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Original Article

Inter- and intra-observer reliability in histologic evaluation of necrosis rate induced by neo-adjuvant chemotherapy for osteosarcoma

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Abstract: Purpose: Tumor necrosis rate following neo-adjuvant chemotherapy is one of the most important prognostic factors for patients with osteosarcoma, and it also provides the basis for selection of postoperative adjuvant chemotherapy. However, reported necrosis rates for the same tumors can vary among pathologists, complicating decision making for further treatment. Methods: Ten H&E stained pathology slides from 10 osteosarcoma patients treated with neo-adjuvant chemotherapy were randomly selected. Six expert pathologists were assigned to analyze the slides for tumor necrosis rate at four time points, with an interval of 3 weeks. Intraclass and interclass correlation coefficients (IntraCC & InterCC) and 95% confidence intervals (CI) were calculated. Results: The overall InterCC among the 6 observers was 0.652 (95% CI 0.294-0.820) for tumor necrosis rate (range: 0-100%), suggesting good reliability. IntraCCs were 0.799, 0.788, 0.867, 0.935, 0.962, and 0.947 respectively. The interCC among higher careers and lower careers were 0.603 (95% CI.: 0.387-0.843) and 0.696 (95% C.I.: 0.487-0.919), respectively, which was not significantly different. Major differences in tumor necrosis estimation were due to interpretation of areas with isolated atypical cells in fibrotic stroma. Conclusion: Low inter-observer and relatively high intra-observer reliability were observed in the histologic evaluation of necrosis rate after neo-adjuvant chemotherapy. The interCCs between the higher career and lower career groups did not vary. These findings suggest that a valid measurement protocol for tumor necrosis rate evaluation after chemotherapy is required to improve the clinical relevance of the quantification of response to neo-adjuvant therapy.

Keywords: Osteosarcoma, tumor necrosis, chemotherapy response, observer variation

Introduction

Chemotherapy for osteosarcoma has dramatically improved the survival rate of osteosarcoma. The 5-year survival ratefor patients with localized disease has improved from 20 to 70% in the last three decades [1]. One of the most important prognostic factors for osteosarcoma is the percentage of tumor necrosis induced by neo-adjuvant chemotherapy [2]. This factor also provides the basis for selecting postoperative adjuvant chemotherapy and estimating the clinical relevance of other evaluation methods for chemotherapy response, such as diffusion MRI or ^{18}F-FDG PET CT. Many studies suggested a cut-off value for tumor necrosis rate of 90%, and patients with a rate over 90% are considered good responders [3]. Recently, many studies raised questions about the belief that the histological response cut-off value of 90% tumor necrosis was correlated with prognosis [4-11]. Li et al. reported that a tumor necrosis rate over 90% was not correlated with better disease-free survival or overall survival, but a rate>70% was significantly correlated with disease-free survival [4]. In their study, only 25% of

Table 1. Careers of the six observers

Observer	Years*	Career in musculoskeletal pathology**	No. of osteosarcoma cases evaluated/year†	Study abroad Y/N (period)‡
#1	1991	> 15 years	4-6 cases	Yes (6 months)
#2	2002	5-10 years	≥11 cases	No
#3	1995	> 15 years	≥11 cases	No
#4	2002	10-15 years	≥11 cases	No
#5	1982	> 15 years	≥11 cases	Yes (2 years)
#6	1986	> 15 years	≥11 cases	Yes (2 years)

*Year professional certification in pathology was acquired. **Career in musculoskeletal pathology. †Number of osteosarcoma cases diagnosed each year. ‡Experience studying abroad for a sub-specialty in musculoskeletal pathology (#1 and #5, Mayo Clinic; #6, Albert Einstein College of Medicine and Hospital for Special Surgery).

