TUMOR CONTAMINATION IN THE BIOPSY PATH OF PRIMARY MALIGNANT BONE TUMORS

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ABSTRACT

Objective: To study factors possibly associated with tumor contamination in the biopsy path of primary malignant bone tumors. Method: Thirty-five patients who underwent surgical treatment with diagnoses of osteosarcoma, Ewing's tumor and chondrosarcoma were studied retrospectively. The sample was analyzed to characterize the biopsy technique used, histological type of the tumor, neoadjuvant chemotherapy used, local recurrences and tumor contamination in the biopsy path. Results: Among the 35 patients studied, four cases of contamination occurred (11.43%): one from osteosarcoma, two from Ewing's tumor and one from chondrosarcoma. There was no association between the type of tumor and presence of tumor contamination in the biopsy path (p = 0.65). There was also no association between the presence of tumor contamination and the biopsy technique (p = 0.06). On the other hand, there were associations between the presence of tumor contamination and local recurrence (p = 0.01) and between tumor contamination and absence of neoadjuvant chemotherapy (p = 0.02). Conclusion: Tumor contamination in the biopsy path of primary malignant bone tumors was associated with local recurrence. On the other hand, the histological type of the tumor and the type of biopsy did not have an influence on tumor contamination. Neoadjuvant chemotherapy had a protective effect against this complication. Despite these findings, tumor contamination is a complication that should always be taken into consideration, and removal of the biopsy path is recommended in tumor resection surgery.

Keywords – Neoplasm Seeding; Biopsy; Sarcoma; Bone Neoplasms; Neoplasm Recurrence, Local; Musculoskeletal System

INTRODUCTION

Malignant tumors of the musculoskeletal system are relatively rare forms of neoplasia, representing only 0.2% of all new cases of cancer[1]. Approximately 80% of them originate from soft tissues, and the remainder originate in bone tissue. On the other hand, they represent a group of very important diseases, given the morbidity and mortality that they cause and their particular incidence among young patients, which gives rise to great impairment to the lives of the individuals affected[2-5].

Dealing with these tumors requires integration of clinical, laboratory, radiographic and histological characteristics in order to achieve a precise diagnosis and management leading to successful treatment. In this respect, biopsy can be highlighted as a fundamental step in dealing with tumors of the musculoskeletal system, and it is indispensable for achieving a definitive diagnosis and for identifying the histological pattern of the tumor[4,6,7]. Biopsies should provide sufficient representative tissue sample for a precise diagnosis, but without excessively manipulating the lesion, so as to avoid modifying the tumor's relationship with the anatomical compartments and contaminating the neighboring tissues with tumor cells[7].

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Many surgeons with experience in treating musculoskeletal tumors advocate removal of the biopsy path at the time of surgically resecting the tumor, taking the view that this path is potentially contaminated by tumor cells[5,6,8-18]. However, no basis for this practice has been found in any scientific studies, and it is based more on personal experience than on the current literature. Even so, many issues are covered in a wide variety of studies, and untested hypotheses have arisen. Among these, there is a hypothesis that attempts to obtain several tissue samples in biopsies are associated with greater dissemination and consequently greater likelihood of contamination of the biopsy path[11]. Another hypothesis that has been published is that biopsies performed using a percutaneous technique are associated with less contamination of the biopsy path because they involve less manipulation of the tumor tissue[8,11,19-21]. It has also been observed that contamination of the biopsy path occurs more frequently in cases of soft-tissue sarcoma than in bone and cartilage lesions[17]. It is also believed that implementing neoadjuvant chemotherapy has a protective effect with regard to controlling tumor infiltration at the biopsy site[20,22] and that this contamination has a negative value in the prognosis for affected patients[23].

In the literature, there is a lack of detailed studies on biopsy paths in cases of musculoskeletal tumors[17,20,21]. Knowledge of the characteristics of contamination of biopsy paths within orthopedic oncology may provide important support for improving biopsy techniques and for following up patients affected by these tumors.

The aim of the present study was to study the factors possibly associated with tumor contamination of the biopsy path in cases of primary malignant bone tumors.

