University Clinic of Orthopaedic Surgery

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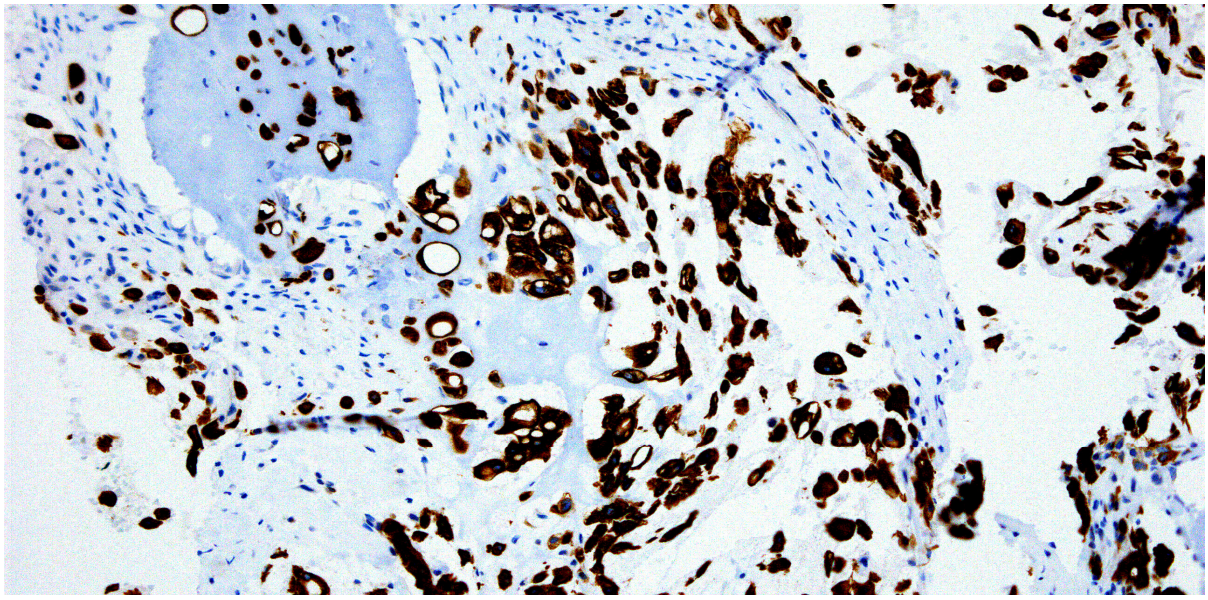
Medical University of Graz

Chordomas: A retrospective clinical and immunohistochemical study



This M.D.-thesis is submitted in partial fulfillment of the requirement of the degree of Doctor medicinae universae at the Medical University of Graz.

Presented by Elke Verena Fröhlich, 12/2008

Contents

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The dissertation, as part of a study, was realisable because of support of the below mentioned participants.

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III. Introduction

III.I. Definition

According to the World Health Organisation (WHO) a *Chordoma* is defined as a “low to intermediate grade malignant tumour that recapitulates notochord”. [71]

Although a plenty of scientific work, dealing with the subject of chordomas, was performed, this neoplasia still riddles. Discussions about its development, prognosis, and treatment as well as future treatment options only give a touch of scientific proceedings. Until that date the biological behaviour of chordomas and the involved patients meaning are not completely understood and clarified.

1. Aetiology

Chordomas are very rare, low to intermediate-grade malignant tumours, which behave locally aggressive and destructive. [34, 109] They develop from remnants of the notochord in the axial skeleton.[34] Notochordal cells are ectodermally originated.

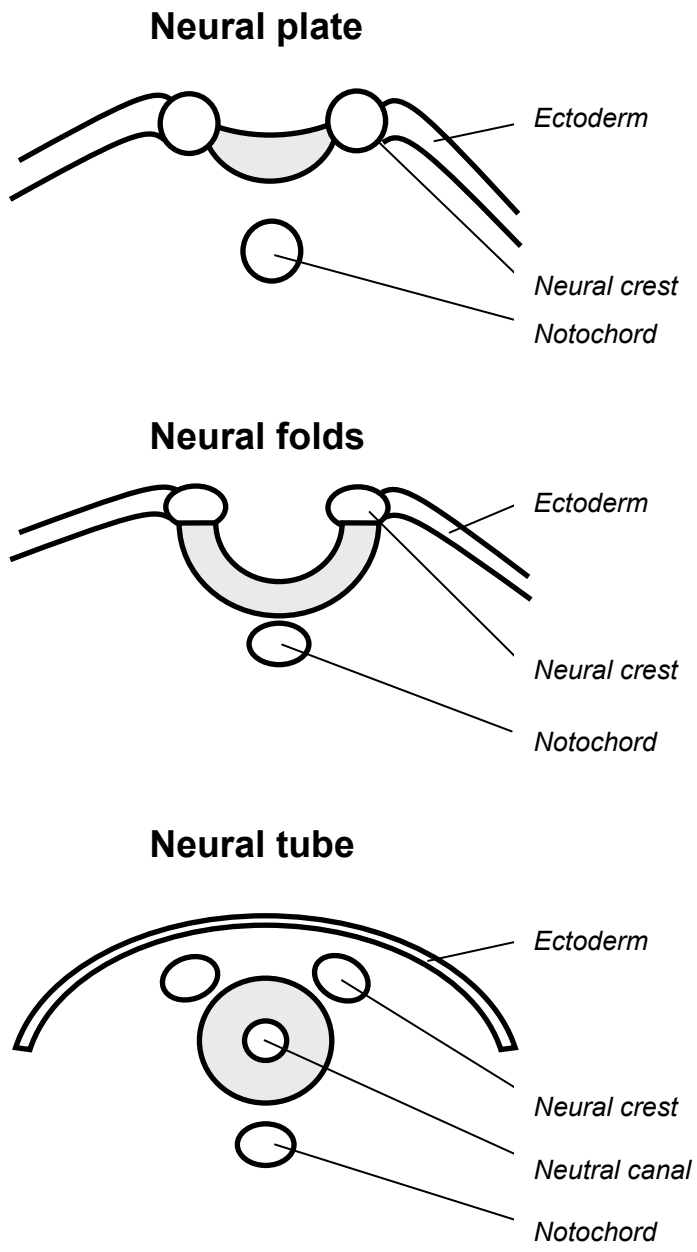


Fig.1: Localisation of the notochord during the formation of the neural tube (according to Salas (1998))[90]

On the 17th day after gestation the human notochord begins to develop, due to the migration of cells, located in the primitive pit. These cells migrate between the endoderm and ectoderm cranially and form the notochord in the midline of these structures. In this phase of development the notochord impresses as an elongated structure containing cohesive epithelial cell nests. [88] Occasionally within the notochord it is possible to find the typical *physaliphorous cells* that are one of the hallmarks of a chordoma. They are characterized by a frothy vacuolated cytoplasm, and a nucleus that is pressed to the fringe. The cell surrounding stroma is a mucinous substance, a mixture of carboxylated and sulfated glycoproteins. [18, 88, 116]

During the embryologic development, when bone and cartilage formation starts, these primitive ectodermal cells become enmeshed by the mesenchymal pericartilaginous cells, as start of the endochondral ossification.

By the 10th week of gestation all notochordal structures are obliterated by ossification centers forming the vertebral bodies. In those areas where discus development proceeds notochordal remnants remain. Now the entire destruction of the fetal notochord depends on the presence of type II collagen, which is jointly involved in the formation of the intervertebral discs. So the time span of activity of the notochord is short.[45, 88, 109]

Usually, the notochordal tissue in the nucleus pulposus of the intervertebral discs regresses totally between the sixth fetal month and second decade of life, although the complete fusion of all ossification centers of the vertebral bodies finally ends within the third decade of life. [45, 88, 109]

Of diagnostic relevance is the development of spinal chordomas out of the vertebral bodies (ectopic notochordal tissue). [34]

If notochord still remains as an asymptomatic, nonneoplastic rest it is denoted as ecchordosis physaliphora, benign notochord cell tumours (BNCT), giant notochordal rests or hamartomas.[27] Studies of Srinivasan A. et al. (2008)[98] and Deshpande V. et al. (2007)[27] show, that the relationship between notochordal rests and the further development to a chordoma still is discussed. Despite this fact there exists a general acceptance that BNCT act as precursor lesions of chordomas. [27, 97, 108, 115] Srinivasan and co-workers (2008)[98] are of the opinion that ecchordosis physaliphora has to be seen as a congenital malformation, whereas the proper chordoma presents a totally different entity. By contrast Deshpande's et al. (2007) study results advert to an association between BNCTs and chordomas.[27] They identified six BNCT that were bordering to sacral chordomas. Desphande and co-authors argue with the unique morphology and appearance of this discussed issue. [27] The acceptance that BNCT are strongly connected with classic chordomas is supported by Yamaguchi et al. (2004)[116]. They performed autopsies of 100 cadavers and investigated 100 vertebral columns but also 61 pieces of the clivus. They found a surprisingly high incidence of intraosseous BNCT (12% in the sacro-coccygeal area, 11,5% in the clival region, 5% in the cervical and 2% in the lumbar vertebrae) correlating with the anatomic distribution of chordomas, what indirectly suggests the presumption that BNCT are precursor lesions of classic chordomas.[116]

However, following histological elaborateness of Yamaguchi and his team showed the differences between notochordal material of fetal and adult intervertebral discs. For example the affected bone trabeculae of the adult tissue presented with sclerotic areas that indicate a slow growing process and give the evidence that these lesions are neoplasms rather than remnants.[115, 116]

2. Epidemiology

2.1. Incidence

In general chordomas are infrequent tumours with an estimated occurrence of 1 to 4% of all malignant bone tumours, although they are the most frequent of the sacral region.

Data from the SEER (Surveillance, Epidemiology, and End Result) program of the National Cancer Institute of the US by MacMaster et al. in the year 2000[65] yielded an annual incidence of chordomas of 0.08 per 100.000 populations.

2.2. Gender

The distribution between the sexes averages male to female at a ratio of 1,8-2:1.[71, 81, 88] In our chordoma series we calculated a distribution between the sexes male to female at a ratio of 2,25:1.

Data of the SEER program show a male predominance in chordomas agreeable to the Mayo Clinic experience.[28, 65] Between 1910 and 1971 they examined data of 155 chordoma patients, 103 were males and 52 were females. [28] This results in a male to female ratio of 2:1.

Matsumoto and his collegues[68] generally confirm the male predominance in chordomas. Further evaluations of the teams' dataset also presented that the localisation of chordomas in the cranium more often appear in young males (children and adolescents) than in young female patients compared to MacMaster's study results.[65, 68] They revealed a female predominance in reference to a cranial

chordoma localisation (p=0,037), and a predominance of sacral chordomas in older male patients.[65]

2.3. Age

Chordomas most commonly appear within the fifth to seventh decade of life, followed by the second to the fourth decade. The mean age at the time of diagnosis is about 35 years with vertebral tumours and spheno-occipital lesions, and 50 years with chordomas localized in the sacro-coccygeal region. [22, 34, 109]

In our chordoma series we analysed 39 patients (27 males and 12 females). We found a mean age of 51 years (16-80) at time of primary diagnosis.

2.4. Localisation

As aforementioned these types of tumours develop in the midline skeleton from remnants of notochordal cells. Out of this knowledge it is possible to deduce the most common localizations of chordomas that are listed in Table 1.[71]

sacral region	60%
sphenooccipital/nasal region	25%
cervical region	10%
thoraco-lumbar	5%

Tab.1: Chordoma localisation according to Mertens F. (2002)[71]

In our chordoma series we found the following tumour localisations (Fig.2) in descending order: The statistical summery (Graph 1, Graph 2) did not show a relevant correlation of chordoma localisation and the patients' outcome.

scull 19 cases (48,7%),

sacrum/coccyx 11 cases (28,2%),

cervical spine 6 cases (15,4%),

lumbar spine 2 cases (5,1%),

thoracic spine 1 case (1,6%).

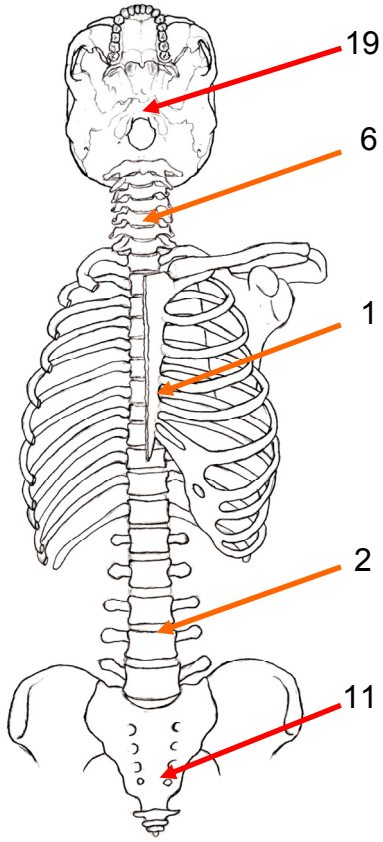


Fig.2: Tumour localisation

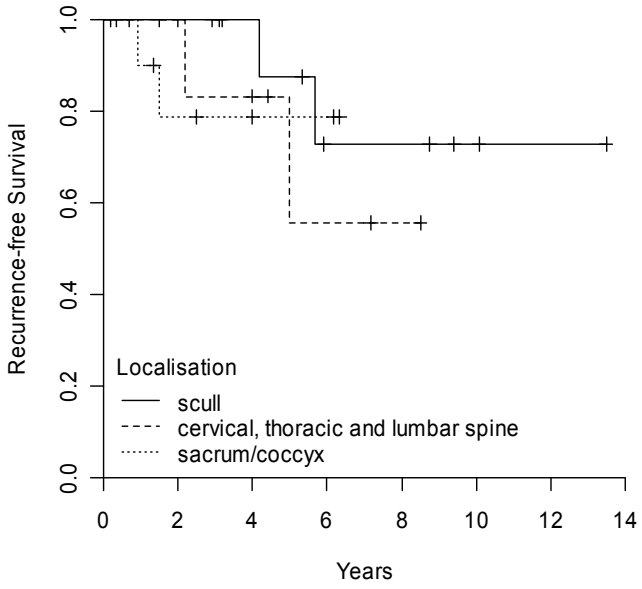
2.5. Tumour volume

Further in 25 patients we were able to calculate a mean tumour volume of 189,7 cm^3 (1,3 cm^3-688,2 cm^3). The calculation was based on the three-dimensional expansion of each lesion (radiological finding) and was made by the use of the following formula: ([x · y · z] / 0,5). Neither localisation nor tumour volume presented with statistically significant p-values in regard of survival analyses (Tab.2).

