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Diagnosis and tumor response in osteosarcoma and Ewing's sarcoma

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Both osteosarcoma and Ewing's sarcoma are highly malignant tumors, usually affecting children and adolescents. Before chemotherapy was introduced in the treatment of these tumors the prognosis was poor, but by intensified pre- and postoperative chemotherapy regimens the prognosis has improved dramatically. It soon became obvious that the tumor response to preoperative chemotherapy also served as a prognostic marker and today the postoperative chemotherapy treatment modality is based on the response rate. Those with a good tumor response receive a less intensive postoperative treatment than those whose tumor response has been poor (Picci 1997 et al., Bacci 2000 et al.).

The Scandinavian sarcoma group (SSG), has since 25 years carried out several studies on both osteosarcoma and Ewing's sarcoma, some of them in collaboration with the Italian sarcoma group (ISG). The protocols for osteosarcoma SSG II, SSG VIII, ISG/SSG I, ISG/SSG II and SSG XIV, as well as the protocols for Ewing's sarcoma SSG IV, SSG IX, ISG/SSG III and ISG/SSG IV are described elsewhere (http://www.ssg-org.net/). In these protocols different methods of assessing the chemotherapy response have been used. Here, we will present and discuss the different methods.

Patients and methods

Diagnosis

592 cases of osteosarcoma and 277 cases of Ewing's sarcoma have been reported to the SSG register, and most of the cases have been enrolled in the different treatment protocols. To ensure that the diagnosis was correct, the diagnostic samples of these cases collected from the individual treatment centers, have been reviewed by an expert pathologist panel. If the panel did not confirm the primary diagnosis the case was excluded.

In most cases the diagnosis is based on histology, where the samples have been obtained by open or needle core biopsy. In some centers the diagnosis is made on fine-needle aspiration biopsy. Today, most centers include molecular methods in their diagnostic panel, especially in Ewing's sarcoma, including immunocytochemistry and/or the demonstration of the typical translocation t(11;22) by karyotyping, FISH or RT-PCR-methods (Meis-Kindblom 1996). A combination of histology/cytology and some of these ancillary methods have been obligatory for the diagnosis of Ewing's sarcoma in the later protocols (ISG/SSG III and ISG/SSG IV).

Tumor response

Osteosarcoma

SSG II and SSG VIII: In osteosarcomas different methods for assessing the response has been established. In the two first SSG osteosarcoma protocols (SSG II and SSG VIII) the criteria defined by Huvos (1991) were used. This method is based on a four-grade system, where the grades are defined as:

Grade I: Little if any identified effect.

Grade II: Areas of acellular tumor osteoid, necrotic, and/or fibrotic material attributable to the effect of chemotherapy, with other areas of histologically viable tumor.

Grade III: Predominant areas with acellular tumor osteoid, as well as necrotic and/or fibrotic material attributable to the effect of chemotherapy, with only scattered foci of histologically viable tumor cells.

Grade IV: No histologic evidence of viable tumor within the specimen.

For the decision of postoperative treatment grade I and II were considered as poor responders and grade III and IV as good responders.

ISG/SSG I and ISG/SSG II: For the ISG/SSG I and ISG/SSG II osteosarcoma protocols a new system was developed in cooperation with the Italian sarcoma group. The purpose was to combine the experience and tradition of the two sarcoma groups to develop a system, which would be easy to use in all individual institutions, and where the response was graded based on an objective number of vital tumor cells. In this method the response was graded either as good or as poor, as follows:

Good response: Total necrosis, or not more than 10 foci containing not more than 30 cells/focus.

Poor response: All other cases, also those with only one area containing vital tumor cells, but with more than 30 cells.

SSG XIV: Based on the preliminary results of the ISG/SSG studies, where the criteria for good responders seemed to be too strict, leading to a low percentage of good responders, a further system was developed for the now ongoing osteosarcoma SSG XIV protocol. In this two grade-system the response was defined as:

Good response, two criteria fulfilled:

1. <10% of examined tumor area reveals unquestionable viable tumor.
2. No single area of unaffected viable tumor exceeds 2.5 mm in largest diameter.

Poor response: Fulfilling one or both of the following criteria:

1. One or more areas of unaffected, viable tumor >2.5 mm in largest diameter.
2. >10% of the examined tumor area show unquestionable viable tumor.

