing an association between the variables local treatment modality and tumor location (p = 0.023). This fact is explained by the difficulty of surgical access and the obtaining of adequate surgical margins in lesions of axial involvement. The apparent superiority of the surgery may represent a selection bias, since most central and larger lesions are often treated with radiotherapy.35

EFTs biological samples used in this study showed 51.5% of specimens with strongly expressed IGFR and 48.5% with weak positivity. When we evaluated the presence of metastases at diagnosis along with the expression of IGFR we found an association between the variables (p < 0.001). The literature, in principle, does not contemplate the association between expression of IGFR and metastasis at diagnosis. Perhaps the strong positivity for the expression of IGFR may be a predictor of metastasis at diagnosis in EFTs.

We found an OS of 45% at 5 years and 30% of EFS at 5 years. The literature shows EFS around 70% with the awareness of the chemotherapy administration concept of interval regimen in addition to local treatment.5 In contrast, studies show that the advanced cases have not presented encouraging results when subjected to the standard treatment approach, with regard to improving survival rates, where the 5-year rate has been recently described as lower than 25%.36 Our study involved a greater portion of patients metastatic at diagnosis than that described in the literature; since metastasis at diagnosis is the worst prognostic factor, we believe that EFS of 30% at 5 years for the patients included in the study is justified.

Although prognostic factors in EFTs are diverse, it is possible to notice discrepancy in these factors in some of the studies. Age is a prognostic factor in literature, where patients younger than 15 years have a better prognosis compared to adolescent patients aged over 15 years and adults.7,10,14,15
However, a review of two German clinical trials for the treatment of ES involving patients older than 40 years at diagnosis had a survival comparable to adolescents treated in the same trial. Our study found that patients aged less than 15 years, the curve shows improved survival in this group compared with older patients, however, there were no statistically significant differences between the curves of OS and EFS (respectively, \( p = 0.202 \) and \( p = 0.417 \)).

Our study suggests better OS and EFS in female subjects with OS of 58% at 5 years and EFS of 32%, but no statistical significance was found (respectively, \( p = 0.555 \) and \( p = 0.587 \)). Studies show the variable gender as a prognostic factor in EFTs, indicating greater survival in females.\(^{34}\)

Our results favor higher OS and EFS in patients with tumors located in soft tissues when compared to those of skeletal site, however, there was no statistical significance in the analysis (respectively, \( p = 0.457 \) and \( p = 0.243 \)). Literature data makes inference to worse prognosis in tumors localized in soft tissue.\(^{32}\) A study using cancer registry-based population found no difference in prognosis with respect to axial or extremity location of the tumor.\(^{25}\) Upon evaluating OS and EFS in our study, the data suggest better OS and EFS in patients with extremity lesions compared with lesions of axial involvement, however, there was no statistical significance (respectively, \( p = 0.256 \) and \( p = 0.502 \)).

EFS was statistically significant when analyzing the variable metastases at diagnosis, with a rate of 48% at 5 years for non-metastatic patients, 33% for those with lung metastases and \( 10\% \) for extrapulmonary metastases (\( p = 0.009 \)). Upon evaluating these data in a univariate Cox regression, we found a worse prognosis in patients with extrapulmonary metastasis (HR = 3.13 [1.33–7.37], \( p = 0.009 \)). Upon categorizing metastatic patients into two subgroups, pulmonary metastasis and extrapulmonary metastasis, we also observed a worse prognosis in patients affected by extrapulmonary metastasis, with EFS of \( 10\% \) at 5 years (\( p = 0.015 \)). The univariate Cox regression showed better prognosis for patients with lung metastases compared to patients with extrapulmonary metastasis (HR = 2.82 [1.18–6.76], \( p = 0.009 \)). Literature shows a worse prognosis for extrapulmonary metastases.\(^{7,10,37}\) The presence of bone metastasis only seems to have a better prognosis compared to patients who, in addition to extrapulmonary involvement, also displayed pulmonary involvement.\(^{38}\)

The local treatment modality demonstrates a better OS and EFS for patients submitted to surgery alone, and 64% at 5 years for OS and \( 48\% \) for EFS, showing a statistical significance (respectively, \( p < 0.001 \) and \( p = 0.008 \)). Univariate Cox regression for local control with surgery was statistically significant (HR = 0.11 [0.03–0.41], \( p = 0.001 \)). As mentioned above, this superiority for the surgery may represent a selection bias, since larger and more central lesions are often treated with radiotherapy.\(^{37}\) We stress the need for randomized clinical trials to better assess the surgical superiority over other local treatment modalities.

Serum LDH level was categorized into 2 groups: <600 mg/dl and \( \geq 600 \) mg/dl. OS in 5 years was \( 46\% \) for the two groups in question, with no noticeable statistical significance for the variable (\( p = 0.710 \)). Seven patients had no serum LDH level at diagnosis, which may have been responsible for the similar results, since increased serum LDH levels before treatment are associated with a worse prognosis, and this increase can also be correlated with large primary tumors and metastasis.\(^{14}\)

Survival analysis considering the variable expression of IGFR was \( 42\% \) at 5 years for OS in case of strong positive and \( 42\% \) for weak positive (\( p = 0.502 \)). When we analyzed EFS, we observed a survival of \( 34\% \) at 5 years for the strongly positive and \( 15\% \) for the weak positive (\( p = 0.628 \)). There was no statistical significance in survival analysis considering the variable expression of IGFR. This fact may be explained by the size of the sample. Nine patients did not have any biological samples in the Pathology Department. The use of biological samples in studies involving cytogenetic evaluation has its limitations. Much of this material consists of fragments of tissue obtained by biopsy by way of needles, thus, scarce. The substance used for fixing such materials can also be a determining factor, especially in molecular studies. The creation of the so-called tumor banks, with the snap freezing of samples, may be the way for the optimization of studies involving cytogenetics.

Several studies seeking targeted therapies that are focused on blocking the activity of the IGF receptor have been performed. A recent review demonstrates that in some types of tumors the use of drugs to block the IGFR1 receptor has worsened the survival of these patients. A Phase II clinical trial involving treatment with drugs blocking the IGFR1 receptor in breast cancer after menopause, with positive receptor, showed worse overall survival. It was also mentioned that the resistance mechanisms to targeted therapy with the blocking of IGFR1 may be involved in combination with other receptors such as EGF, and concluded that the signaling pathways involved in IGFR are more complex than originally thought.\(^{39}\)

### Conclusions

We conclude that patients with a diagnosis of EFTs, treated at Barretos Cancer Hospital, show a higher incidence of metastases at diagnosis when compared to data from current literature. The strong expression of IGFR is more prevalent in patients with metastases at diagnosis. Extrapulmonary metastases were associated with worse prognosis. The exclusive surgical management of the primary site was associated with better prognosis compared with other local treatment modalities. At first, the expression of IGFR did not behave as a prognostic factor in EFTs.

### Conflicts of interest

The authors declare no conflicts of interest.

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