METHODS

A retrospective study was conducted on the medical files of all patients who underwent surgical treatment with a diagnosis of osteosarcoma, Ewing's tumor or chondrosarcoma at Hospital das Clínicas, Federal University of Pernambuco (HC-UFPE), between June 2005 and July 2011. The analysis was conducted independently of gender and age, biopsy technique used (whether open or percutaneous), institution (whether at HC-UFPE or elsewhere), team performing the biopsy and whether neoadjuvant chemotherapy was administered. The following patients were excluded: those whose biopsy path was not removed during the operation to resect the tumor; those whose biopsy path had not been examined from an anatomopathological point of view to define whether tumor cell contamination was present or absent; and those whose records did not present complete data for the required analyses.

At HC-UFPE, whenever possible, it is preferred to perform biopsies by means of the percutaneous technique, except in cases in which there is a risk of injury to prime structures, or in some cases of repetition of the biopsy because the previous examination was inconclusive. In addition, biopsies are performed by the same team that will perform the surgical treatment of the lesion. With regard to the biopsy path, it is routinely removed at the time of tumor resection surgery. To study the path, after this has been collected from the surgical specimen, the normal histological technique is used: fixing in 10% formol, dehydration in a series of alcohols, diaphanization, impregnation and embedding in paraffin, sectioning using a microtome and staining with hematoxylin and eosin); followed by analysis under an optical microscope to identify the presence or absence of tumor cells in the sample, which defines the presence or absence of tumor contamination, respectively.

All the patients in this study were operated by the same surgeon, who was one of the authors of this study (PMAL), and the anatomopathological evaluations were performed by the same pathologist, who was also one of the authors of this study (RJVM). Some of the patients evaluated came with biopsies already performed at another clinic, and this variable was not controlled for in this study.

It was observed that a total of 46 patients underwent surgical treatment with the abovementioned diagnoses during the study period. Of these, 11 were excluded because their data were incomplete, thereby impeding analysis. Thus the sample available for this study comprised 35 patients, of which 19 were female and 16 were male, with a mean age of 30.7 years (ranging from eight to 77 years).

The sample was analyzed to characterize the biopsy technique used (open or percutaneous), histological type of tumor (osteosarcoma, Ewing's tumor or

chondrosarcoma), neoadjuvant chemotherapy used (yes or no), local recurrence and tumor contamination of the biopsy path (present or absent) (Table 1). The data were catalogues in contingency tables and were subjected to statistical analysis. Hypotheses were analyzed by means of Fisher's exact test and the G test with Williams correction. The descriptive level (p value) was taken to be 5%. The BioEstat 5.0 software was used for analyzing the data.

RESULTS

Out of the 35 patients studied, 15 cases (42.86%) were osteosarcoma, 10 cases (28.57%) were Ewing's tumor and 10 cases (28.57%) were chondrosarcoma. Contamination of the biopsy path was observed in four of the 35 patients evaluated, representing 11.43% of the sample. Among these, there was one case of osteosarcoma, two of Ewing's tumor and one of chon-

Table 1 – Patient distribution according to diagnosis, biopsy technique, use of neoadjuvant chemotherapy, presence of local recurrence and occurrence of tumor contamination in the biopsy path.