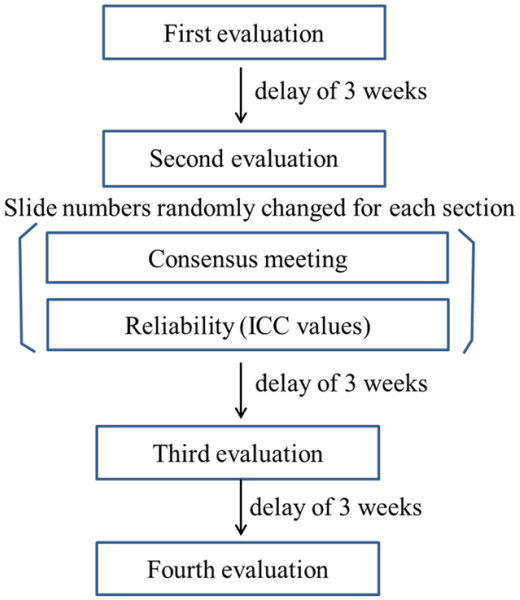

Figure 1. Evaluation the flowchart of this study.

patients achieved 90% or greater tumor necrosis, which was much lower than in prior studies showing 50% or more good responders. However, their 5-year overall survival rate was 65.9%, which is similar to those of previous studies [4, 12, 13]. Several studies reported the discrepancy between tumor necrosis and survival rate and have concluded that histologic response to chemotherapy has lost its prognostic importance, despite the contribution of chemotherapy to osteosarcoma treatment [5, 7-11, 14-16]. These studies recommended further prospective randomized and controlled studies with large samples to investigate the relationship between percentage of tumor necrosis and survival of osteosarcoma patients.

Large discrepancies have been reported in necrosis rates for the same tumors among pathologists. We presumed that the loss of the clinical relevance of necrosis rate might have originated from disagreement in the histologic interpretation of tumor necrosis rate. To the best of our knowledge, there has been no study evaluating this issue.

The purpose of this study was to evaluate the inter- and intra-observer reliability in histopathological estimation of the necrosis rate after neo-adjuvant chemotherapy for osteosarcoma and to revisit the clinical relevance of the necrosis rate induced by neo-adjuvant therapy.

Methods

Patients and pathology

The Institutional Review Board of our hospital approved this study. Ten consecutive patients who underwent two cycles of neo-adjuvant chemotherapy and sequential surgery for conventional osteosarcoma at Seoul St. Mary's Hospital between 2012 and 2014 were enrolled. Histologic subtypes were as follows: osteoblastic (n=6), fibroblastic (n=2), and mixed osteoblastic and chondroblastic (n=2). A single representative hematoxylin and eosin-stained pathology slide of the osteosarcoma was selected from each case. One pathologist (CKJ) was responsible for slide selection. The glass slides were assigned random case numbers and sent to six pathologists.

Observers

Six expert pathologists were assigned to analyze the slides for tumor necrosis rate (**Table 1**). They majored in musculoskeletal oncology, and four of six observers diagnosed 10 or more