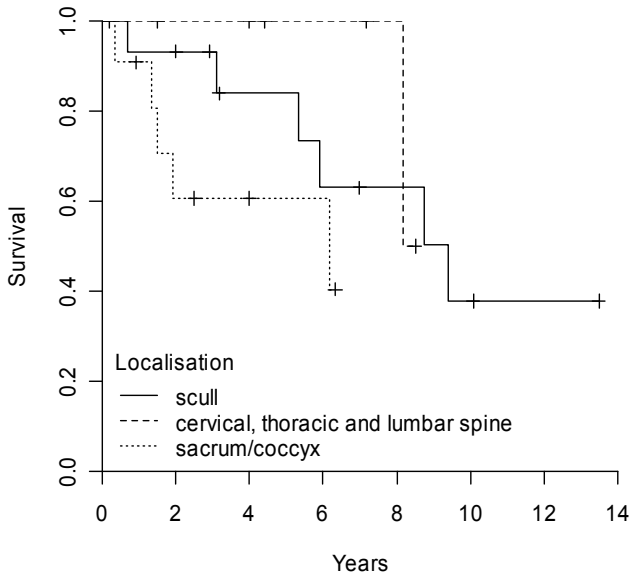
Generally the tumour volume presents with a wide span of extension, depending on the localisation (Fig.3).

Risk factor	*p-value OS*	*p-value (RFS)*
localisation	0,14	0,7
log (volume)	0,079	0,391

Tab.2: p-values of chordoma localisation and volume



Graph 1: Recurrence free survival in regard to chordoma localisation



Graph 2: Overall survival in regard to chordoma localisation

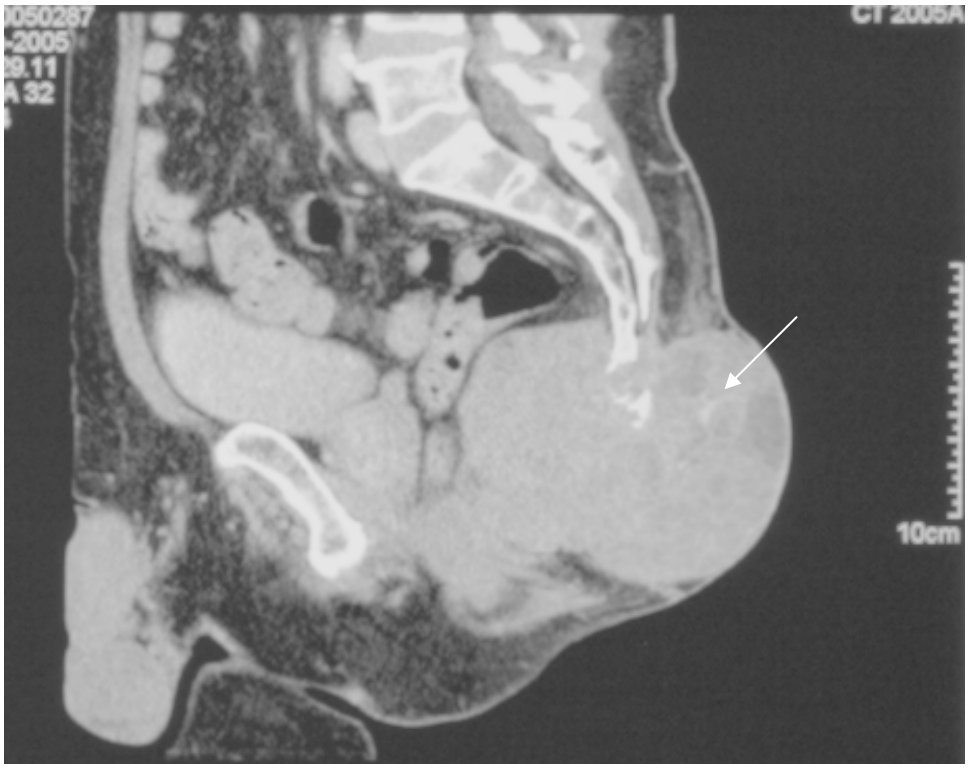


Fig.3: CT-image of a 62-year-old male. The sacro-coccygeal chordoma (arrow) presented with an extension of 12,7 (sagittal) x 10 (cranio-caudal) x 9,1 (transversal) cm. The calculated tumour volume resulted in 577,8 cm^3.

3. Pathology

3.1. Macroscopy

Macroscopically, in the intersection chordomas are sleeking, greyish-white to bluish-white tumours (Fig.4), which often show a pseudocapsule. Their consistence differs from soft, muco-gelatinous to hard, crumble and cartilaginous (Fig.5), whereas even intratumoural calcifications may appear particularly in sacral chordomas. Most of the chordomas are lobulated. Depending on the localisation, the diameter varies from 2 to 15 cm. Due to their extension they contain bone fragments, haemorrhagic areas, and cystic changes [34, 42, 71, 81, 105, 108]

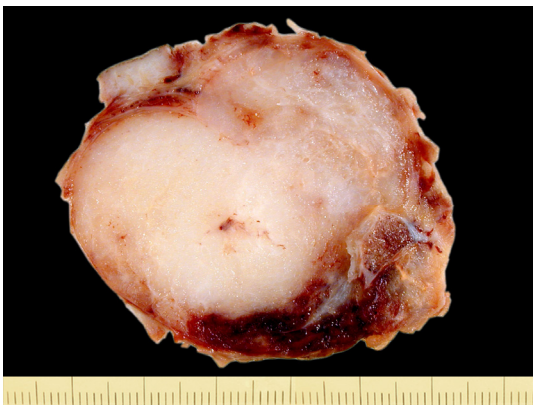


Fig.4: Macroscopic view. Greyish-white intersection with a smooth surface

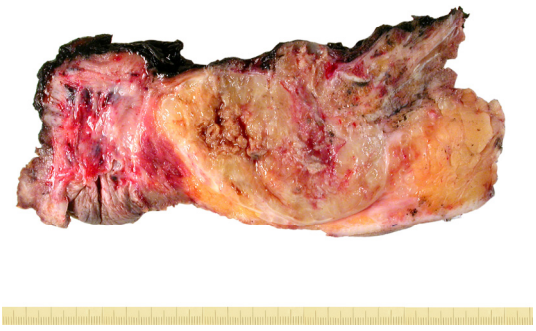


Fig.5: Macroscopic view. Huge sacro-coccygeal chordoma mass with a crumbling surface

3.2. Microscopy

Microscopically chordomas show dense fibrous bands that lead vascular channels and separate the tumorous tissue into small lobules. The cells themselves are interconnected with spidery tails.[34] Tumour cells are called “physaliphorous cells”. They consist of round or oval cells of pale appearance, containing frothy vacuolated cytoplasm, which relegates the bland, round nucleus to the fringe (Fig.6). Chordoma cells show an epithelial character as evidenced by a strong immunoractivity for cytokeratins (Fig.7). They are arranged in columns, sheets or even float singly and are imbedded in mucinous substance, a mixture of carboxylated and sulfated glycoproteins. In eosin-haematoxylin stain these cells impress luminously eosinophilic.[18, 116] Although chordomas are malignant tumours they only evince mild to moderate nuclear atypia and even mitoses are relatively infrequent.[18, 71]

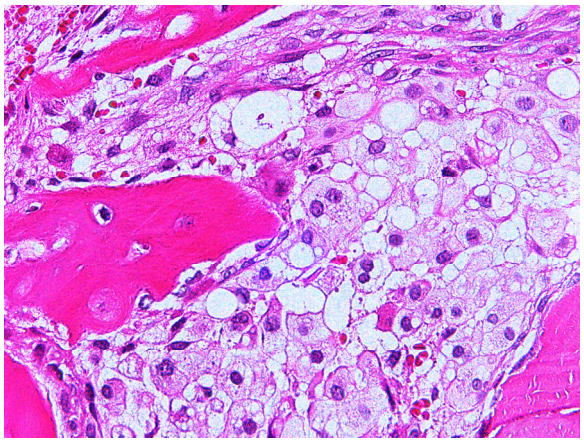


Fig.6: Microscopic view. “Physaliphorous cells”. HE-staining, x400

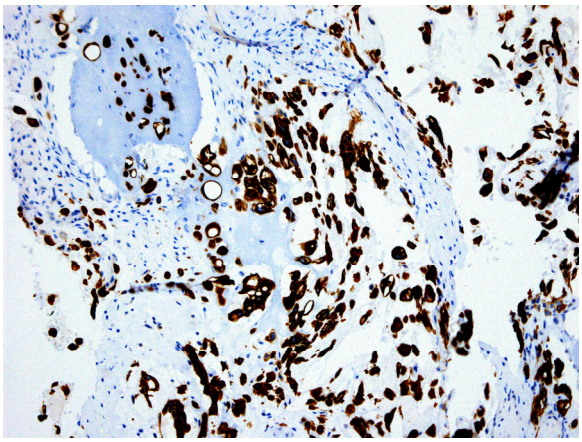


Fig.7: Microscopic view. Cytoplasmic immunstaining for cytokeratins, x400

According to the WHO[71], chordomas can be classified in different histopathologic types:

1) conventional or classic chordoma (ICD-O code 9370/3)

2) chondroid chordoma (ICD-O code 9371/3)

3) dedifferentiated chordoma (ICD-O code 9372/3)

The classic chordoma variant stands out due to the lack of mesenchymal components and is the most common chordoma type.

Dedifferentiated chordoma is known as the most aggressive one, but appears very seldom. This tumour metastasizes early, which certainly is connected with a fast, fatal course of illness, although patients younger than 40 years have a better prognosis than older ones.[20, 109]

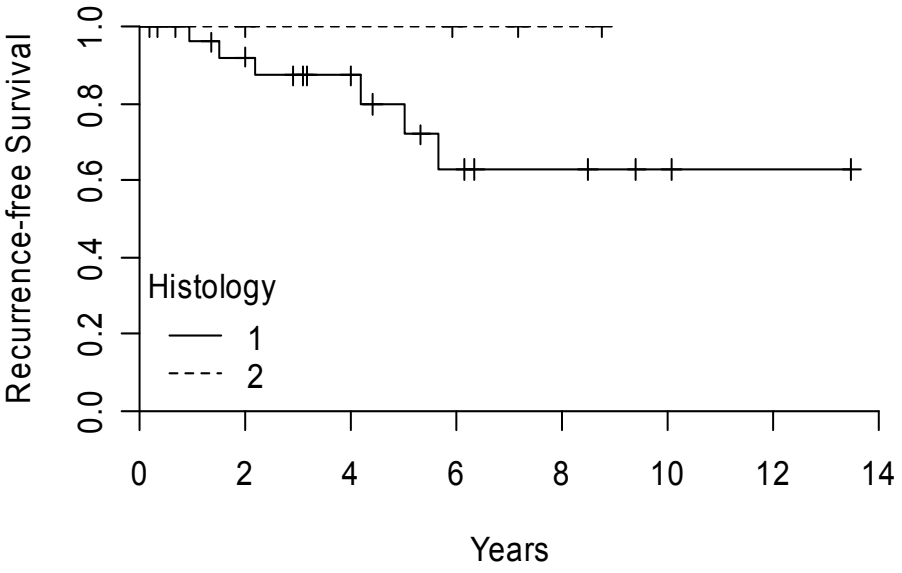
The chondroid variant of chordoma seems to appear more often in women and generally younger patients. They can contain chondromatous and chordomatous hallmarks and show predominance for spheno-occipital localisation. Chondroid chordomas seem to have the better prognosis. [18, 22, 71, 106]

In our chordoma analysis the histological features pictured 34 classic chordomas, 4 showed chondroid hallmarks and one chordoma emerged as dedifferentiated type.

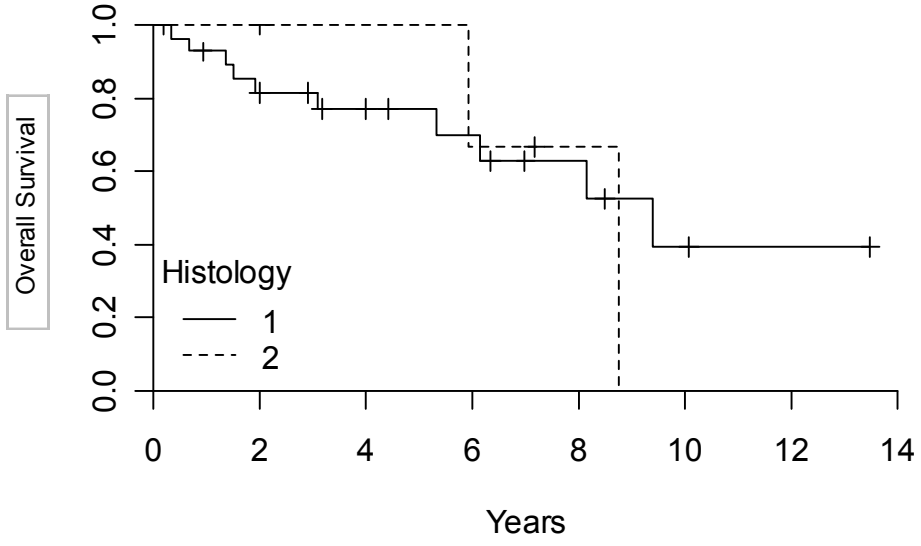
Unfortunately no statistically significant p-values in regard to chordomas' histologic type and the patients' outcome, recurrence free survival and overall survival could be achieved (Tab.3, Graph 3, Graph 4).