Case	Diagnosis	Biopsy technique	Neoadjuvant chemotherapy	Local recurrence	Contamination of the biopsy path
1	Osteosarcoma	Percutaneous	Yes	No	No
2	Osteosarcoma	Percutaneous	Yes	No	No
3	Osteosarcoma	Percutaneous	Yes	No	No
4	Osteosarcoma	Percutaneous	Yes	No	No
5	Osteosarcoma	Percutaneous	Yes	No	No
6	Osteosarcoma	Percutaneous	Yes	No	No
7	Osteosarcoma	Percutaneous	Yes	No	No
8	Osteosarcoma	Percutaneous	Yes	No	Yes
9	Osteosarcoma	Percutaneous	Yes	No	No
10	Osteosarcoma	Percutaneous	Yes	No	No
11	Osteosarcoma	Percutaneous	Yes	No	No
12	Osteosarcoma	Open	Yes	No	No
13	Osteosarcoma	Percutaneous	No	No	No
14	Osteosarcoma	Percutaneous	Yes	No	No
15	Osteosarcoma	Percutaneous	Yes	No	No
16	Ewing's tumor	Percutaneous	Yes	No	No
17	Ewing's tumor	Open	Yes	No	No
18	Ewing's tumor	Percutaneous	Yes	No	No
19	Ewing's tumor	Percutaneous	Yes	No	No
20	Ewing's tumor	Open	Yes	No	No
21	Ewing's tumor	Percutaneous	Yes	No	No
22	Ewing's tumor	Open	Yes	No	No
23	Ewing's tumor	Open	No	Yes	Yes
24	Ewing's tumor	Open	No	Yes	Yes
25	Ewing's tumor	Open	Yes	No	No
26	Chondrosarcoma	Percutaneous	*	No	No
27	Chondrosarcoma	Percutaneous	*	No	No
28	Chondrosarcoma	Open	*	No	Yes
29	Chondrosarcoma	Percutaneous	*	No	No
30	Chondrosarcoma	Open	*	No	No
31	Chondrosarcoma	Percutaneous	*	No	No
32	Chondrosarcoma	Open	*	No	No
33	Chondrosarcoma	Percutaneous	*	No	No
34	Chondrosarcoma	Percutaneous	*	No	No
35	Chondrosarcoma	Percutaneous	*	No	No

* For the cases of chondrosarcoma, neoadjuvant chemotherapy did not apply.

drosarcoma. No association between the tumor type and the presence of tumor contamination in the biopsy path was observed (p = 0.65).

In analyzing the sample as a whole, it was observed that out of the four cases that presented contamination, three were biopsied by means of the open technique and one by means of the percutaneous technique. There was no statistically significant difference (p = 0.06) in relation to the cases without contamination, regarding the biopsy technique. The variable of chemotherapy could only be evaluated in relation to the cases of osteosarcoma and Ewing's tumor, which are the types of tumor to which this therapeutic method applies. Thus, there were three contaminations in 25 cases. One of these three cases received neoadjuvant chemotherapy and two of them did not. Among the cases without contamination, 21 received neoadjuvant chemotherapy and one did not. There was a statistically significant difference between the groups (p = 0.02), thus showing an association between not administering neoadjuvant chemotherapy and occurrence of contamination in the biopsy path. Regarding local recurrence, it was observed that out of the four patients who presented contamination, two evolved with this complication. None of the cases without contamination presented local recurrence. In relation to this variable, a statistically significant difference was observed between the groups with and without contamination (p = 0.01).

In evaluating the data relating to each type of tumor individually, it was observed that in the cases of osteosarcoma, contamination occurred in 15 of the patients studied (6.67%). In relation to the biopsy technique used, the case that presented contamination was biopsied using the percutaneous technique. In the cases without contamination, one was biopsied using the open technique and 13 using the percutaneous technique. It was not possible to detect any association between the biopsy technique used and occurrences of contamination in the osteosarcoma cases (p = 0.93). It was also observed that the case with contamination in the biopsy path and 13 out of the 14 cases without contamination received neoadjuvant chemotherapy, while one of the cases without contamination did not receive it. Again, no association was detected between this variable and occurrences of contamination in the biopsy path (p = 0.93). In relation to local recurrence, none of the patients with a diagnosis of osteosarcoma presented this complication.

Regarding the cases of Ewing's tumor, two cases of contamination occurred among the 10 patients studied (20%). These two cases occurred in patients who had not received neoadjuvant chemotherapy. On the other hand, the other eight patients who received neoadjuvant chemotherapy did not present contamination. It was observed that in these cases, not administering neoadjuvant chemotherapy was associated with occurrences of contamination in the biopsy path (p = 0.02). Regarding the biopsy technique used, no statistically significant difference was observed between the patients who underwent open biopsy and those with percutaneous biopsy in relation to occurrences of contamination of the path. There was contamination in two of the six cases of open biopsy and no contamination in any of the four cases of percutaneous biopsy (p = 0.33). Regarding local recurrence, the two cases with contamination presented this complication, while the other eight cases without contamination did not; there was a statistically significant difference between the patients with and without contamination, in relation to local recurrence (p = 0.02).