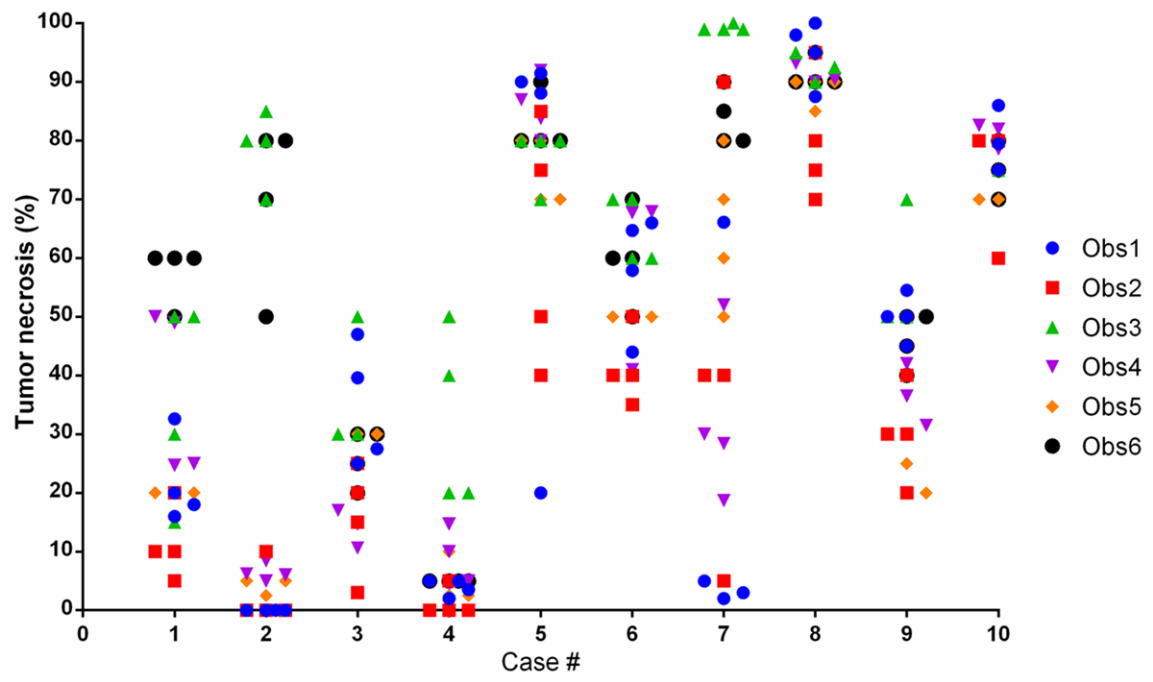

Figure 2. Dot plot showing a dataset from four evaluations of six pathologists. The most discrepant data were from slide #2 and slide #7, ranging from 0 to 85%, and from 2 to 100%, respectively.

cases of osteosarcoma every year with careers of over 10 years in musculoskeletal pathology. The mean pathology career length was 22.8 years (range, 14 to 34 years). Three observers experienced abroad study of musculoskeletal oncology as a sub-specialty.

Evaluation flow design

Observers evaluated the tumor necrosis rate of each slide at 4 time points with a delay of at least 3 weeks to minimize recall bias. Every time the slides were evaluated, randomized numbers were assigned to each slide. After the second evaluation, the six observers met to share opinions and establish a consensus for estimating tumor necrosis rate. Until matched evaluation sheets of each pathologist were obtained for statistical analysis, pathologists and other authors were blinded to randomized numbers of slides and patient information (**Figure 1**).

Statistical analysis

Statistical analyses were conducted with SPSS 18.0 software for Windows (IBM, Armonk, NY, USA). The means, standard deviations, intraclass and interclass correlation coefficients (IntraCC and interCC), and 95% confidence intervals (CIs) were calculated [17]. To evaluate the effect of experience with histologic diagnosis of osteosarcoma on estimation of necrosis rate, the observers were divided into two groups (higher career and lower careergroups). The interCC and intraCC of those two groups were compared. ICC values were interpreted as follows: ICC between 0.81 and 1.0 was considered 'excellent', between 0.61 and 0.8 was considered 'good', between 0.41 and 0.6 was considered 'fair to moderate', and below 0.4 was considered 'poor' [18]. Significance was set at a p value of<0.05.

Results

The ICC across 6 observers was 0.652 (95% CI, 0.455-0.865) through all 4 time evaluations, suggesting good reliability; ICC was 0.659 (95% CI, 0.447-0.871) at the first and second evaluations and 0.631 (95% CI, 0.414-0.858) at the third and fourth evaluations after a consensus meeting, which was not a significant improvement. Furthermore, percentages of necrosis ranged from 0 to 85% for slide #2 and from 2 to 100% for slide #7 (**Figure 2**). This is sufficiently different to confuse clinicians and create difficulty in determining further chemotherapy pro-

tocols in a clinical situation. The InterCC limiting those two slides among the 6 observers was 0.268 (95% CI, 0.054-0.997). From the first to the sixth observer, intrarater data were 0.799 (95% CI, 0.583-0.937), 0.788 (95% CI, 0.563-0.933), 0.867 (95% CI, 0.703-0.960), 0.935 (95% CI, 0.844-0.981), 0.962 (95% CI, 0.906-0.989), and 0.947 (95% CI, 0.845-0.985), respectively. At the first and second evaluations, intrarater data were 0.595 (95% CI, 0.051-0.877), 0.867 (95% CI, 0.570-0.965), 0.842 (95% CI, 0.485-0.958), 0.952 (95% CI, 0.831-0.988), 0.984 (95% CI, 0.938-0.996), and 0.969 (0.887-0.992), respectively. At the third and fourth evaluations after a consensus meeting, intrarater data were 0.998 (95% CI, 0.991-0.999), 0.852 (95% CI, 0.429-0.963), 0.834 (95% CI, 0.486-0.956), 0.988 (95% CI, 0.954-0.997), 0.972 (95% CI, 0.889-0.993), and 0.911 (95% CI, 0.545-0.979), respectively. There were no differences in intraCC before and after the consensus discussion.