Unaffected means a morphologic appearance closely resembling that of the pretreatment biopsy. Unquestionable viable means various degrees of response, including decreased cellularity and signs of maturation with bone and cartilage matrix production, but with remaining clearly viable tumor cells.

Ewing's sarcoma

SSG IV and SSG IX: In the two first Ewing's sarcoma protocols (SSG IV and SSG IX) the tumor response evaluation was based on a modification of the Huvos system. Grade I and II response were regarded as poor response, grade III and IV as good.

ISG/SSG III and ISG/SSG IV: In the joint protocols with the Italian sarcoma group the response was graded according to the method developed at the Rizzoli institute (Picci et al. 1997). By this method the response is evaluated in three grades:

Grade 1: The specimen contains at least one "macroscopic" nodule of viable tumor tissue, defined as an individual nodule larger than one 10× objective magnification field or scattered nodules that individually are smaller than one 10× field, but the total areas of these nodules exceed one 10× field.

Grade 2: The specimen contains only isolated microscopic foci of viable tumor tissue (totally not more than on field at 10× magnification).

Grade 3: The specimen contains no viable tumor tissue.

In the ISG/ SSG III and IV protocols grade 2 and 3 responses are considered as good responses, whereas grade 1 response is considered poor.

Results

Osteosarcoma

SSG II and SSG VIII: The SSG II study included 92 cases and the SSG VIII study 121 cases. The diagnosis was confirmed in all cases of the SSG II study, but 2 of the cases in the SSG VIII study were excluded, as the diagnosis was changed (Holmström et al. 1999).

The chemotherapy response could be determined in 85 (92%) of the cases of SSG II, and the response was good (Huvos grade III or IV) in only 19%. Compared to the response grade reported from the individual institutions the grade was

changed in over 25% of the cases when reevaluated by the pathologist panel. Many of these changes were minor (i.e. grade IV changed to grade III) but in 9 cases it lead to a change from good response to poor response.

Of the cases included in SSG VIII 115 (97%) could be reevaluated for the response. The number of good responders increased dramatically compared to the SSG II study, as 51% of the cases were considered as such. Also in this series there was a major change in the response evaluation (i.e. from poor responder to good or vice versa) in almost 10% of the cases.

ISG/SSGI: Of the 177 cases enrolled in the ISG/SSGI protocol (120 from Italy and 57 from Scandinavia) 154 have so far been reviewed for diagnosis and tumor respons. The primary diagnosis of osteosarcoma was confirmed in all cases. Only slight changes were made concerning the subtype of the tumors (classical osteoblasitc, chondroblastic, fibroblastic, teleangiectatic or a combination of these). Of the 154 cases only 13 (9%) were graded as good responders using the new response grading system, whereas the respons was poor in the remaining 141 (92%). In 13 (9%) of the 154 cases the response grade was changed at review. Of these 10 had primarily been graded as good responders but were classified as poor responders at reevaluation and, vice versa, 3 cases were classified as good responders although they had primarily been classified as poor responders. At review all 154 cases were also graded using the Huvos system giving 42 cases with good response (Huvos grade III or IV) and 112 with poor response (Huvos grade I or II).

SSG XIV: This study is still active, and so far 49 cases have been reported. Of these, 32 have been reevaluated by the panel and the diagnosis has been confirmed in all cases. Using the response grading system developed for this protocol the number of good responders is 15 and of poor responders 17. Of the primary grading made at the individual centers, only 1 case was reclassified (i.e. from good response to poor response). 23 of the cases have also been classified according to the Huvos system, and in most cases there was a good correlation between the SSG XIV and Huvos grading. In 2 cases, however, a poor responder according to SSG XIV was a good responder (grade III) according to the Huvos system.

Ewing's sarcoma

SSG IV: In this study 52 patients were enrolled. From 50 of these material was available for reevaluation of the diagnosis. 3 cases were excluded due to inaccurate diagnosis or lack of appropriate material. Of the remaining 50 cases 35 were operated on and of these 32 were available for reevaluation of the chemotherapy response. Using the Huvos grading system 14 were good responders, whereas 18 were poor responders.

SSG IX: Of the 104 cases included in this study material from 96 were available for histologic reevaluation of the diagnosis. Of these 8 were excluded due to other diagnosis than Ewing's sarcoma (2 osteosarcomas, 2 alveolar rhabdomyosarcomas, 3 malignant small round cell tumors and 1 breast cancer) (Elomaa et al. 2000).

