PROGNOSTIC FACTORS
AND NEW MEDICATION POSSIBILITIES
IN MALIGNANT MUSCULOSCELETAL TUMORS

Ph. D. Theses

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INTRODUCTION

The malignant musculoskeletal tumors make out 2-3% of the malignant neoplasms. Their rare occurrence, the peculiar diagnostic and treating methods, the high treatment costs and the fact that the average age of osteosarcoma patients is around 20 years explain why it is important to deal with these relatively rare tumors, in order to further improve diagnostic and treatment results.

With the development of chemotherapy, following the introduction of the pre- and postoperative medication, the 5-year survival increased to 60-80% from the earlier 15-20%, and that was reached by surgical intervention. The application of limb-saving surgery has come to the forefront.

In the framework of a retrospective clinical research, while evaluating the patients having osteosarcoma connected in the Osteosarcoma Register of the Department of Orthopedics at the Semmelweis University of Medicine between January 1986 and June 1999, beside the clinical, radiological and histological evaluation, we also examined the p53 and mdm2 expression from the biopsy material and from the tissue removed at the operation. We also examined the cell proliferation by an immunohistochemical method and the p53 gene amplification by molecular hybridization.

The aim of our research was the analysis of the prognostic factors known from the literature on our own patient material, and to search new methods and factors, which could help to design a better treatment of these tumors.

Significant changes are to be observed in the diagnosis and treatment of soft tissue sarcomas in the past 10 years. While earlier the treatment of these tumors was exclusively surgical, the role of radio- and chemotherapy has increased vastly in the past years beside the primary ablation of the tumor. The life expectancy of patients suffering from advanced soft tissue sarcoma are rather poor in spite of the sufficiently radical surgical methods and the radiotherapy. The 5-year survival of patients is around 10%, the survival of patients with metastases or local recurrences is 1.5 years on average. A successful medication is limited.
These observations led to a prospective research between January 1990 and June 1999, where we examined the efficiency of a new, not yet applied drug combination, the VIP protocol (Vepesid, Ifosfamid, Cisplatin) on 134 patients suffering from advanced soft tissue sarcoma.

**OBJECTIVES**

**Osteosarcoma**

**The prognostic role of clinical-radiological factors:**

**Parameters examined:**
- patients’ age, sex, radiographic appearance of the tumor, location, size, histological subgroup, stage, significance of the safe surgical margin, role of the type of surgery (amputation, limb-saving), response to chemotherapy, role of pre- and postoperative chemotherapy.

**Questions examined:**
- which of the parameters listed above can be used as prognostic factors;
- does the application of chemotherapy influence the survival: the role of pre- and postoperative, as well as only postoperative chemotherapy with regard to the survival;
- does the tumor-free surgical margin and the type of surgery (amputation or limb-saving surgery) influence the appearance of local recurrences;
- are the above examined factors significant separately or collectively in the prognosis of the osteosarcomas.

**p53 and mdm2 gene expression as a possible prognostic factor:**
- can the p53 and mdm2 expression be traced in the biopsy and surgical sample;
- is there a connection between the p53 and mdm2 expression and the response of these tumors to chemotherapy;
- is there a connection between the p53 and mdm2 expression (detected in the biopsy material) before chemotherapy and the survival.

Loss of heterozygosity of the p53 gene as a possible prognostic factor:
- is there a connection between the allelic loss of the p53 gene and the prognosis.

Proliferative activity of the tumor as a possible prognostic factor:
- does the Ki67 proliferative index change in the biopsy material;
- does the knowledge of the proliferative activity help to prognose the outcome of the tumor beside other prognostic factors.

Soft tissue sarcoma

Questions examined:
- how effective is the new VIP protocol (Vepesid, Ifosfamid, Cisplatin) in the treatment of advanced soft tissue sarcoma;
- grade of appearing side-effects;
- can the overall and disease-free survival time be increased by applying the VIP combination.

MATERIALS AND METHODS

Osteosarcoma

Clinical examinations
- we evaluated the prognostic role of the parameters detailed within the objectives through the treatment results of 121 osteosarcoma patients;
- the diagnosis was established at all patients by exploratory surgical biopsy; to determine the stage and to plan therapy we carried out the following examinations: two-directional chest radiographs, bone radiographs, chest CT and magnetic resonance images (MRI) of the tumorous region, bone scans and abdominal ultrasound scans;
- we applied laboratory examinations and imaging modalities at the treatments and during the follow-up of the patients;
- clinical documentation and the surgical protocol was evaluated by standard statistical tests and methods;

**Experimental examinations**
- we determined the protein products of the genes (p53, mdm2) by immunohistochemical method from the biopsy materials and the removed tumors of 42 patients. We examined the changes in the p53 and mdm2 protein expression by antihuman monoclonal antibodies (DO 7, DAKO-clone, HK 090-5K BioGenex);
- we determined the proliferative activity on sections of paraffin embedded material using Ki-67 immunohistochemistry;
- deletions of p53 was detected by polimerase chain reaction from blood and tumor tissue.

**Soft tissue sarcoma**
- for determining the stage we applied chest radiographs, chest CT scans, abdominal ultrasound, bone scans, CT and MRI examination of the tumors region;
- before each treatment cycle laboratory tests for renal and liver functions, ions, full blood-picture and ECG were carried out;
- treatment according to VIP protocol (Vepesid, Ifosfamid, Cisplatin) was given for 5 days in every 28 days until progression, maximum six times;
- response to the treatment was evaluated in every two months, CT and MRI examinations were applied according to the location of the tumor;
- patients were followed after surgery (with osteosarcoma and soft tissue sarcoma) for 10 years. Average follow-up period: 54 months (12-120 months).
RESULTS AND CONCLUSIONS

On the basis of examining prognostic factors at patients with osteosarcoma we conclude:

1. There was no difference between the survival of the individual age groups. The survival chances of patients above or under 30 years are equal, if they receive chemotherapy according to strict protocol guidelines (p=0.85).