Among the patients with a diagnosis of chondrosarcoma, contamination occurred in 10 patients studied (10%). This contamination occurred in one patient who underwent open biopsy. In the nine cases without contamination, two received open biopsy and seven, percutaneous biopsy. No association was detected between the biopsy technique and occurrences of contamination among the patients with a diagnosis of chondrosarcoma (p = 0.30). In relation to local recurrence, none of the patients with a diagnosis of chondrosarcoma presented this complication.

DISCUSSION

The perception that the biopsy path in cases of musculoskeletal tumors might be contaminated by tumor cells seems to have been reinforced within the orthopedic community through the study by Cannon and Dyson[18], who reported that there was statistically significant lower occurrence of local tumor recurrence in cases in which the biopsy path was resected, in comparison with the cases in which it was not. The literature pertinent to this topic reveals that local recurrence was constantly observed in a series of reports on cases in which the biopsy path had not been resected[19,22-27]. On the other hand, in studies by Kaffenberger *et al*[21] and Saghieh *et al*[28], in which biopsy

paths produced using the percutaneous technique had not been resected, no local recurrence was observed.

In our sample, it was seen that among the four cases that presented contamination, there was local recurrence in two cases, thus showing a statistically significant difference in relation to local recurrence in the group without contamination ($p = 0.01$). When the sample was individualized according to the histological type of the tumor, it was observed that there was no local recurrence among the cases of osteosarcoma and chondrosarcoma. On the other hand, among the cases of Ewing's tumor, it was observed that the two cases that presented contamination evolved with local recurrence, while none of the eight cases without contamination presented this complication, and this was a statistically significant difference between the groups ($p = 0.02$). Taking into consideration both the results found in the present study sample and the analyses in the literature, the possibility for local recurrence in patients whose biopsy path had not been removed was considerable. Thus the practice of resecting the path is recommendable despite what was shown in the studies by Kaffenberger *et al*[21] and Saghieh *et al*[28].

Some authors believe that because biopsies performed using the percutaneous technique involve less manipulation of the tumor tissue, they are associated with lower occurrence of path contamination[8,11,13]. In our sample, out of the four patients with contamination, three underwent open biopsy and one, percutaneous biopsy. However, despite this difference, no statistically significant difference was observed in relation to patients without contamination, with regard to the biopsy technique used ($p = 0.06$). When the sample was individualized according to the type of tumor, again there was no association between the biopsy technique and occurrences of contamination in the cases of osteosarcoma ($p = 0.93$), Ewing's tumor ($p = 0.33$) or chondrosarcoma ($p = 0.30$).

In analyzing a set of eight cases of tumor contamination in biopsy paths made in the musculoskeletal system that were reported in the literature[19,22-26], it was observed that percutaneous biopsy was performed in seven of them[19,22-25] and open biopsy in one case[26]. Also analyzing the literature, Mohana *et al*[20] observed two cases of contamination among six cases of open biopsy (33.3%) and three cases of contamination among 20 cases of percutaneous biopsy (15%). No reference was made to the criteria for choosing the biopsy technique, and it was not stated whether there was homogeneity between the two groups. Although the authors believed that biopsies using the percutaneous technique presented lower risk of contamination of their path, compared with the open technique, no statistical method was used to test this hypothesis. In the study by Ribeiro *et al*[17] four cases of contamination occurred among seven open biopsies (57.1%) while there were another four cases of contamination among 18 percutaneous biopsies (22.2%). These authors also did not perform statistical tests to evaluate the significance of these differences. It should be noted that they studies both bone tumors and soft-tissue tumors, and that all the bone tumors underwent percutaneous biopsy, while all the soft--tissue tumors underwent open biopsy by means of mini-incisions. Thus, in comparing the occurrences of contamination between the open and percutaneous techniques in their study, it needs to be borne in mind that the biopsy technique chosen was different for the different tumor types, thus making the two groups very heterogenous. In the studies by Kaffenberger *et al*[21] and Saghieh *et al*[28], all the biopsies were performed by means of the percutaneous technique. In these two studies, no contamination of the biopsy path occurred. Although the results shown in the present study sample and the observations made in the literature indicate a tendency for biopsies using the percutaneous technique to be associated with less local recurrence than with open biopsy, this cannot be considered statistically. The main remark that should be put forward is that tumor contamination in biopsy paths is a reality even in biopsies performed using percutaneous techniques, thus reinforcing the need for path removal at the time of tumor resection.