The InterCC of the higher career group was 0.603 (95% CI, 0.387-0.843), and that of the lower career group was 0.696 (95% CI, 0.487-0.919). The intraCC was 0.799 (95% CI, 0.583-0.937), 0.867 (95% CI, 0.703-0.960), and 0.962 (95% CI, 0.906-0.989) in the higher career group and 0.788 (95% CI, 0.563-0.933), 0.935 (95% CI, 0.844-0.981), and 0.947 (95% CI, 0.845-0.985) in the lower career group. There were no statistically significant differences between higher and lower career groups in both interCC and intraCC ($P>0.05$).

Throughout the consensus meeting, there was a major difference in whether bizarre atypical cells with nuclear and cytoplasmic aberrations in necrotic or fibrotic stroma were identified as viable cells or non-viable cells. When pathologic slides show typical histological findings of active tumor cells or necrosis after neo-adjuvant chemotherapy, the tumor necrosis rate coincided well (**Figure 3**). However, if there were isolated atypical cells with nuclear and cytoplasmic aberrations in necrotic or fibrotic stroma (**Figure 4**), some pathologists considered the area surrounding these cells a viable tumor area. This would indicate the ineffectiveness of chemotherapy and include these areas in the measurement of viable tumor. However, other pathologists considered these areas more conservatively and felt it was a spectrum-like change indicating partial chemotherapy effectiveness and partial tumor necrosis.

Discussion

The histologic percentage of tumor necrosis after neo-adjuvant chemotherapy in osteosarcoma patients is the most important factor for predicting prognosis and determining further chemotherapy regimens [2, 3, 13]. It also used as a basis to estimate the clinical relevance of other evaluation methods for chemotherapy response, such as diffusion MRI or PET CT. Many studies have defined a good response as 90% or more tumor necrosis [1-3]. A good responder is usually given the same chemotherapy regimen postoperatively, while other salvage chemotherapy regimens should be considered for poor responders [1, 5, 6, 8-10]. Because chemotherapy drugs used in osteosarcoma are limited compared to other carcinomas, the histologic response to standard chemotherapy has considerable importance in osteosarcoma treatment. Clinicians feel quite differently about even a 10 to 20% difference in necrosis rates, which may significantly change treatment strategy [1].

Histological evaluation of tumor necrosis is considered the gold standard method for determining response to preoperative chemotherapy agents. In addition to the histological necrosis rate, many studies have introduced tools such as ^{18}F-FDG PET/CT, MRI diffusion-weighted imaging (DWI), and bone scan as novel methods to evaluate chemotherapy response in patients with osteosarcoma [19-23]. All studies compared their results with histopathological response rate because it has a confirmative meaning for clinicians, radiologists, and radiation oncologists. However, if histopathological response rates lose their consistency, they will not have a standard value and will lose their usefulness.

The tumor necrosis rate is evaluated on the basis of the amount of remaining viable tumor in the resected specimen. Rosen and Huvos et al. described a method for evaluating tumor necrosis and divided tumor response into four grades [24, 25]. Salzer-Kuntschik et al. divided response into six grades. Those methods are based on random sampling of slides, which is thought to be representative of the entire tumor [26]. Each slide was analyzed and a mean per-