Risk factor	*p-value OS*	*p-value (RFS)*
histology	0,86	0,237

Tab.3: p-value of histological differentiation



Graph 3: Recurrence free survival in regard to the histological differentiation in chordomas (1:classic chordoma; 2: chondroid and dedifferentiated chordoma)



Graph 4: Overall survival in regard to the histological differentiation in chordomas (1:classic chordoma; 2: chondroid and dedifferentiated chordoma)

4. Metastases

Depending on the histopathologic presentation and their localization chordomas can eventually metastasize, although it is seldom and more often observed in terminal stages of disease.[28, 42, 109]

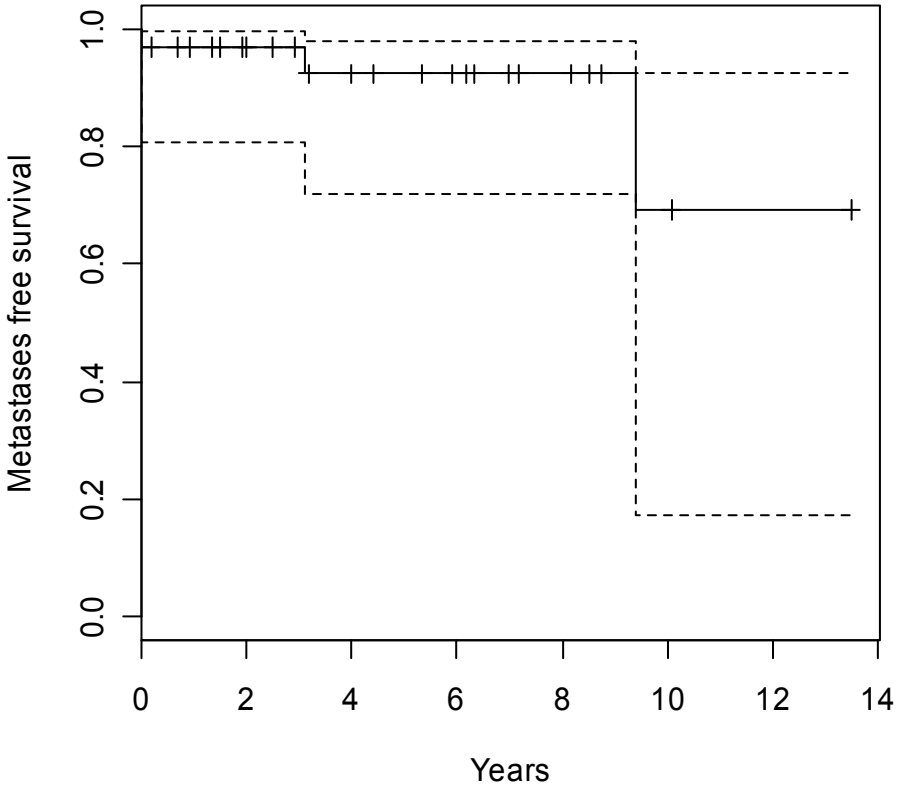
Metastases occur in 10 to 40% of chordoma patients, about 3 years after the initial diagnosis of the neoplasia. It may affect lungs, liver, bones, lymph nodes, skin, soft tissue, muscle, peritoneum, heart, pleura, retroperitoneum, spleen, adrenal glands, kidneys, urinary bladder, and the pancreas. Generally the age in men with metastatic chordomas is 45 years, and in women 37,3 years. [28, 70, 109]

According to McPherson et al. (2006)[70], who performed a study on 37 patients suffering from spinal and sacro-coccygeal chordomas, seven patients (19%), six men and one woman, developed metastases. They were located in the lung in three cases, in two cases each in lung, liver in the distant spine. McPherson et al. (2006) found that filiae exclusively appear in patients with local recurrent tumours (28%), compared to those without local tumour recurrences (0%), though the authors did not attain statistically significant results (p=0,07). McPherson and his colleagues calculated a median survival time of 106 months after the first surgery. But this result did not show a significant difference to the non-metastatic control group (p=0,93). [70]

In our series of 34 patients (follow-up population), two males and one female (8,8%) developed metastases from sacro-coccygeal tumours. The metastases were located in the pelvic lymph nodes, lung, and the thoracic spine. One patient additionally

presented with a residual tumour, a second patient with a tumour recurrence, and a third patient still suffered from the whole primary tumour.

The following Kaplan-Meier graph (Graph 5) demonstrates the statistical summary of metastases free survival in our chordoma series:



Graph 5: Metastases free survival (continuous line: steps describe the appearance of metastases and the fraction of metastases free survival; broken line: confidence-interval of metastases free survival per time)

5. Genetic background

Considering the genetic background one can separate spontaneous chordomas, which appear definitely more often, from familial neoplasias. The karyotypes in both familial and spontaneous chordomas are hypodiploid or near diploid.[25, 71, 93]

All sporadic chordomas, genetically heterogeneous, present frequent chromosomal imbalances of large regions.[39] Dalpra et al. (1999)[25] found that losses as well as structural rearrangements of 1p36 present common genetic changes in sporadic chordomas, but also in hereditary chordomas. This finding is in agreement with studies of Bayrakli et al. (2007)[9], who analysed different copy number changes in 7 primary and 11 recurrent chordoma samples. The number of gains for chromosome 1p36 accounted 60% in recurrent chordoma samples, admittedly just 14,2% in primary tumour samples. 66,6% of gains were found for chromosome 1q25 in recurrent, but 14,2% only in primary chordomas. [9]

Hallor and et al.[39] found a minimally deleted region in 1p36.31-p36.11. The particular of this finding is that this region contains, inter alia, the gene of the transcription factor *RUNX3*. This factor, also transcriptionally silenced or deleted in various cancer types has been suggested to encode an important tumour suppressor protein, comparable to *CDKN2A* and *CDKN2B* loci in the chromosomal band 9p21. Also in this region frequent losses are described in chordomas, but also in other tumour types as chondrosarcomas. An inactivation of CDKN2A could present an important factor in the development of chordomas. [39]

A study of Henderson et al. (2005)[40], however, suggests that genes involved in cartilage development might be of importance for chordoma oncogenesis. They found that the overall expression patterns are closely related to those of chondrosarcomas and chondroblastomas. The assumption of Hernderson and co-workers is based on the finding of the transcription growth factor *brachyury*, which is strongly expressed in notochordal tissue. [40]

According to these results Hallor et al. (2008) [39] found a gaining of the locus for TGFBI on chromosome 5 in five out of 30 classic chordoma samples. The corresponding protein product is also proposed to encode a part of cartilage development by stimulating the growth of prechondrogenic cells. In six further cases Hallor et al. [39] found a gaining of the chromosomal region 6q27, where the gene encoding brachyury is located. Brachyury, detected as an important transcription growth factor, is exclusively expressed in chordomas and notochord-derived tumours.

The unique expression of this specific transcription factor in chordomas and notochordal tumours was discovered by Vujovic and co-authors (2006)[110]. They analysed 323 samples of different tumours, including 53 chordoma samples, embryonic notochord and nucleus pulposus samples. The immunohistochemical examinations of Vujovic et al. did not show the expression of brachyury in all non-chordomatous tumours. Also the investigation of nucleus pulposus samples did not show an expression of this very specific marker, completing the enduring “chondroid-chordoid dilemma”. [87, 110]

These findings support the generally accepted theory that chordomas derive from embryonic notochordal tissue.

Tirabosco et al. (2008)[107] confirmed the results of Vujovic et al. (2006).[110] The former authors examined the expression of brachyury in 428 samples of different malignancies (including eight extra-axial skeletal and four soft tissue chordomas) and normal tissue of different organs.[106] Tirabosco and co-workers found the expression of brachyury in ten chordomas, but also in the 14 examined hemangioblastoma samples, in two of 14 germ cell tumours of the testis and in two out of 13 non-neoplastic testis tissue samples. [110]

A case report by O'Donnell and co-authors (2007)[76] also indicates brachyury as an important specific marker of notochordal differentiated tumours. They were able to diagnose an extra-axial chordoma of the proximal tibia by using CK19, CAM5.2, MNF116, AE1/AE3, CEA, EMA and HBME1 antibodies, though the imaging features proposed a benign intracortical neoplasia such as chondroblastoma, osteoid osteoma or osteoblastoma. Two years after surgery O'Donnell et al. (2007)[76] examined respectively their case using an antibody against brachyury. They found a strong, nuclear located, immunostaining of the tumour cells.

In conclusion the expression of brachyury in axial and extra-axial chordomas shows that this transcription factor is of diagnostic value, especially in the question of differential diagnosis.[76, 107]

Another approach in chordoma development is suggested by Denize et al. (2005)[26]. The authors performed an analysis investigating 14 cranial base chordoma tissues of patients with and without tumour recurrences. They examined the relationship between recurrent chordomas and the expression of growth factors *TGF-α* (transforming growth factor-α), *VEGF* (vascular endothelial growth factor), and *bFGF*

(basic fibroblast growth factor), *collagen IV*, *collagen III*, and *fibronectin*. Denize et al. found a positive correlation of the expression of bFGF and TGF-α with tumour recurrence, but no correlation for VEGF. Also a strong expression of fibronectin was suggested to be a marker of aggressive biological behaviour of chordomas.[26]

These results propose that increasing levels of TGF-α and bFGF, eventually combined with high expression levels of fibronectin, are strongly connected to higher rates of chordoma recurrences. Angiogenesis, triggered by VEGF, does not seem to play a crucial role in chordoma development and progression. [26]

Bayrakli et al.(2007)[9] also investigated the VEGF locus 6p12 and the TGF-α locus 2p13. In primary chordomas both chromosomal loci presented deletions, whereas chromosomal aberrations of 2p13 were observed in recurrent chordomas. This finding may suggest that defects in the VEGF pathway could influence chordoma genesis, but does not lead to the development of recurrences, whereas 2p13 deletions are crucial elements in the progress of chordomas. [9]

6. Clinical background

The clinical presentation of chordomas not only depends on their localisation and their spread, but also on their behaviour of growth. Most of these neoplasms are slowly growing, why, for a long period of time, signs and symptoms are usually non specific before diagnosis is made. According to studies of Ebersold et al. (2003) [28] the time slot between the occurrence of the first symptoms and diagnostic workup averages 3,44 years.[28]

The most common sacro-coccygeal chordomas produce, after a long indolent, asymptomatic while, lower back and pelvis pain as major symptom, followed by neurological deficits, such as anaesthesia and paresthesia at advanced stage of disease. Depending on their spread chordomas in the sacral area may lead to disturbance of defecation and/or miction, because of compression and dystopia of bowel and/or bladder, or even because of nerval lesions faecal and urinary incontinence may appear. [18, 28, 34, 42, 44, 71, 81, 109]

Cranial and cervical chordomas induce clinical symptoms earlier. In general it concerns of chronic headache, endocrine disturbances and signs of chiasmal compression like diplopia or visual-field defects. If the mass spreads inferiorly nasal obstruction, bleeding or even a nasal-pharyngeal mass may be presented. A displacement of trachea and oesophagus might lead to breathlessness and swallowing disturbances. [18, 28, 34, 42, 44, 71, 81, 109] Most chordomas cases of the lumbar and thoracic spine imitate a prolaps of a discus. Lesions in the area of L1-L2 can also

induce pain in the hip, knee and groin. Furthermore an involvement of the nerve roots or spinal cord may lead to paraparesis and spasticity.[18, 28, 34, 42, 44, 71, 81, 109]

Seldom chordomas can present acutely and fatally or even contrary with regression. For example Franquemont et al. (1989)[33] presented a case report of a 49 year old female, who had just a 10-day history of headache and died within a few hours due to an acute pontinocerebellar hemorrhage of a chordoma located in the spheno-occipital region. [33]

A totally different case was reported by Radl et al. in 2005.[86] The authors presented a 24 year old male patient with the histological confirmed diagnosis of a cervical chordoma (2,5 x 2 x 4cm) that suddenly regressed after an open biopsy was performed. The patient developed febrile temperatures, although no evident signs of infection were detected. Therefore he was treated with dexamethasone. Two weeks after an en bloc resection of the supposed chordoma should be performed. During the surgery an abscess, located at the biopsy wound was detected. The microbiological examination of the lavage and drainage resulted in “Escherichia coli”, why the patient received ciprofloxacin (iv.), for three weeks. A magnetic resonance imaging followed after further 2 weeks and detected the disappearance of the chordoma. To the authors knowledge no basic cause was found. It seemed to be the combined application of corticosteroids and the gyrase inhibitor ciprofloxacin. Further studies will be necessary to evaluate the systemic effect of these agents in regard of chordomas. [86]

According to the update database information the patient developed a recurrent chordoma after 24 months of the first biopsy. As further treatment a resection of C2,

followed by ventral and dorsal stabilization was performed. A local radiotherapy followed.

7. Diagnosis

Diagnosis is made by an exact anamnesis, clinical examination, x-ray, MRI, CT, biopsy and histological examination. Also laboratory parameters (hemogram, C-reactive protein (CRP), calcium balance, alkaline phosphatase, iron, protein metabolism) can provide an indication of pathological bone alteration by a chordoma. [18, 34, 42, 58, 59, 60, 71, 81, 109]

7.1. Imaging procedures

7.1.1. Radiographic examination

Radiographic examinations still are the most important diagnostic measures in the detection of tumours. Chordomas, depending on their volume, localisation, patients' age, and histological differentiation present variably. Especially in the early phase of tumour growth the detection of chordomas can be difficult. [18, 34, 42, 71, 81, 109]

In general in the sacro-coccygeal region located tumours are large, often impress with extended soft tissue masses and several osteolytic lesions of the involved bone and joints (Fig. 8). Therefore, especially in the sacral area chordomas do not present clear margins. Chordoma extension in the pelvic cavity or infiltration of gluteal muscles argue for their local aggressive and destructive biological behaviour.

Further calcifications, preferable in advanced stages of disease, can be detected. [18, 34, 42, 71, 81, 109]

Intracranial located Chordomas present clinically mostly as small lesions, which predominantly lead to bony destructions of the clivus, the floor and dorsum of the sella, sphenoid and petrosus bone and also the posterior clinoid processes. Generally, these unspecific osteolytic lesions can be associated with or even without reactive ossifications.

The classic variant of a chordoma is characterized by a slow growth and therefore an irregular sclerotic fringe develops. In comparison to the dedifferentiated chordoma is characterized by a faster growth and leads to an image presenting with a broad, irregular and blurry sclerotic fringe.