2. Sex of patients showed no significant difference regarding survival (p=0.15).

3. There was no statistical connection between the radiographic appearance of the tumor (and the presence of the pathologic fracture at the time of the diagnosis) and the survival.

4. The 5-year survival of the patients group with axial location (spine, pelvis, rib) of osteosarcoma was 62-63%, while at the group with distal location (originating from wrist, ankle, metatarsus) the survival was 92%. The prognosis of tumors with distal location proved to be significantly better by a uni- and multivariate analysis (p=0.03).

5. Survival rate of patients decreased with the increase of tumor size, statistically significant difference was observed only between groups below and above 60cm³ (p=0.061). According to the proportional regression hazard model of Cox the volume may be a prognostic factor.

6. There was no significant connection between the histological subgroups and the survival (p=0.32). However, in cases where the cartilaginous ground substance in the tumor remained under 20%, the 5-year survival was above 80%, while in other cases survival was only 50%. With other words, if the cartilaginous ground substance is above 20% in the tumor, we noticed a significantly worse survival in both the uni- and multivariate analysis (p=0.006).
7. There is a significant difference between the surgical stages of the osteosarcoma according to Enneking with regard to survival. While the average 5-year survival is 60-75% in stages II/A and II/B with the appropriate complex treatment \((p=0.3)\), in stage III when et the recognition of the osteosarcoma lang metastases are also present we experienced significant differences \((p=0.04)\) only 10-20%.

8. In case of preoperative chemotherapy the grade of necrosis (as an indicator of chemosensitivity) was a strong prognostic factor both in uni- and multivariate statistic analysis. There was a significant difference considering survival of patients who received praeroperative chemotherapy, 5-year survival was 81%, in contrast to patients receiving only postoperative chemotherapy 58%, \((p=0.02)\).

9. We found a significant difference between those patients having osteosarcoma who responded well to chemotherapy and the others (who showed less response or did not respond at all) \((p=0.043)\). However, when we examined the three groups separately, the well-responding and moderately well-responding groups behaved almost exactly while the non-responding group showed a significantly worse survival rate than the first two groups \((p=0.001)\). The response of osteosarcomas to chemotherapy, was the most important prognostic factor in the multivariate statistic analysis.

10. On the basis of the examination of the surgical margin there is also a close connection between the local recurrences and the 5-year survival. The 5-year survival of radically operated patients proved to be 80%, while that of the patients operated marginally and intralesionally was only 44%. There was a significant difference in the survival of patients operated with different radicality \((p=0.017)\).

11. In case of appropriate indication there is no significant difference between the different surgical methods (amputation or limb-saving surgery) concerning the survival \((p=0.67)\).
12. The local recurrence proved to be an independent negative prognostic factor.

13. Positive (++, +++) p53 reactions were detected by immunohistochemistry only in stage II/B tumors. This indicates a connection between the stage and the intensity of the p53 expression (p=0.032).

14. Connection appeared between the p53 expression, the response to chemotherapy and the survival (p=0.03). None of the p53 positive cases responded to preoperative chemotherapy and these patients showed a significantly shorter survival period.

15. On the basis of our results the connection can be proved between the p53 expression and the survival (p=0.02). Patients, with a positive (++, +++) p53 expression had a shorter survival period than the patients with negative p53 status. Furthermore, the survival period of the ++ positive cases was somewhat longer than that of the +++ patients.

16. Allelic loss of the p53 gene was found only in samples of stage II/B, while LOH in tumors in stage II/A was missing.

17. Inverse connection was found between the changes of mdm2 and p53 expression, mdm2 positivity appearance in p53 negative cases. The response to chemotherapy and survival period in mdm2 positive cases correlated to p53 negative cases, but as a prognostic factor it did not give more information than the p53.

18. The Ki-67 proliferative index was above 20% in any degree of p53 expression. In accordance with the p53 expression we experienced a higher proliferative index in non-responding tumors with worse prognosis.

On the basis of our experience in the treatment of advanced soft tissue sarcoma we can conclude:

19. In the hope of increasing efficiency we introduced a new drug combination before not yet applied, the VIP
protocol (Vepesid, Ifosfamid, Cisplatin). The treatment proved to be successful, the remission rate (CR+PR=45%) was higher than the efficiency of the combinations applied earlier in Hungary in this patient group.

20. The side-effects of the treatment were of medium severity.

21. The average duration of remission was 4.6 months, the average survival period 10 months (4-30 months). We could not lengthen the average survival period by the applied treatment (VIP), but during the remission time the life quality of the patients responding to the treatment improved significantly, which is an important factor in this patient group.

**SUMMARY**

*The age and sex of the patient, the radiographic appearance of the tumor and the time of anamnesis did not* prove to be significant prognostic factors in our patients with osteosarcoma, not even by univariate statistic analysis.

Positive prognostic factors are the tumor volume under 60 cm$^3$, the wide or radical surgery, the distal location, and cartilageous ground substance under 20%, and the response to chemotherapy. These factors proved to be significant in course of the multivariate analysis.

Increased expression of the p53 protein can be a further prognostic factor, we recommend its practical application.

Further research is needed for the application of mdm2 and Ki-67 as prognostic factors.

In osteosarcoma patients the examined factors are important collectively in judging the prognosis.

VIP combination as a treatment choice is applicable in the daily routine for the treatment of patients with advanced soft tissue sarcoma due to its appropriate efficiency and the tolerable side-effects.
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