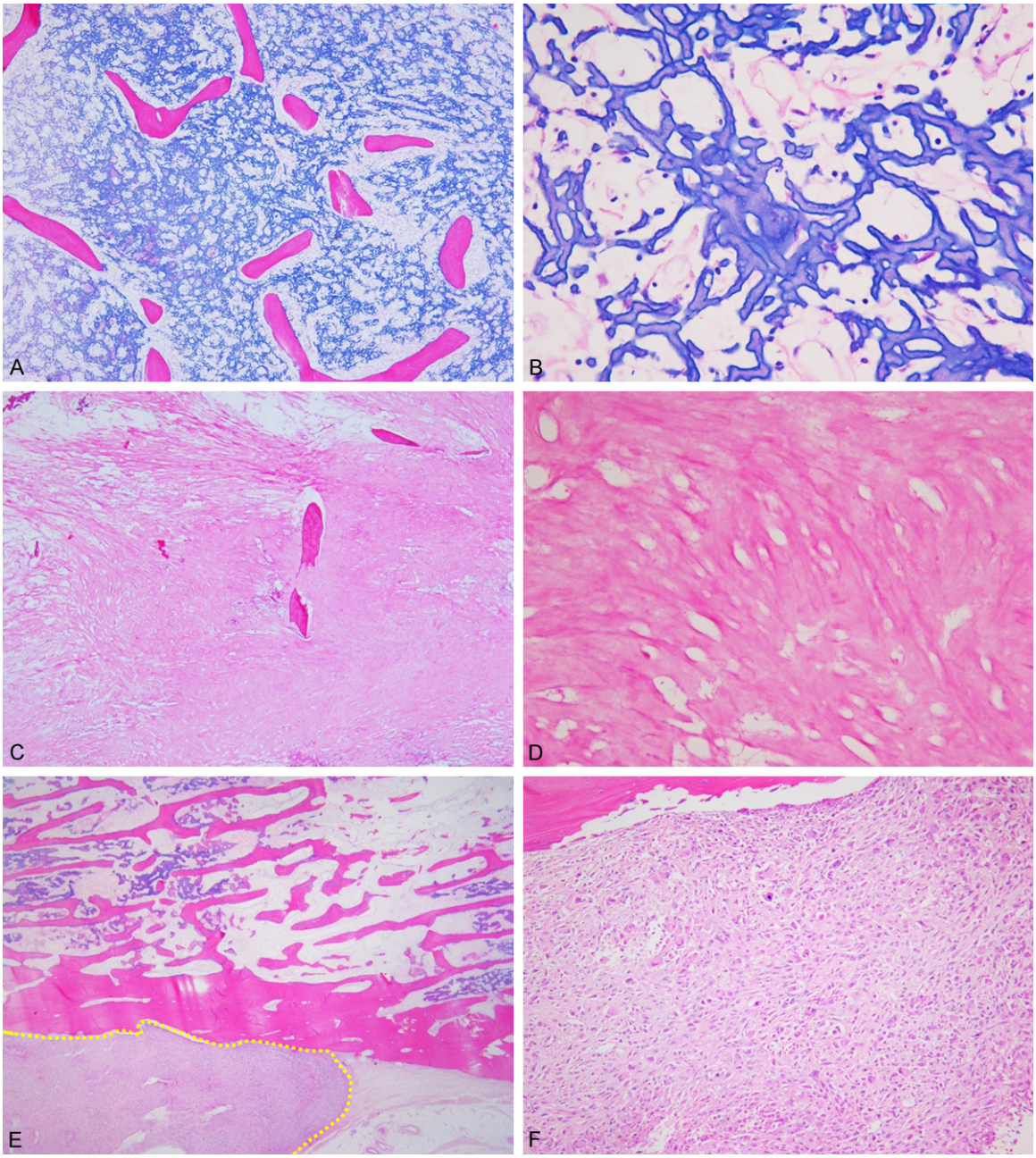

Figure 3. Typical histologic findings of necrotic and viable areas in osteosarcoma after neo-adjuvant chemotherapy. (A-D) Complete tumor necrosis showing acellular osteoid, necrotic or fibrotic materials. (A) Osteoblastic osteosarcoma after chemotherapy shows residual tumor osteoid and normal eosinophilic trabecular bone (×40). (B) At high power magnification (×400), there is complete cell dropout with acellular osteoid and necrotic stroma. Post-chemotherapy findings of fibroblastic osteosarcoma show diffuse coagulative necrosis (C, ×40) without viable cells (D, ×400). (E) The yellow-dotted line reveals residual viable tumor area (×40). (F) There are numerous viable tumor cells resembling highly cellular osteosarcoma on pretreatment biopsy (×400).

centage of necrosis was mathematically calculated by mapping on X-ray or gross specimen pictures. Raymond et al. suggested that the process by which specimens are analyzed is crude and subjective [3]. It is possible that slide selection and necrosis judgment are subjective processes.