Spinal chordomas also present with osteolytic lesions and may narrow the intervertebral space by discus destruction, imitating an intraspongious prolaps of the discus.[2, 18, 34, 42, 71, 81, 109]

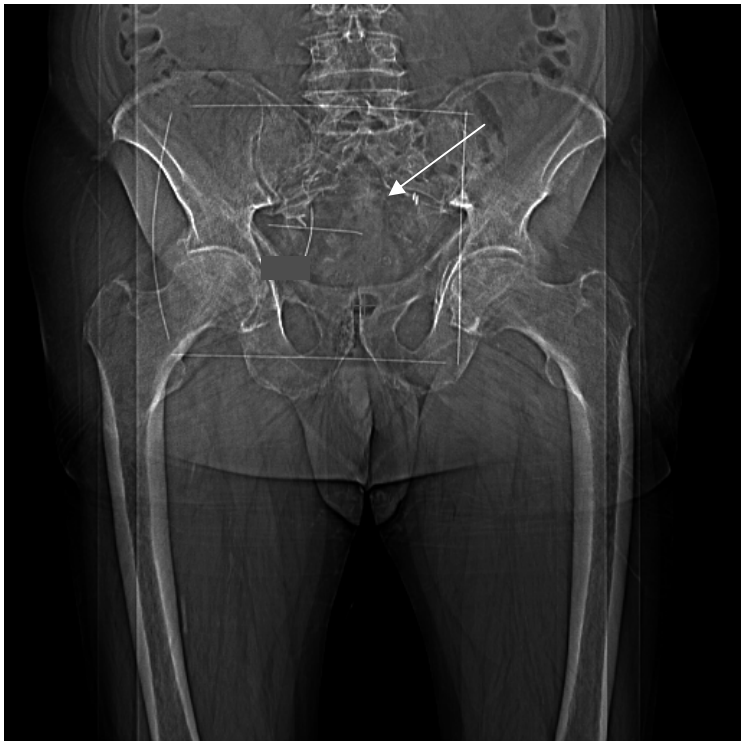


Fig.8: Osteolysis of the sacrum (arrow) of a 73-year-old male

7.1.2. Computer tomography and magnetic resonance imaging

Followed by a radiographic examination further imaging procedures like computer tomography and magnetic resonance imaging are mandatory (Fig.9, Fig.10). Out of the multifaceted applications of these procedures it is possible to detect cortical destructions and assign the exact tumour extension, and therefore an accurate planning of surgical resection, after biopsy, or radiotherapy can be performed. [2, 18, 34, 42, 59, 60, 71, 81, 109]

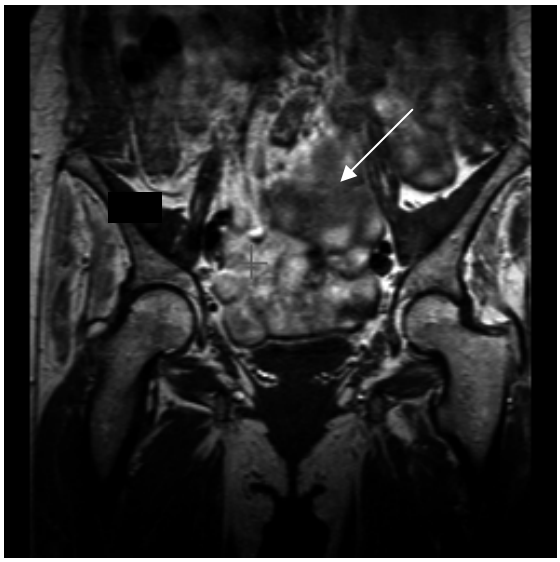


Fig.9: MR-imaging of a 59-year-old, female patient; sacro-coccygeal chordoma mass (arrow)

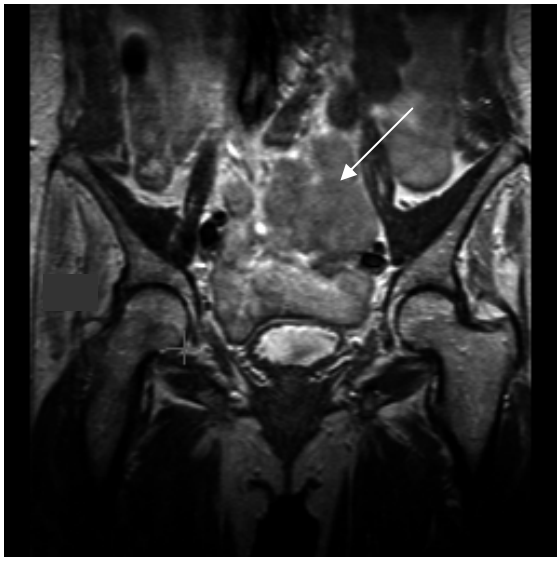


Fig.10: MR-imaging of a 59-year-old, female patient; sacro-coccygeal chordoma mass (arrow)

7.2. Biopsy

Basically a biopsy always is required if there exists a strong suspicion of a malignant tumour. The purpose of such an intervention is the extraction of tissue samples ($1cm^3$) suitable for histopathologic examinations. In this process it is necessary to pay heed to some basic rules to avoid "sampling errors".[58, 59, 60]

1) The localisation of the biopsy-tract has to be elected according to an adequate preoperative imaging and the following definitive surgical approach (Fig.11,12 show an intra-operative demonstration of a biopsy-tract).

2) It is necessary to choose the shortest, direct way to the tumour, without opening any further compartment. Thereby a safety margin towards joints, vessels and nerves should be preserved.

3) An accurate haemostasis also presents one of the major items to avoid haematoma and further spreading of malignant cells. In this process the use of a drainage-system is important.

4) The biopsy technique has to be chosen as atraumatic as possible.

5) The biopsy specimen should be removed by an experienced surgeon and histopathologically investigated by a specialized bone-/soft tissue pathologist.

Generally it is necessary to separate the *closed*- from the *open-biopsy technique*. The *closed method* is a fine needle aspiration biopsy (FNAB), eventually CT-guided, and especially is useful in spinal lesions with vague genesis. The advantage of this technique certainly is given by the exact intraoperative imaging but also by the minimal invasiveness, whereas the risks of complications like intra- and postoperative bleeding may present a disadvantage. Also the possibility to extract few utilisable tissue samples reduces the value of this biopsy option. [58, 59, 60]

If a closed biopsy is performed, it is of urgent need to mark and even document the biopsy canal entry point, to be able to resect the whole canal in a consecutive surgery, if the histological result confirms a malignancy.[60]

The *open-biopsy technique* can be separated in *incisional-* and *excisional-biopsy.* An *incision-biopsy* primarily is used if tumour type and biological behaviour is insecure, whereas the *excision-biopsy* exclusively is applied in benign tumours, the nature of which is evidenced by imaging procedures. In line with the biopsy act a marginal resection can be performed. [58, 59, 60]

7.3. Staging

If the diagnose of a malignant tumour, especially in the case of a chordoma, is secured by a bone pathologist, further staging is indispensable to exclude metastatic spreading. Therefore a computer tomography imaging of the thorax, abdomen and pelvis is applied.

In case of spinal and sacro-coccygeal tumour location further surgical staging, has to be performed by the Enneking staging system (see chapter 8). [58, 59]

7.4. Differential diagnosis

Possible differential diagnoses not only depend on tumour localisation, radiographic imaging, but also on histological aspects.

Although the histopathological pattern of chordomas is typical, the following differential diagnoses have to be considered (Table 4).

Differential diagnosis	Localisation	Histology
myxoid chondrosarcoma	skull, column, sacrum	myxoid parts impress as chondroid chordoma type
myxoid liposarcoma	sacrococcygeal area, lumbar spine	myxoid parts impress as chondroid chordoma type (lipoblasts↔physaliphorous cells)
carcinoma metastases	skull, column	renal clear cell carcinoma
parachordoma	soft tissue	chordoma like
craniopharygeoma	skull	no similarity to a chordoma
multiple myeloma	skull, column	no similarity to a chordoma
giant cell tumour of the bone	sacro-coccygeal area	no similarity to a chordoma

Tab.4: Histopathological differential diagnoses to chordoma [7, 28, 34, 81]

8. Treatment

8.1. Surgery

The treatment of chordomas is essentially based on tumour size, localisation and the clinical stage of disease depending on the aggressiveness of the neoplasia.[14, 42, 109] The most common and effective therapy with the best results of long term survival-rate is a wide local excision with tumour-free margins, either via anterior, posterior, or combined approach, and a postoperative radiation-therapy to reduce the risk of a possible tumour recurrence.[2, 18, 48]

William F. Enneking developed a staging system for musculoskeletal neoplasias that was first published in 1968.[29] Assumption for the use of the Enneking staging system is a total preoperative work-up containing clinical hallmarks, radiographic imaging, CT and MRI data describing the localisation and the exact tumour extension. Staging furthermore includes CT scans of the chest, radioisotope scans as well as a biopsy, to achieve the histopathological diagnosis.[12] Generally the Enneking surgical staging system (SSS) contains three planes of tumour assessment (surgical grade (G), surgical site (T), regional or distant metastases (M)).[29]

1. Surgical grade (G)

For surgical planning it is necessary to separate benign lesions (G0) from low-grade (G1; Stage I) and from high-grade malignant (G2; Stage II) neoplasias (Tab.5), because G2-lesions demand a more aggressive surgical procedure then G1-lesions

or even G0-lesions to achieve local tumour control. Accordingly the incidence of metastases is higher for patients suffering from a G2-lesion. This schedule line therefore gives information about surgical margins that will be required intra-operatively. [29, 30]

Surgical Grade	
G1	*G2*
parosteal osteosarcoma	Classic osteosarcoma
endosteal osteosarcoma	Radiation sarcoma
Fibrosarcoma	Fibrosarcoma
Chordoma	
Giant-cell tumour, bone	Giant-cell sarcoma, bone
other and undifferentiated	other and undifferentiated

Tab.5: Abstract of the table "Surgical Grade (G)".[30]

2. Surgical site (T)

Among the biological behaviour of a neoplasia also the anatomic setting and its extension are important hallmarks in planning a surgical procedure.

Therefore the aforementioned surgical grading further is divided by whether the tumour is encapsuled and does not extend (T0), or if the lesion presents with extra-capsular expansion into the reactive area, but still is intracompartmentally (A; T1) located. A further possibility is presented by more aggressive tumour types that develop an extracompartmental (B; T2) unit. [29, 30]

This classification depends on natural barriers that form the anatomic compartments. Skin and subcunateous tissue are seen as one compartment that is separated from deeper tissues by the superficial fascia. In the soft tissues the boundaries are generated by major fascial septae, tendinous origins and the insertions zones of

muscles, whereas corticalis and articular cartilage present the barriers of the bone. Joint capsule and articular cartilage form the compartment in joints. [29, 30]

If neurovascular structures, interfascially sited, are already infiltrated by tumour tissue, the primary lesion has to be treated as extracompartmental. [29]

3. Regional or distant metastases (M)

As aforementioned G2-lesions more often present a metastatic spread of the primary tumour then G1-lesions. Therefore even the presence (M1) or absence (M0) of filiae is a further important factor for the surgical planning and is strongly connected to the patient's outcome (Tab.6). [29]

Surgical stages		
Stage	*Grade*	*Site*
IA	G1	T1
IB	G1	T2
IIA	G2	T1
IIB	G2	T2
III	any (G); metastases;regional or distant	any (T)

Tab.6: Surgical stages. Synoptical table. [29]

Concerning tumour extension and the therefore planned type of resection, the surgical procedures are classified as intralesional, marginal, wide, and radical. [29]

The terms *curettage* and *intralesional* describe the piecemeal excision of the tumour. Thereby microscopic or even macroscopic tumour tissue is left in the surgical area. Intralesional interventions are mainly performed as diagnostic measure, for example as *incisional biopsy*. *Intralesional resection* of malignant tumours (Fig.11, 12, 13) may

provide functional palliation and pain relief, but is connected with a very high incidence of local recurrence. [12, 29]

Curettage procedures are used to remove intracranially located chordomas. [12, 29]

The term *marginal* is appropriate if the neoplasia is removed as a whole. The dissection plane runs along the peripherally located pseudo-capsule; this layer of tumour-free reactive tissue has to be confirmed histologically. [12, 29] For example a marginal procedure is an *excisional biopsy*, especially in presumed benign lesions.

The term *wide* is used if the tumour is removed with a continuous cover of healthy tissue, but still is located within the involved compartment. This procedure can be called *wide en bloc resection*. Wide margins definitively are the required resection boundaries in stage IA lesions. [12, 29]

Radical procedures are achieved, if the whole compartment is removed. The resection block contains pseudo-capsule, reactive zone and involved muscles or bone. [12, 29]

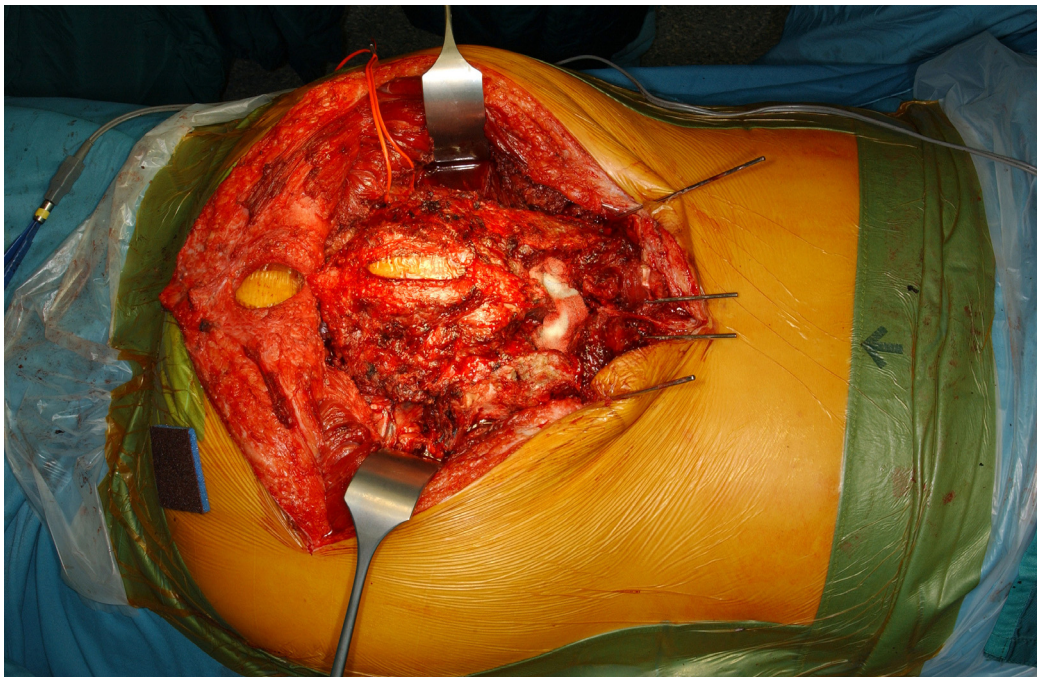


Fig.11: 75-year old male patient. Subtotal resection of the sacrum (intralesional) down from L1