Another issue raised in the literature is the influence of the type of tumor on occurrences of tumor contamination in the biopsy path[17,29]. Ribeiro *et al*[17], who studied both bone and soft-tissue tumors, found four cases of contamination (57.1%) among seven cases of soft-tissue tumors and four cases of contamination (22.2%) among 18 cases of bone tumors. These authors suggested that greater cellularity and lower quantities of matrix, which are particular characteristics of soft-tissue sarcomas, would be related to greater cell dissemination, in comparison with bone tumors. However, they stressed that no statistical test was performed to assess the significance of this difference.

Among the 35 patients in our study, there were four cases of contamination: one out of the 15 osteosarcoma cases, two out of the 10 Ewing's tumor cases and one out of the 10 chondrosarcoma cases. No association between the type of tumor and occurrences of contamination ($p = 0.64$).

Some authors believe that neoadjuvant chemotherapy has a protective effect with regard to controlling tumor infiltration at the biopsy site[20,22]. Mohana *et al*[20] observed that the occurrence rate of tumor contamination among patients who received neoadjuvant chemotherapy was 12.5% (three out of 24 cases). In their sample, the only two cases that did not receive neoadjuvant chemotherapy evolved with contamination in the biopsy path. However, it should be noted that the three patients who received neoadjuvant chemotherapy and presented contamination had poor responses to chemotherapy. In the study by Saghieh *et al*[28], in which neoadjuvant chemotherapy was administered to all the patients, there was no contamination in the biopsy path. In our sample, the effect of neoadjuvant chemotherapy could only be evaluated for the cases of osteosarcoma and Ewing's tumor, since these are the tumor types to which this therapeutic method applies. Thus, there were three cases of contamination out of 25 cases studied (12%). Among these three cases, two did not receive neoadjuvant chemotherapy while one did. Among the cases without contamination, 21 received neoadjuvant chemotherapy and one did not, and thus there was an association between occurrence of contamination and not administering neoadjuvant chemotherapy ($p = 0.02$). When the osteosarcoma cases were analyzed separately, this statistically significant difference was not observed ($p = 0.93$), thus differing from the cases of Ewing's tumor ($p = 0.02$). It should be noted that we did not evaluate the tumor response to the chemotherapeutic regimen used, although this is a factor that could be taken into consideration in evaluating the effect of neoadjuvant chemotherapy as a protection against tumor contamination. Analysis on our sample and observations on the results from the studies by Mohana *et al*[20] and Saghieh *et al*[28] reinforce the idea that this therapeutic method has some protective influence against occurrences of tumor contamination in the biopsy path.

The limitations of our study include the small sample size, which may have compromised the analysis on the phenomena studied. However, in studies that deal with tumors of the musculoskeletal system, this is a frequently occurring reality, because of the relative rarity of these tumors. In addition to this issue, the diversity of the diagnoses studied and the heterogeneity of the cases may be limiting factors regarding the observations made. Moreover, there was a lack of well defined criteria for choosing the biopsy technique to be used, and there was no control over the clinic where the biopsy had been performed. Another matter that deserves to be taken into consideration is that the staging of the tumors was also not controlled for in this study, and the tumors were also not divided into their subtypes, which are known to present differentiated behavior.

CONCLUSION

The presence of tumor contamination in the biopsy path of primary malignant bone tumors was associated with local recurrence. On the other hand, it was not shown to be influenced by the type of biopsy performed or the type of tumor studied. Neoadjuvant chemotherapy was shown to be an effective protector against this event. Despite these findings, tumor contamination is a complication that should always be taken into consideration, and removal of the biopsy path is recommended in cases of tumor resection surgery.

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