Our study shows relatively high intra-observer reliability for histological evaluation of necrosis

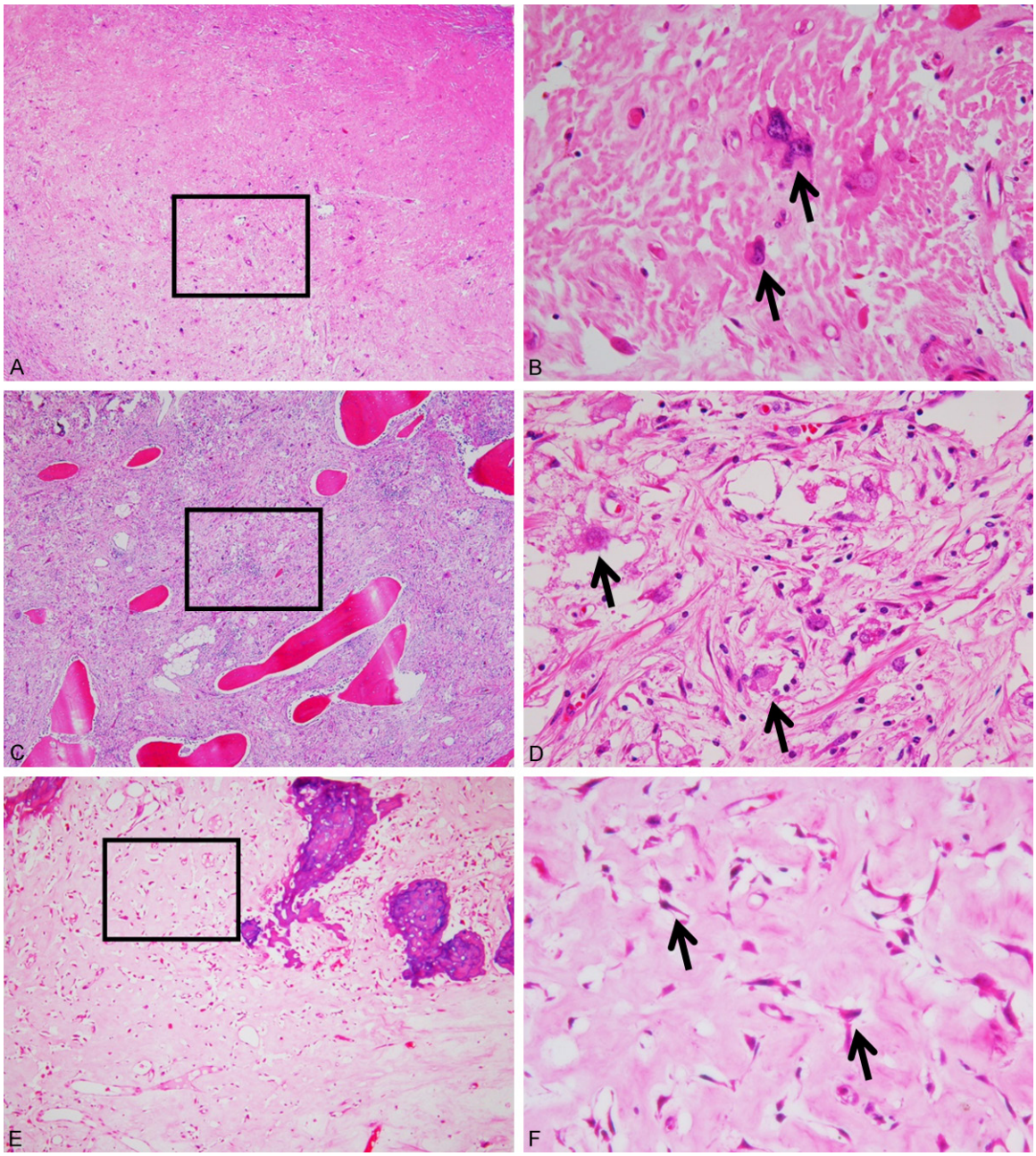

Figure 4. Isolated atypical cells with nuclear and cytoplasmic aberrations in necrotic or fibrotic stroma after osteosarcoma chemotherapy. A-F. Images in right column show high-power magnification (×400) of the boxed areas in the left column. Tumor cells are significantly dropped out. There are no mitotically active cells; however, there are a few atypical scattered cells (arrows). Some pathologists consider these atypical cells viable tumor cells and include this area in measurements of viable tumor.