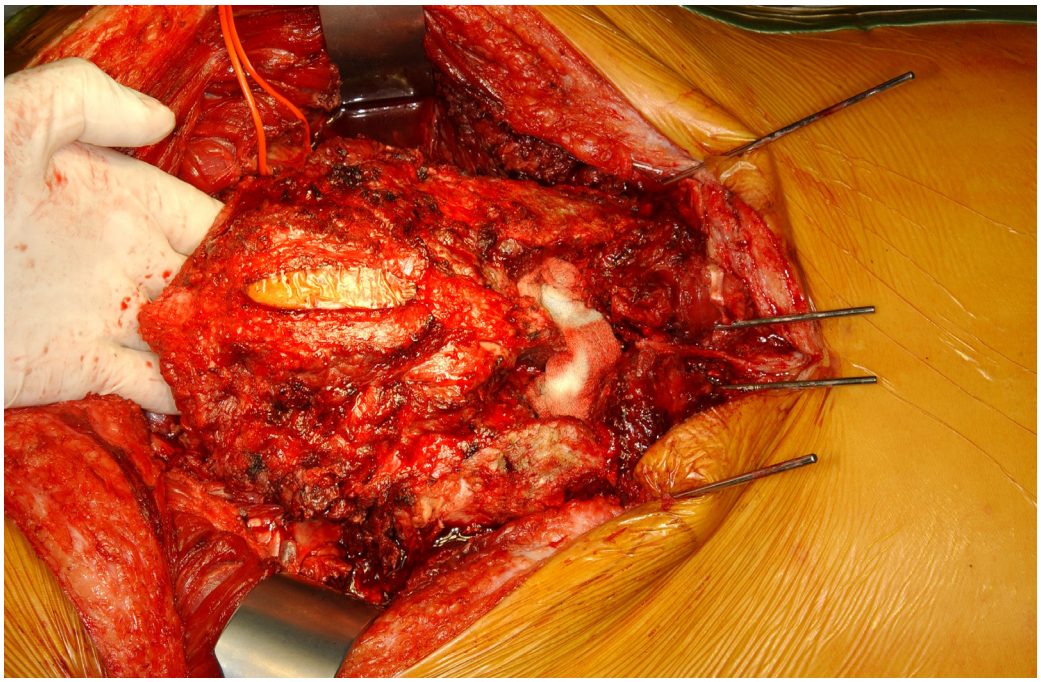


Fig.12: Demonstration of the biopsy tract

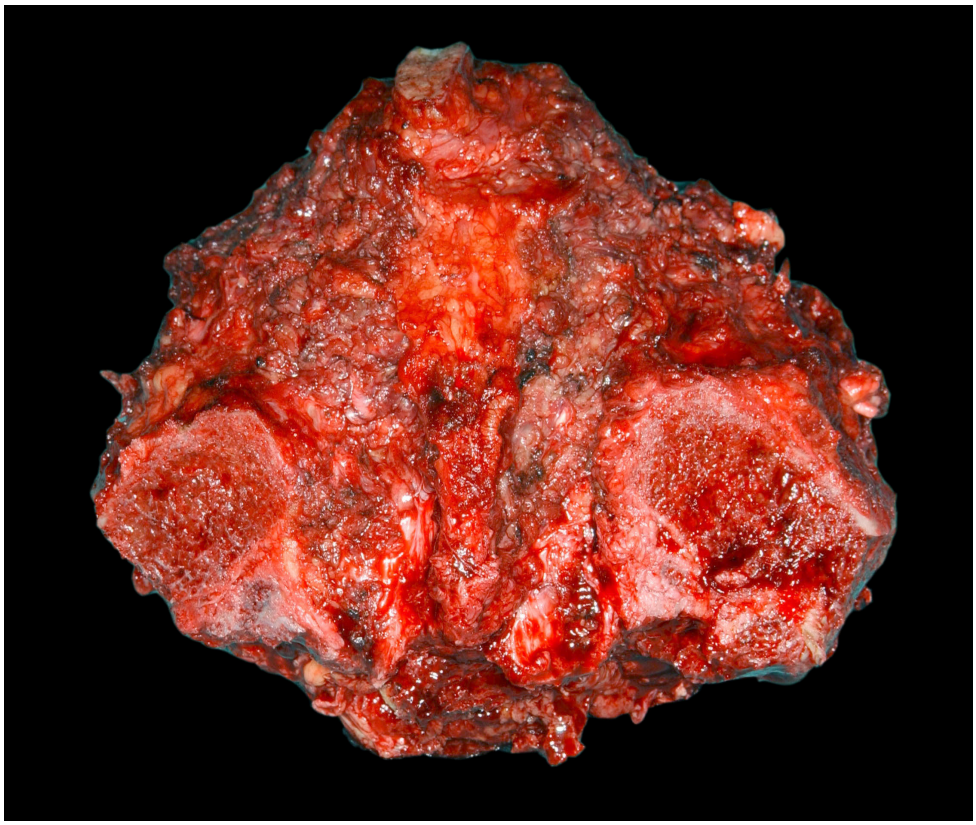


Fig.13: Macroscopic view of the resected area. The sacral chordoma presented with an expansion of 13,5 (transversal) x 11 (craniocaudal) x 9 (sagittal) cm in imaging.

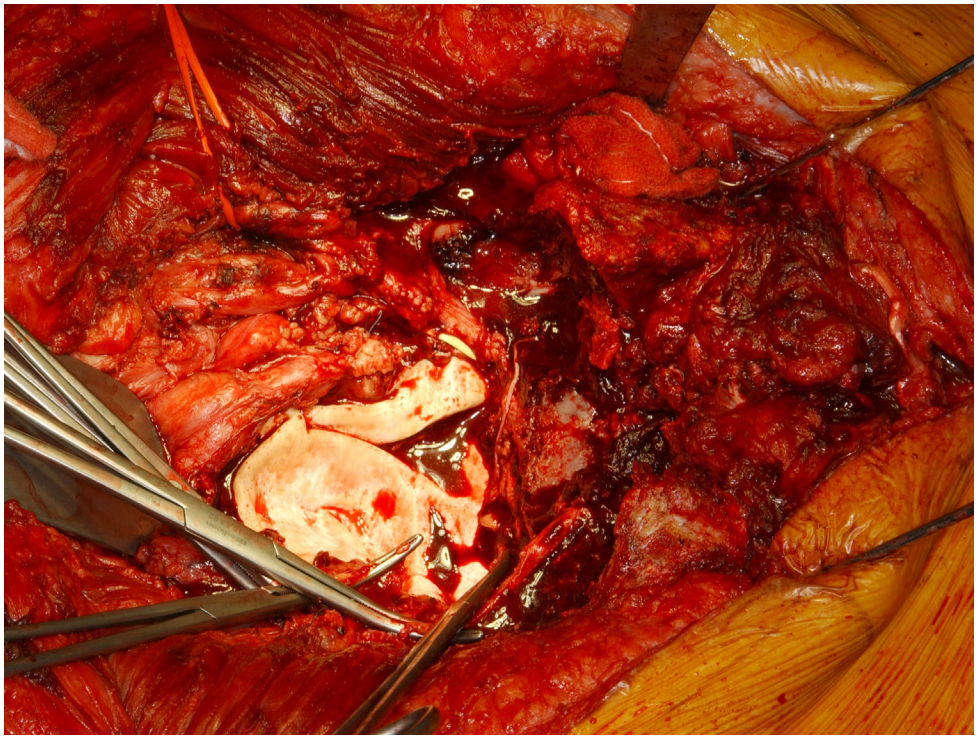


Fig.14: Intra-operative status after a subtotal resection of the sacrum

One of the most important principles in oncologic surgery is to include the tract of biopsy with an adequate margin of healthy tissue in the en bloc excision to avoid tumour spreading. [12]

Osaka et al. (2006)[80] showed in a survival analysis of 12 surgically treated patients with sacro-coccygeal chordomas, that a wide excision, performed via anterior-posterior procedures, by using the modified threadwire saw, leads to acceptable results in the recurrence free and overall survival. Osaka and co-workers were able to conduct microscopically wide margins in 11 patients. In one patient an R1 resection was performed, due to a sacral fracture. This patient postoperatively underwent radiotherapy with a dose of 70 Gy. [80] In ten patients they achieved recurrence free survival rates of 100% and 66,7% after 5 and 10 years. The overall survival rate for all 12 patients resulted after a period of 5 and 10 years was 83,3% and 55,6%.[80]

Also Bergh et al. (2000)[10] showed with their Göteborg series the importance of wide excision margins. The authors examined 39 cases. Twenty-nine patients were treated primarily at the Muskuloskeletal Tumour Center, Göteborg. In 17 patients wide margins could be achieved, in five marginal ones and in seven patients an intra-lesional resection was necessary. [10] The lacking ten patients were treated primarily outside of the centre – all with intra-lesional resection margins. Reoperations could be performed by Bergh and his team that led to six wide resections, one marginal and three intra-lesional resections. Local recurrences were found in 17 patients (44%). Among these patients 16 received intra-lesional or marginal resections. Further 11 patients (28%) developed metastases. Bergh and co-authors calculated an estimated overall survival of 84%, 64%, 52% after 5, 10 and 20 years.[10]

At the E.M.S.O.S. meeting in 2008 Bramer et al.[15] presented a clinical chordoma study. They evaluated the local recurrences and the overall survival rate in 30 patients who had suffered from chordoma. From these patients seven received a palliative treatment and 23 underwent surgical procedures (7 wide resections, 12 marginal and 4 intra-lesional resections). Local recurrences developed in 52% of the treated patients, of which the incidence rate presented 60% after intra-lesional resection, 57% after marginal resection and 25% after wide resection status (p=0,49).[15] The overall survival of the operated patient group was accounted for 67% after 5 years and 47% after 10 years. [15]

Similar high recurrence rates for intra-lesional resection margins appeared in the Mayo Clinic series by Kaiser et al. (1984).[46] They examined 63 cases, of which nine patients with recurrent tumours were primarily treated outside of the centre. In 50 patients a complete excision could be performed. Among this group of interest 25 patients were operated with wide resection margins what led to a recurrence rate of 28%. In contrast a second group with further 25 patients with an intra-lesional resection status revealed a recurrence rate of 64%.[46]

Boriani et al. (1996)[13] performed a study with 21 patients suffering from a chordoma of the mobile spine. The surgical treatment was defined by Enneking's criteria and 15 patients additionally underwent radiotherapy. Out of the 21 patients nine received a conventional radiotherapy, sometimes combined with palliative surgical treatment. Only one patient resulted in local disease control after a follow-up interval of 69 months. Further seven patients presented with slow tumour progression and in one case a recurrent chordoma was observed. In two patients an intra-lesional resection only was performed. Both cases showed recurrences after 18 and 48 months.

Further six patients underwent an intra-lesional resection and adjuvant radiotherapy. Four out of these six patients developed recurrent chordomas after a mean time of 52 months and further two patients were free from disease after an observation period of 41 and 42 months. In four patients it was possible to perform an en bloc resection, followed by an adjuvant radiation. These patients presented with the longest mean survival time of 77 months. Boriani et al. calculated an incidence of recurrence or progression in this series of 21 patients of 62% (13/21).[13]

The aforementioned studies clarify the importance of tumour-free-margins which definitively lead to lower local recurrences and therefore result in a better and prolonged survival.

8.2. Radiotherapy

Unfortunately, as experiences show, wide surgical treatment is not always save to perform because of tumour volume and localization. Hence palliative treatment options exist in surgery as well as in radiotherapy.

Although chordomas compared with other malignant tumours are relatively radio-resistant, megavoltage radiation therapy can be utilised in curative or palliative ways and can lead to tumour regression. Radiotherapy is also used after incomplete surgical excision out of inaccessibility, or just to alleviate pain in patients with widespread recurrent tumours.[18, 42, 81]

A combination of megavoltage CT scanner and therapeutic linear accelerator is used in helical tomotherapy.[100] Therewith it is possible to achieve high-dose-distribution for different tumour entities, but also different locations. The tomotherapy system

represents a special advantage for patients with extensive malignancies, or multiple lesions and organs at risk by neoplasia's proximity. Since 2006 this combined therapeutic method is available at the Department of Radiation Oncology, University Hospital of Heidelberg, Germany.[100]

Generally proton beam radiation, a specific type of megavoltage therapy is used to avoid tissue destruction beyond the neoplasia by application of high local concentrations [81] Rutz et al. (2007)[89] performed a retrospective analysis of 26 chordoma patients treated with spot-scanning proton beam radiation after function-keeping surgical treatment. Hadron beams (protons, helium, carbon or neon ions) for radiation therapy permit the use of high doses that concentrate in the target volume to treat. It presents a conservative treatment option because of low-dose entrance and steep fall-off after passing the target volume. [89] The results of Rutz et al., after a follow-up period of three years, showed a higher local control rate compared to studies that examined the effect of photon based radiotherapy. These authors achieved an overall survival of 84% after three years and a progression free survival of 77%, whereas a residual tumour volume of more than 30ml after surgery showed a negative association regarding the overall survival (p=0,013) and the progression free survival (p=0,025). [89]

Similar results were attained by Schulz-Ertner (2002)[94] et al.. They treated 24 skull base chordoma patients with carbon ion radiotherapy within a phaseI/II trial, with a median tumour dose of 60 GyE (=Gy x relative biologic effectiveness). Schulz-Ertner et al. achieved both an overall survival of 81% and a progression free survival of 83% after 2 years [94]

An analysis by Zorlu et al. (2000)[120] revealed that conventional radiotherapy, under application of standard doses of 50 to 60 Gy, in patients with clival chordomas led to a poor outcome. Zorlu and co-workers treated 18 patients (11 with subtotal surgery, seven had a biopsy only) and calculated an overall survival after 5 years of 35% and a progression free survival after 5 years of 23%. These low survival rates suggest that a conventional external radiotherapy is useful in palliative treatment of clival chordomas but has no effect concerning the long-term progression free survival. [120]

Park et al. (2006)[83] examined the treatment efficiency of high-dose proton/photon-beam radiotherapy alone and in combination with surgery for sacral chordomas. Their study included 27 patients (10 females, 17 males) with primary chordomas as well as with recurrent tumours. 21 patients were treated with surgery and radiotherapy compared to six patients, who underwent radiation exclusively. [83] Out of the 21 patients 14 with primary tumours and seven with recurrent chordomas were treated. Park et al. calculated a disease-free survival for the primary chordoma group of 90,9 ± 8,7% after 5 and 10 years and an overall survival of 92,9 ± 6.9%. Those group with recurrent chordomas showed an overall survival after 5 and 10 years of 66,7 ± 19,3% and 44,4 ± 22,2%. The disease-free survival resulted in 42,9 ± 18,7% after 5 years and 14,3 ± 13,2% after 10 years. These study results led to the authors' current policy to perform a maximal surgery and radiation at time of primary treatment. [83]

8.3. γ-Knife Treatment

A further option of treatment is the use of Gamma Knife SRS (stereotactic radiosurgery), a technique developed by the two swedish scientists, Lars Leksell and Björe Larsson, in 1968. Normally this kind of treatment is only applied in the area of the neurocranium; therefore only small skull base chordomas, where a high dose selective radiosurgery can be given, can be treated.