60 patients underwent operation, and sufficient material for assessing the chemotherapy response was available from 52. Using the modified Huvos grading system 24 were graded as poor responders (grade I and II) and 28 as good responders (grade III and IV). Interestingly, the response grade was changed in 16 of the cases by the reevaluation panel.

ISG/SSG III and ISG/SSG IV: These two Ewing's sarcoma protocols are still open, and there is very little data available. 106 patients have been registered in the ISG/SSG III protocol. The diagnosis or responses have not been reevaluated. The individual institutions have reported the response to chemotherapy from 46 cases, and the number of good responders (Picci grade 2 or 3) and poor responders (Picci grade 1) is equal, 23 cases are graded as good responders and 23 as poor responders.

Comments and conclusions

Regarding osteosarcomas, the accuracy of the primary diagnosis must be considered good, as the diagnosis was changed by the reevaluation panel in only few cases. The diagnostic methods seem thus to be adequate for this diagnosis in most centers participating in SSG. On the contrary, there were several cases in the early protocols were the diagnosis of Ewing's sarcoma was changed at review. This fact further emphasizes the importance of using supplementary methods for diagnosing Ewing's sarcomas. In the ongoing ISG/SSG III

and IV protocols this has already been added to the protocol, as immunohistochemistry using CD99 antibodies and/or cytogenetic studies are obligatory for making the diagnosis. As only a few cases of Ewing's sarcoma are annually diagnosed at each Scandinavian center, the diagnostics should be centralized to a few places, where the above methods are in use.

The chemotherapy response could not be evaluated in all operated cases of either osteosarcoma or Ewing's sarcoma. This is partly due to the fact that there was not sufficient material available. From some cases only a few samples had been taken for examination. In the ongoing protocols it is therefore stressed in the pathology guidelines that sufficient material has to be sampled.

In osteosarcomas the different methods used for grading the chemotherapy response had their shortcomings. When using the Huvos system there were many cases where the response grade changed at reevaluation. The definitions of viable tumor and especially the size of areas/foci are unspecified, leading to a variation of interpretation between investigators. In order to make a more objective system for the ISG/SSG I and II protocols a new grading system was therefore established. In that grading system a tumor focus was clearly defined. However, the criteria were too strict, leading to a low percentage of good responders. Furthermore, there were still differences in interpretation of viable cells. In many cases single bizarre cells were seen among otherwise completely fibrotic/necrotic tumor tissue, which by some investigators were considered as viable tumor cells. Also chondroid tumor areas and reactive new bone formation caused variation in interpretation and subsequently response grading.

In the SSG XIV protocol the above shortcomings are dealt with. The size of an area is clearly defined, and also the problem of single bizarre cells has been taken in account. So far, this system has proven to function well, as only 1 case has been reclassified, and the number of good responders is nearly 50%. However, it is clear, that there are differences in the grading between individual centers and therefore the evaluation of chemotherapy response should be centralized. In fact, for the future protocol (EURAMOS 1) it has been decided that all cases should be evaluated by only few centers in Scandinavia (perhaps only one), as in the other participating European and American countries.

In osteosarcomas the chemotherapy response still serves as a prognostic factor, but the significance of it is not as strong as in Ewing's sarcoma. Thus, many attempts have been made to develop prognostic molecular tools. Several interesting studies have been published, including the overexpression and/or amplification of P-glycoprotein (Baldini et al. 1995), Her2/erbB-2 (Gorlick et al. 1999) and specific chromosomal changes (Tarkkanen et al. 1999a). To further study the possible use of such markers the well documented SSG material will serve as an excellent study object.

Also in Ewing's sarcoma there were many differences in the interpretation of tumor response when the Huvos system was used. To study whether the Picci system could be better the SSG IX material was evaluated also using this method (Åkerman and Stenwig 1998). The prognostic value of the Picci grading system was confirmed also on the SSG IX material. The study also showed, that the Picci method was easier to apply and more informative than the Huvos method. The Picci grading system is therefore now used in all ongoing Ewing's sarcoma SSG protocols. Although the tumor response proved to be a strong prognostic factor for Ewing's sarcoma, also other molecular prognostic markers should be developed. This is important since many centrally located tumors are not operated and the histological response can not be assessed. Molecular prognostic markers and perhaps also treatment targets could include growth factors, such as insulin-like growth factor (Scotlandi et al. 1996) or specific chromosomal changes (Tarkkanen et al. 1999b). The well documented SSG material is excellent for the search of such biological markers in Ewing's sarcoma

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