rate after neo-adjuvant chemotherapy. However, inter-observer reliability was lower. In some cases, the difference was high enough for clinicians to arrive at different treatment strategies. Despite long discussions, pathologist decisions did not coincide with each other on some points. Tumor necrosis is generally defined as an absence of neoplastic cells. It is usually represented by pathological findings such as cell dropout with residual matrix, or so called "ghost cells" [24-26]. However, pathologists are confused by some situations in which a few cells containing cytoplasmic vacuolization with bizarre change were surrounded by an area

largely replaced by fibrosis and granulation tissue. If these areas are considered viable, chemotherapy response may be underestimated.

Michael et al. recommend considering only dense areas of mitotically-active atypical cells as viable [27]. In their study, disease-free survival significantly correlated with tumor necrosis rate when counting only dense mitotically-active atypical cells as viable ("stringent method") rather than widely distributed, rare, mitotically inactive atypical cells. The stringent method may be one of the possible solutions for improving the reliability of estimating tumor necrosis rate.

Li et al. urged caution in interpreting tumor necrosis after neo-adjuvant chemotherapy as an all-or-nothing phenomenon. They also suggested that a 10% viability cut-off does not indicate an absolute border beyond which chemotherapy is no longer effective. They concluded that tumor necrosis showed a gradual effect on survival rate [4].

Some pathologists have suggested that it is better to select a method for interpreting tumor necrosis as an all-or-nothing phenomenon that does not acknowledge partial effectiveness just to improve inter-observer reliability. Many studies reported that tumor necrosis for chemotherapy response had a gradual effect on survival rate, so some have recommended interpretation on a spectrum that acknowledges partial effectiveness. However, this method of interpretation is more time consuming and there is no definite measurement protocol to reflect the degree of partial effectiveness. Many clinicians expect histologic evaluation of tumor necrosis to be more informative as a definitive report, and to make comparable with quantitative evaluation methods such as ^{18}F-FDG PET/CT.

All six observers in this study agreed that a consistent consensus is needed to improve inter-observer and intra-observer reliability. They also suggested that the creation of a valid measurement protocol for evaluating tumor necrosis is urgently needed. Some observers preferred to interpret tumor necrosis as a spectrum; however, these pathologists thought that this method made it difficult to objectively calculate the ratio of tumor necrosis. Other observers considered it better to interpret tumor necrosis as an all-or-none phenomenon because there are no unequivocal criteria for determining the viability of bizarre cells. They thought these cells might represent degenerative changes secondary to chemotherapy, but could also represent regenerative changes.

There is a recommendation that the best way to ensure reliable and consistent data on chemotherapy response is to continuously refer pathological specimens to the same pathologist. Some have argued that this is simple because most sarcoma patients are concentrated in tertiary care hospitals where clinicians and pathologists have worked well together over a long period.

There were several limitations to this study. First, it included a small number of slides and observers. Given the rarity of expert musculoskeletal pathologists and the geographical distance between them, it is difficult to obtain a large number of observers. However, to the best of our knowledge, this is the first attempt to evaluate the validity of histological evaluation of tumor necrosis. The second limitation of this study was that observers had only one consensus meeting to share their knowledge and experiences. Further meetings would be helpful for reaching a consensus to estimate necrosis rate. Another limitation was that the response rates were not correlated to patient survival rates.

In summary, we observed lower inter-observer reliability regarding histological evaluation of necrosis rate after neo-adjuvant chemotherapy. At a consensus meeting, major differences among pathologists did not easily converge. These findings suggest that a valid measurement protocol for evaluating tumor necrosis rate is urgently needed in musculoskeletal tumor pathology. Pathology training programs should consider this issue to restore the clinical relevance of necrosis rate evaluation in osteosarcoma treatment.

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Disclosure of conflict of interest

None.

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