Martin et al. (2007)[67] examined data of 18 chordoma patients, who underwent SRS, but also resection, re-resection, and radiotherapy. With a median follow-up period of 88 months after a single SRS therapy they calculated a tumour control of 53,4 ± 9,7% after 5 years. In three patients a second radiosurgical session followed and showed an overall and tumour control survival after 5 and 10 years of 62,9 ± 10,4%.[67]

8.4. Chemotherapy

Chemotherapy has virtually no role in this disease; however, molecularly targeted therapy is showing significant promise and may present an area of great potential in future therapy strategies of chordomas.[80]

9. Results of the clinical part of the study

Within our study we also elicited the status of resection, the effectiveness of radiotherapy regarding overall survival, and recurrence free survival. Baseline data were obtained from the hospital database of the Medical University of Graz and patient charts including age, sex, date of diagnosis, tumor histology, grade, tumor stage, sites of metastases, and treatment (surgery, radiotherapy, gamma knife). The used methods were Cox proportional hazards regression with score test criterion.

The resection status we investigated in our population of 34 cases, showed 25 intra-lesional resections, two marginal, and two wide resections. In five patients biopsy exclusively was done (summarized in Tab.7). Out of these five patients two underwent radiotherapy.

Number of patients	Status of resection
25	intralesional
2	marginal
2	wide
5	biopsy only

Tab.7: Resection status

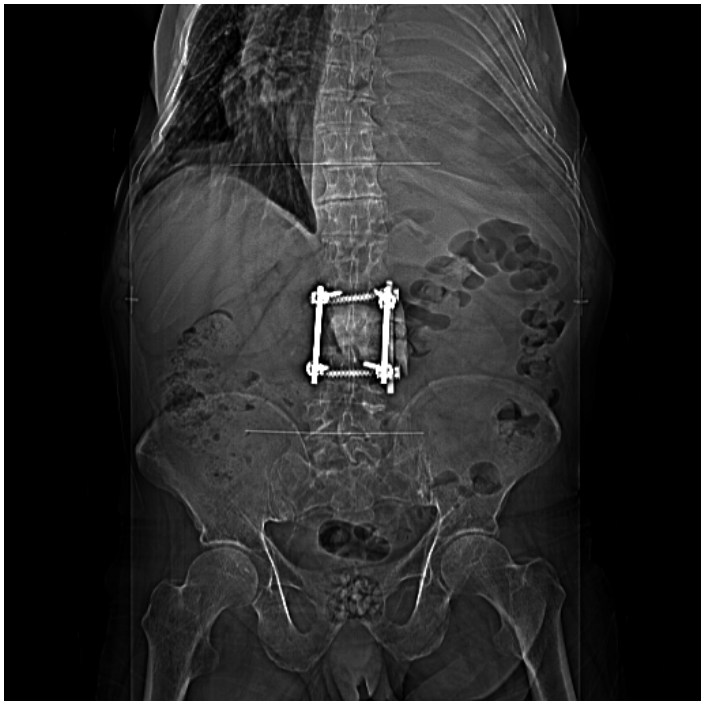


Fig.15: Postoperative x-ray a.p. of a 68-year-old male patient. Reconstruction and stabilization (ventral/dorsal) of L2

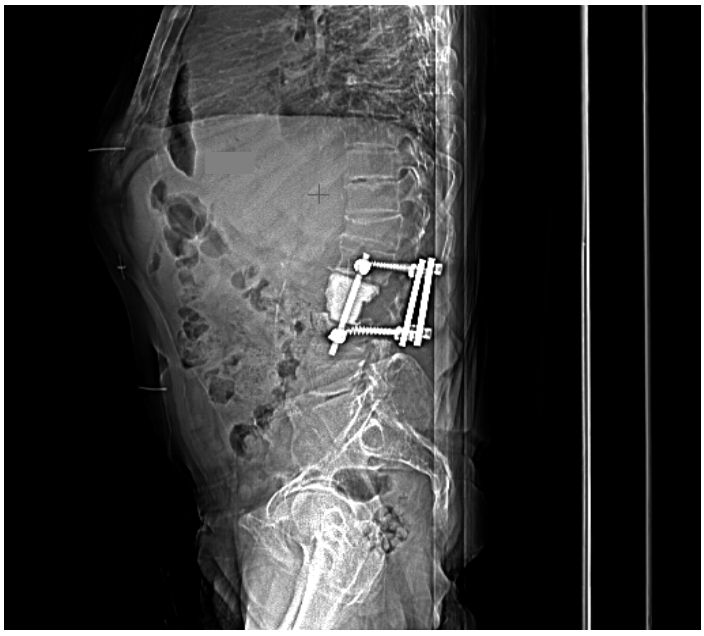
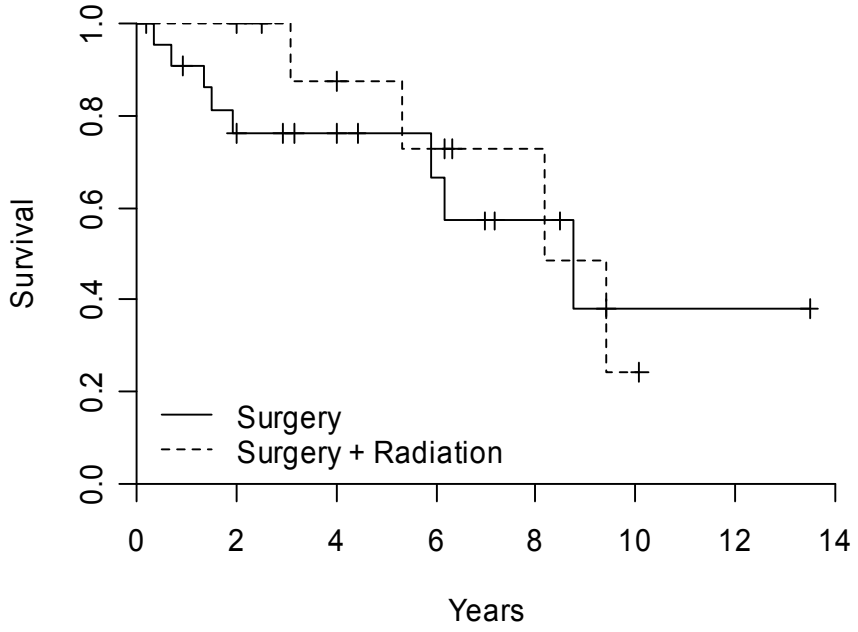


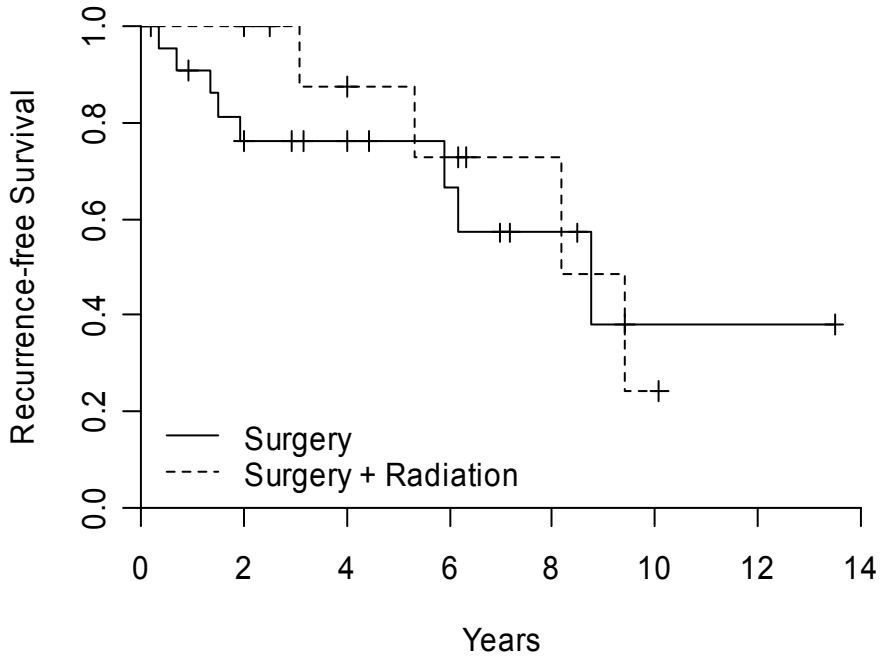
Fig.16: Postoperative x-ray lat. of a 68-year-old male patient. Reconstruction and stabilization (ventral/dorsal) of L2

In 13 patients a residual tumour remained after intralesional resection. Moreover six patients developed local tumour recurrences – all after intralesional resection margins. The overall survival after 5,3 years resulted in 74,7% and after 9,4 years 32,5%. The recurrence free survival after 5 years was 77%.

The following Kaplan-Meier graphs (Graph 6, 7) illustrate the aforementioned statistical results:



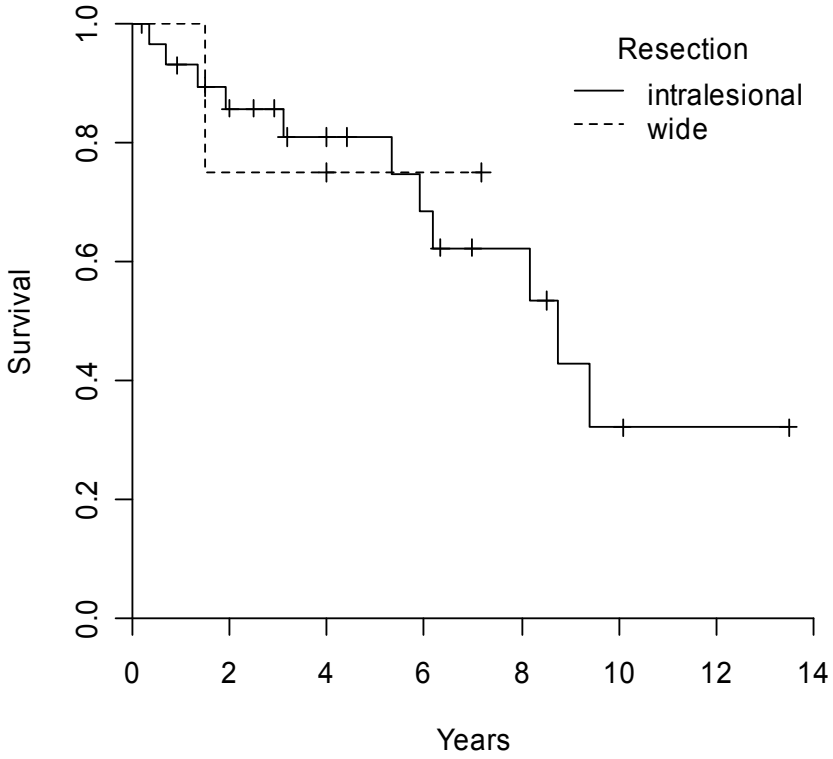
Graph 6: Overall survival in regard to resection with or without radiotherapy



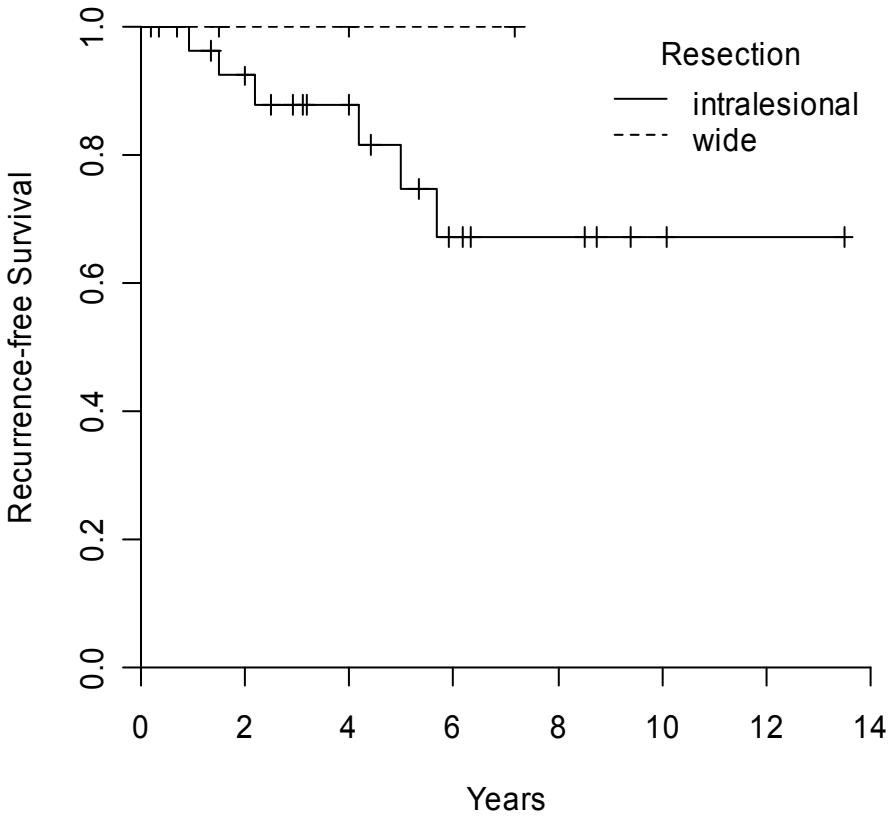
Graph 7: Recurrence free survival in regard to resection with or without radiotherapy

12 patients died due to disease. Among these four survived 78 months after intralesional resection followed by radiotherapy. In other five patients surgery alone was done. They survived on average 45 months. Two patients survived 16 and 4 months respectively, after rejecting any therapy. Just one patient, who suffered from a clival chordoma, underwent gamma-knife-therapy and survived 7 months.

The following Kaplan-Meier graphs (Graph 8, 9) illustrate the study results:



Graph 8: Overall Survival in regard to resection status



Graph 9: Recurrence free survival in regard to resection status

10. Conclusion

As the aforementioned data of publications show, there exists a multitude of factors influencing the survival rate of patients suffering from chordomas. This is the reason why the first part of the analysis presents the evaluation of “survival factors” in terms of “tumour localisation”, “histopathological features of the neoplasia and evaluation of resection margins”, “metastases and recurrence rate”, “tumour volume”, “age and sex”, and “treatment”.

Though “chordoma science” lasts for more than 30 years in the majority of clinical studies the number of chordomas, including our series, is low. The resulting tumour rarity therefore raises the problem of little understanding of chordomas’ biological behaviour.

A number of studies showed that surgery is the primary modality to achieve the best long-term control in patients suffering from a chordoma.[10, 15, 46] However, due to the localisation and the extension of these malignancies an en bloc excision, often presents a challenge to a surgeon and his team to achieve adequate negative margins. Further, the prevention of local recurrences is mainly achieved by marginal or wide resections, which show generally a better survival. [10, 15, 46]

A further option in chordoma treatment is the use of conventional radiotherapy. It still presents an adjuvant or palliative therapy option and therefore has a proven role.[119] However, the high doses necessary for treatment of this radioresistant tumour entity leads to significant toxicity to surrounding normal tissues and limits the therapeutic value.

Newer techniques like hadron beam radiation presented in studies by Rutz et al.[89] and Schulz-Ertner et al.[94] permit the use of high ray doses that concentrate in the targeted area exclusively. The toxic effect to the surrounding healthy tissue lacks and therapeutic results present better disease control.

Different studies show that local control of chordomas is one of the most important factors in regard to the development of metastases and further tumour related death. [10, 15, 46]

The progressive course of chordoma disease also depends on the histological differentiation. In our chordoma series the classic variant was the most common type, whereas only one dedifferentiated type could be found. Dedifferentiated subtypes are known to show the most aggressive biological behaviour and tend to an early development of metastases. [20, 109] McPherson et al.[70] found that metastases exclusively appear in chordoma-patients with local recurrent tumours (28%), whereas patients without local tumour recurrences showed no metastatic disease.

Especially in our series it is evident that even an interdisciplinary work is a determining factor not only for the patients' outcome but also for the better understanding of the biological behaviour of chordomas. Due to the different tumour localisations and therefore heterogeneous therapy modalities we did not achieve significant p-values in relation to the elected prognostic factors (summarized in Tab.8).

Risk factor	OS	RFS
Localisation	0,14	0,7
Resection	0,998	0,4
Log(volume)	0,079	0,391
Therapy	0,65	0,486
Histology	0,86	0,237
Resection within Therapy	0,9	0,91

Tab.8: Synoptical table of p-values (OS: overall survival; RFS: recurrence free survival)

Despite this fact we draw on already published analyses that a marginal or wide resection status in combination with radiotherapy leads to the longest recurrence free and overall survival.

11. Analysis of immunohistochemical features

11.1. Classical immunohistochemical profile of chordomas

The second part of this study concentrates on analysing immunohistochemical features of chordomas. In these days standard antibodies like S-100 protein, low molecular weight cytokeratin, pan-keratin, vimentin, and Epithelial Membran Antigen (EMA) and very recently brachyury are used to identify this neoplasia. Despite, it is possible that other malignant tumours, for example chondrosarcomas, present similar genetic and immunohistochemical characteristics like chordomas. Thus it is necessary to examine and define specific markers to facilitate the histological diagnosis and to find further strategies in the therapy of chordomas. [71, 81, 95]

The following table (Table 9) shows basic antigens which are typically expressed by chordomas.

Author/Year	*Relevant antigens for chordoma-diagnosis*
Nakamura Y. et al. (1983)[74]	S-100 protein
Salisbury J.R. et al. (1985)[92]	Cytokeratins (CK 8, CK 18, CK 19)
Salisbury J.R. et al.(1985)[92]	EMA (epithelial membrane antigen)
Vujovic S. et al.(2006)[110]	Brachyury

Tab. 9: Diagnostically relevant antigen expression in chordomas.

11.2. “New markers” for biological behaviour in chordomas

11.2.1. Survivin

Survivin, in general, has a bifunctional role in influencing the cell cycle. On the one hand it suppresses apoptosis, while inhibiting further processing of procaspase-3 and procaspase-7, as well as the enzymatic activities of caspase-3, -7 and -9; on the other it regulates the main part in promoting cell division by interacting with B tubulin, microtubules, centrosomes and kinetochores.[4, 46, 62, 63, 66, 112] This specific communication between survivin and the mitotic apparatus is just possible because of the chemical structure of this anti-apoptotic protein. The gene product, survivin, presents a lacking of the COOH-terminal RING finger domain also a single baculovirus (IAP) repeating domain (BIR), which is essential in inactivating the aforementioned caspase-3 and caspase-7.[4, 66] Furthermore the suppression of apoptosis and cancer cell viability is to put down to an increased activity of $p34^{cdc2}$ kinase. This enzyme is elevated during spindle checkpoint activation and leads to enhanced levels of survivin expression.[75]

Survivin belongs to the IAP (inhibitor of apoptosis) gene family. So far few studies have examined the role of survivin in different types of malignant tumours to figure out, if there exists a key role of survivin in matters of histological tumour grading, the histological type, patients‘ clinical features and even the survival rate.[1, 79, 96, 103, 112]

Li et al. (1998)[62] found that a gap of the interaction of survivin and the microtubule apparatus results in a deprivation of survivin’s anti-apoptotic feature. This leads to a boosted activity of caspase-3 what is tantamount to an increased level of mitosis. This study suggests, that an overexpression of survivin in malignant tumours induces

a fall through of the apoptotic checkpoint and yield to further mitosis of neoplastic cells.[62]

In this regard it has to be mentioned that there exist three splicing variants of survivin, “survivin-ΔEx3”, “survivin-2B” and “survivin-3B”, which have varying anti-apoptotic properties. While survivin-2B shows a clearly reduced potential in inhibiting methotrexate-induced apoptosis in HepG2 cells, survivin-ΔEx3 acts similar to survivin. Exclusively survivin is able to confirm all mitotic functions. Unfortunately it is not known, if these anti-apoptotic effects are cell type-specific or general phenomena, in all tissue types appearing. Maybe an exact elucidation of the controlling mechanisms of the expression of survivin and its splicing variants could give information about the anti-apoptotic mechanisms in neoplastic cells.[57, 66]

Normally survivin is expressed during the embryonic and fetal development, but is untraceable in most normal, terminally differentiated tissues expect the thymus, CD34+ haematopoietic stem cells, placenta, and the basal part of the colonic epithelium.[6, 45, 77, 79, 112] Moreover, survivin also is overexpressed in various types of malignant tumours, like osteosarcomas[78, 112], soft tissue sarcomas[47], neuroblastomas[1], cancer of breast [102, 6], lung, liver, colon and stomach [76], oesophagus, pancreas, bladder, uterus, ovarian, prostate, and kidney, melanomas or nonmelanoma skin cancer, and lymphomas or leukaemia.[45] Whereas cancer cells express the “surviving protein” just in the G2/M phase, the CD34+ cells express it during the whole cell cycle.[4, 45, 112] This overexpression in neoplastic tissue suggests a reactivation of the survivin gene, localized on chromosome 17q25 - complementary to the effector cell protease receptor-1 (EPR-1). EPR-1 accumulates in malignant neoplasias, and is associated with higher tumour aggressiveness and a poor survival

rate, although a patient's poor outcome also depends on the level of expression of survivin and it's localisation within the cell.[1, 5, 6, 45, 47, 77, 79, 96] In this context a study by Stauber R. et al. (2007)[99] has to be mentioned that describes the prognostic relevance of nuclear and cytoplasmic survivin. Data indicate that cytoplasmic survivin demonstrates a form of "cytoprotective survivin", while a nuclear localised expression represents the "affected survivin" function.[99] These findings lead to the assumption that a reduction of cytoprotective survivin and therefore an increase of nuclear survivin is associated with a better clinical outcome.[99] This dual "shuttle activity" of survivin is tracing back to the so-called Crm1/survivin axis. Crm1, a nuclear export receptor, creates a cytoplasmic survivin gradient that is countervail by passive diffusion. To build the gradient, Crm1 needs an impulse, which is given by NES (nuclear export signal). In ordinary dividing cells NES is essential for the binding of survivin to the apoptotic machinery that in turn is necessary for a further cell division. Though survivin's cytoprotective function is explained by high survivin levels in the cytoplasm it facilitates an interaction with the apoptotic machinery even in malignant cells. [99]

Adida et al. (1998)[1] studied the expression of survivin and bcl-2 in neuroblastomas in view of the patient's prognosis. They analysed 72 cases by immunohistochemistry and immunoblotting and referred it to the clinical stage of disease and the histological classification. Out of 72, 34 samples (47%) presented with a positive staining for survivin. Adida and co-authors evaluated a significant overexpression of survivin in stages 3-4 (p=0,001), but not in the stages 1-2. Two cases of neuroblastomas showed a spontaneous regression and did not express survivin. In conclusion the results of Adida et al. lead to the assumption that survivin-positive neuroblastomas

are strongly connected to biologically unfavourable tumours and present with a more aggressive course of disease. [1]

A low apoptosis index (<0,52,%) in breast carcinomas, explained by the expression of survivin, but also bcl-2, was found in analysis by Tanaka (2000) et al.[103] They were able to find a significant correlation between the individual expression of survivin and bcl-2, in 118 out of 167 cases irrespective of a simultaneous co-expression suggesting that these two proteins may mediate anti-apoptotic mechanisms. [103]

Okada et al. (2001)[77] performed an analysis including 133 gastric cancer specimens and recognized a nuclear localisation of survivin in 109 cases (82%), but also a cytoplasmic staining in 117 samples (88%). The nuclear expression of survivin thereby was associated with a lower incidence of vascular invasion (p=0,0239), younger mean age (58,8±11,8 years; p=0,0038) and poorly differentiated carcinoma (p=0,0586). A significant better outcome in regard to survival showed the Kaplan-Meier survival analyses (p=0,0485), while cytoplasmic staining did not show significant correlations. Independent prognostic factors were evaluated by Cox proportional hazard (p-value for nuclear staining of survivin 0,0463, depth of cancer invasion 0,0104, lymph node status 0,0088). According to the author's results survivin expression of the nucleus of gastric cancer cells has a positive bearing on tumour progression as it seems to interfere growth. [77]

As aforementioned also different expression levels of survivin seem to exert influence on the patient's clinical outcome.

This result is in accordance with analysis of Kappler et al. (2001)[47], who analysed 98 frozen soft tissue sarcoma samples by quantitative RT-PCR from 94 patients. Kappler et al. detected that a perspicuously increased survivin expression in soft

tissue sarcomas is significantly related to a poor prognosis in soft tissue sarcoma patients (p=0,009). A further evaluation of specimens of patients without tumour recurrence (57/94) also showed a poor outcome. Twentynine patients, who presented with an intratumoral survivin mRNA overexpression, died 29 months (mean value) earlier than further 28 patients who presented with low transcription levels of survivin. Thus, as aforementioned, the analyses result in a significant association between increased survivin levels and poor prognosis. [47]

Even colorectal tumours show increased levels of survivin and a lower value of the apoptosis index (AI). This result is to put down to analysis of Kawasaki et al. (2001)[49] who found different levels of survivin in colorectal neoplasies according to their biological behaviour. Adenomas with low dysplasia, for instance, presented an explicitly lower survivin level in contrast to adenomas with high grade dysplasia or even carcinoma in adenoma.[49]

These results correlate with a study of Sharma et al. (2004)[96]. During the analysis of 50 head and neck squamous cell carcinoma samples they found a survivin overexpression (p<0,001) in 86% of the cases. They also detected a significant association with a loss of differentiation (p=0,021) and correspondingly a worse prognosis. [96]

Osaka et al. (2006)[79] found, that patients, suffering from an osteosarcoma present different expression levels of survivin. Those who already developed metastases or had died within 5 years of the follow-up period showed significant higher levels of this protein (p<0,01) than the others, what makes survivin to a meaningful prognostic marker in respect of osteosarcomas. Further, Osaka and co-authors found a significant negative correlation between initial biopsy specimen and survivin

expression levels (p=<0,01), similar to samples examined after chemotherapeutic treatment (p=0,01). [79]

An analysis by Knauer et al. (2007)[56] tested the hypothesis that patients with predominantly nuclear located survivin present with better results of overall survival. Therefore, they immunohistochemically examined the intracellular localisation of survivin in colorectal cancer samples. Out of 263 cases, 24 (9,2%) presented with nuclear survivin only, leading to statistically significant results in terms of survival (p=0,005). These clinically relevant findings, suggest that nuclear located survivin strongly is associated with increased survival in patients suffering from colorectal cancer. Further, Knauer and co-authors were able to produce a gap of suvivin's export into the cytoplasm via NES-specific antibodies what led to a nuclear accumulation of this IAP. Consecutively survivin's cytoprotective function was prevented. According to Knauer et al. survivin specific transport blockers may present a future option in cancer treatment. [56]

Hypothesis

Out of the fact that the role of the IAP protein, "survivin", in chordomas still is unclear, we hypothesise, based on the mentioned studies and their results, that this protein also is overexpressed in chordomas and may present a crucial prognostic factor concerning the survival rate in patients.

11.2.2. TRAIL/TRAIL R1-R4

Apoptosis (programmed cell death) is induced by several different biochemical and physiological signalling pathways. One essential group that is taking part in these mechanisms, are the so called “death receptors” and their dedicated ligands building a death-inducing signalling complex (DISC).

In general, death receptors rank among the tumour necrosis factor (TNF) receptor gene superfamily, a growing group of cytokines, which consists of numerous members like CD 95 (also called Fas or Apo1), TNFR1 (homonymous p55 or CD120a), which are the best studied components in this family, furthermore *DR3* (homonymous Apo3, WSL-1, TRAMP or LARD), *DR4*, and *DR5* (also called Apo2, TRAIL-R2, TRICK 2 or KILLER) etc.[8, 38, 64]

TRAIL (homonymous Apo-2L), the ligand itself, is among the type II membrane proteins and expressed by various human tissues with predominance of lung, spleen and prostate. The TRAIL gene is localized on chromosome 3 in band 3q26 with the utmost probability in region q26.1-q26.2.[64, 114] Typically this protein is made up of a short intracellular, cytoplasmatic N-domain and an extracellular conserved C-terminal domain.[114]

Surprisingly TRAIL, a apoptosis-inducing protein, just presents its effect in different tested tumour cell lines, but not in normal, sane cells, what leads to the assumption that it can be utilized in cancer treatment.[110] In addition it should be recognized that TRAIL induces apoptosis, while binding TRAIL-Rs, through various effects, like a diminution of the metabolic activity, blistering of the cellular membrane, a

disconnection of the cytoskeleton, as well as a fragmentation of DNA after a release of cytochrome c with consecutive activation of caspase-8 and caspase-3.[25, 31, 96, 111]

In these days four human TRAIL receptors are identified. TRAIL-R1 (DR4), -R2 (DR5, Killer, TRICK2), -R4 (DcR2, TRUNDD) present classical type-I transmembrane proteins, that show a similarity in their chemical structure to further members of the TNFR family. Especially TRAIL-R1 and -R2 are characterized by an intracellular, cytoplasmatic “death domain” (DD), showing a significant concordance to TNFR1 and CD95, while TRAIL-R4 just features an incomplete DD, and TRAIL-R3 (DcR1, LIT) completely lacks the intracellular domain.[12, 21, 35] Especially the extracellular domain, mediating the receptor-ligand binding, presents similar sequences to the TNF family cytokines.[85]

Although the biological role of TRAIL and the TRAIL-Rs is not completely understood as yet there exist different argumentative studies concerning the mediation of death signals. TRAIL-R3 and -R4 are regarded as so-called antiapoptotic “decoy receptors” (DcRs) vying for TRAIL’s binding with TRAIL-R1 and -R2. The both receptors seem to mediate the apoptotic signal by recruiting endogenous FADD, caspase-8, caspase-3 (Fig.17).[17, 31, 55, 111] This finding is also approved by other analyses, using cell lines like BJAB, BL60, and Jurkat. It was emphasized that FADD and caspase-8 present as essentially integrated constituents of the TRAIL-R2-DISC.[111]

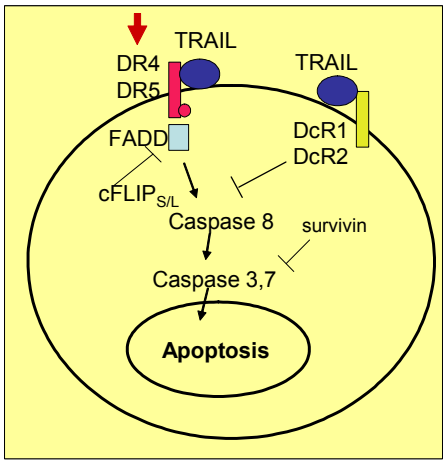


Fig.17: Pathways of DR4 and DR5 and TRAIL (with permission from Leithner, 2007)[61]

In addition, it is known that the receptors are consistently co-expressed in normal cells as well as in cancer cells, although their level of expression differs. Furthermore they are capable to mediate the apoptotic signal independently.[55, 119]

It is well known that most part TRAIL receptors are expressed in different cancer cell lines. Griffith et al. (1998)[38] studied TRAIL-induced apoptosis in human melanoma cell lines. They detected that five out of eight cell lines present a significant sensitivity towards the cytotoxic effect of TRAIL and additionally an increased level of TRAIL-R1 and TRAIL-R2. Griffith et al. also found a simultaneous coexpression of DcR1 and DcR2. Their results also suggest that TRAIL could be established in cancer treatment. [38]

Even in TRAIL-sensitive breast cancer cell lines an augmented expression of DR4 and DR5 was observed.[54] Keane et al. (1999).[51] also analysed the effect of TRAIL and doxirubicin, but also 5-fluorouracil, methotrexate, and paclitaxel on 16 breast cancer cell lines. The authors found that the TRAIL-induced apoptosis in breast cancer cell lines, auxiliary treated with doxorubicin and 5-fluorouracil, was significantly increased in contrast to other cell lines that were treated with other

common chemotherapeutic agents. This study suggests that chemotherapy agents lead to caspase activation in affected TRAIL-sensitised cells ending in apoptosis. [51]

Kischkel (2000) et al.[55] even found a significant expression of DR4 and DR5 in B-cell lymphoma cell line BJAB, while the decoy receptors DcR1 and DcR2 just could be detected in a minor degree or completely lacked. This could mean a minor importance for DcR1 and DcR2 in death regulation with regard to TRAIL. [55]

Evdokiou et al. (2002)[31] concentrated on osteosarcoma cells and their behaviour in presence of TRAIL and/or chemotherapeutic agents. They found that treatment of osteosarcoma cells with TRAIL induced in only one out of six cell lines significant cell death, but a combination of TRAIL and doxorubicin, for instance, led to an increased level of apoptotic cell death. This was associated with an up-regulation of DR4 and DR5 mRNA, while normal human bone cell cultures did not present any effect under same conditions. So this study indicates too that TRAIL and chemotherapeutic agents build a synergism concerning cancer cell killing. [31]

A study of Yoshida et al. (2003)[119] presented different expression levels of DR4, DR5, DcR1, and DcR2 in six malignant rhabdoid tumour cell lines. They detected a low expression of DcR2 in TRAIL-sensitive cell lines, while the expression levels of DR4 and DR5 were high. In addition, Yoshida et al. found a significant up-regulation of DR5 and a not marginal up-regulation of DR4 and DcR2 after a treatment of the TM87-16 cell line with doxorubicin too.[119] At the same time they noticed that the pro-apoptotic effect of TRAIL was increased, leading to a low survival cell rate. [119]

According to a study of Van Valen et al. (2003)[108] osteoscarcoma (OS) cells enter in a marginal programmed cell death after TRAIL-treatment, compared with normal primary hOB (osteosarcoma cell) cultures, which presented a resistance towards TRAIL's apoptotic effects. They also tested the implication of combined TRAIL and doxorubicin (also other chemotherapeutics like ActD, CDDP) treatment in OS cell lines, what led to the result that both human bone tumour and sane human cells show sensitivity to TRAIL-induced apoptosis. [108]

As mentioned before several in vitro studies have examined the role and function of TRAIL and its apoptosis-inducing receptors. In contrast Spierings et al. (2003)[97] performed an immunohistochemical analysis examining 87 specimens of NSCLC (non-small-cell lung cancer) in regard to the expression of DR4, DR5 and TRAIL. The authors achieved positive cytoplasmic staining results for DR4 in 99% of the samples, for DR5 in 82% and for TRAIL in 91%. The staining intensity for DR5 generally presented lower than for DR4. DR4 and TRAIL as well as DR5 and TRAIL highlighted significant correlations (p=0,02, p=0,034; n=74). In regard to different histological NSCLC types (squamous cell carcinomas or adenocarcinomas) no significant associations between DR4 and DR5 were recognized.[97] Poorly differentiated areas of this cancer reflected a very intense staining for the examined receptors and the dedicating ligand. Moreover Spierings et al. evaluated a correlation between DR5 positivity in NSCLC and a demanding risk of death (p=0,045). They did not find any association between the expression of DR4 and DR5. Because of unresectable stage III NSCLCs express death receptors and their ligand TRAIL, using rhTRAIL in combination with chemotherapy, may present a future therapy option. [97]

Also a study of Mirandola et al. (2006)[72] dealt with TRAIL and its effect on osteosarcoma cells in consideration of IAP family proteins. They found that the U2OS cell line shows a significant expression of TRAIL-R2 and enters in TRAIL-induced programmed cell death, while they observed a lower expression of TRAIL-R1 and -R4, and a completely lack of TRAIL-R3 on cell surface. These findings suggest that DR5 presents the main component in signalling TRAIL-induced apoptosis in U2OS cells. Furthermore Mirandola et al. ascertained that the aforementioned specific cell line steadily expresses c-FLIP, X-IAP and survivin. By treating the cells with DOX they detected a down-regulation of survivin mRNA, and in absence of X-IAP it was able to induce sensitivity of U2OS cells towards TRAIL's apoptotic impact.[72]

Hypothesis

Since the role of TRAIL and its dedicating TRAIL receptors, similar to survivin, in chordomas still is unknown, the aim of the study is to examine, (i) if TRAIL-R1/DR4 and TRAIL-R2/DR5 are expressed in chordomas and can be compared to the expected overexpression of survivin, and (ii) if the TRAIL receptors, TRAIL-R1 and TRAIL-R2, can also act as prognostic markers concerning the survival rate in patients.

11.2.3. Ezrin

Eucaryotic cells are composed of a meshwork of cytoskeletal proteins ensheathed by the plasma membrane. These proteins give structural support to the plasma membrane and therefore, inter alia, define the cell's shape. Further the cortical cytoskeleton facilitates dynamic processes as cell migration/invasion and division, cell-cell interaction and cell adhesion via transmembrane signalling pathways, but also endocytosis and exocytosis, growth regulation and cell differentiation.[16, 24, 69]

The protein *ezrin*, also known as *cytovillin or villin2*, is a member of the actin-binding ERM (ezrin-radixin-moesin)-family. It plays not only a crucial role in cell structure-bearing measures, but is also impressive by the regulatory function in the formation and stabilization of specialized plasma membrane domains. Ezrin, predominantly located and best studied in epithelial cells, is the product of the Vil2-gene.[16] It is strongly connected to the band 4.1 protein superfamily, by meaning an interaction with membrane proteins and the actin cytoskeleton. This kind of cooperation is only possible if ezrin is existent in its activated, "undormant" form. Amino- and carboxy-terminal sites also were identified in ezrin. In the cytoplasm all ERM members are existent in an inactivated, dormant conformation, while the respective N- and C-terminal ends undergo intramolecular head-to-tail bindings or attach to further members of the ERM family. Thus the possible biological binding sites are masked. Threonine and tyrosin phosphorylation via PIP2 (phosphatidylinositol 4,5-bisphosphate) convicts ezrin in the active, opened conformation. PIP2, in turn, is phosphorylated by an effector kinase that is activated by the GTPase RHOA. [16, 24, 41, 52, 69, 91] The activated ezrin, ordinarily subcellular located, moves, due to the conformation dependent change, to the cell membrane and directly binds F-actin via

the carboxy-terminal domain to the cell membrane. The actin-binding region of ERM proteins resides in the tail and is localized to the last 34 amino-acids in the sequence. [16, 24, 41, 52, 69, 91] Furthermore ezrin directly interacts with the cytoplasmic tail of the transmembrane receptor for hyaluronic acid, a gene product of CD44.[16, 69] CD44 belongs to a family of cell surface glycoproteins that are strongly involved in the development of metastases, tumour growth and invasion. A stabilization of CD44 complexes via ERM-family members lead to the assumption that they control tumour growth and invasion. [16, 24, 41, 52, 69, 91] Study results by Peng et al. (2002)[84] support this acceptance. They found that increased levels of CD44 expression correlate with a high expression rate of ezrin and leads to an intensified invasion in osteosarcomas. [84] A further study confirms the results of Peng et al.. [52]

Khanna and co-authors (2004)[52] identified a significant association between increased levels of ezrin expression and the poor survival of patients suffering from osteosarcoma. Within a 120-day in vivo study they used wild-type K7M2 (K7M2-WT) mouse osteosarcoma cells and suppressed ezrin expression or disrupted ezrin phosporylation by a steady transfection with antisense ezrin. Khanna et al. generated an ezrin-T567A mutant which can not become phosporylated. This caused, that the mutant presented as negative inhibitor of ezrin. Via plasmid the authors placed the mutant into K7M2-WT cells that afterwards were transfected to mice. This led to the significant result that mice receiving untreated wild type K7M2 cells developed metastases in the majority of mice, compared to mice receiving T567A clones infected K7M2-WT cells. They did not present gross or microscopic metastases. Therefore, the study results of Khanna et al. led to the assumption that the ezrin is essential in the development of metastases in the mouse model. [52] To confirm these result Khanna et al. also evaluated ezrin expression levels in dogs that developed

osteosarcomas naturally, because osteosarcoma cells of pet dogs present with a similar biological behaviour and tumour histology like human ones. Khanna et al. also found that increased ezrin expression levels are connected to an early development of metastases in osteosarcomas of pet dog. [52] This animal model induced Khanna and co-workers to collect, prospectively, pediatric osteosarcoma tissues. In 92% of the samples they found an ezrin expression. An evaluation of the disease free survival proved to be significantly shorter in patients with high levels of ezrin, compared to those presenting with lower expression levels (p=0,01). [52]

Ezrin, therefore was suggested to be an important predictor of survival, but also a future approach for antimetastatic therapy strategies. [52]

A further comparative study was performed by Park et al. (2006).[82] They investigated ezrin expression levels in 32 high-grade and 21 low-grade osteosarcoma patients, and maintained negative staining for ezrin in all low-grade osteosarcoma samples, whereas 43,7% of high-grade tissues presented with an ezrin positive heterogeneous immunoreactivity of the cytoplasm. [82] In comparison results of Salas et al. (2007)[91], showed a positive ezrin expression in both the central low-grade and high-grade osteosarcoma. Also metastatic tissue of six patients with pulmonary filiae of high-grade osteosarcomas was examined. [82] Thereby one case presented with ezrin expression in primary bone and metastatic samples. The results of Park and co-authors clearly indicate that ezrin has an increased level of expression in high-grade osteosarcomas, compared to the low-grade malignancies, suggesting a boost of ezrin expression during tumour progression. The protein may be involved in the development of pulmonary osteosarcoma metastases. [82]

The role of ezrin expression also was examined in other malignancies. Weng et al. (2005)[113] ascertained the prognostic impact of ezrin expression in primary high

malignant soft tissue sarcomas (grade 3 or 4). Therefore they analysed 50 samples immunohistochemically and found a total of 25 cases (50%) with positive homogenous staining of the cytoplasm and membrane. During a follow-up period of 4 years Weng et al. detected that ezrin-positive patients more frequently develop metastases (17 cases, p=0,031), compared to ezrin-negative tumour patients (nine cases, p=0,023). No significant correlation was found for local recurrence and the expression of ezrin. Further statistical evaluations presented ezrin as independent prognostic factor of survival and marker for the genesis of metastases. The p-value of 0,014 confirmed the association of ezrin positivity and death from disease. The overall survival also presented a significant value of p=0,007. In conclusion, findings of Weng and co-authors also confirm the strong correlation of increased ezrin expression and the development of metastases and therefore also a poor patients outcome in terms of survival.[113]

A completely different cancer type was examined by Ilmonen et al. (2005)[43]. The authors performed an immunohistochemical analysis of 95 cutaneous melanoma samples, inclusively 12 metastatic tumours. They investigated the ezrin expression, intensity of immunoreactivity, the association to other tumour characteristics as sex, age, tumour ulceration or localisation and the clinical outcome. In 76 cases out of 95 the immunostaining was nearly homogenous positive. Metastatic tissue samples, generally positively stained, presented with a more intensive staining, compared to primary melanoma samples. Ilmonen et al. found that tumour thickness (according to Breslow-classification) but also the tumour invasion level (according to Clark-classification) significantly correlate with intensity of immunoreactivity (Breslow, p=0,0008, Clark, p=0,004). The higher the invasion level and the thickness, the stronger presented the immunoreactivity. The evaluation of an association between

sex, age, tumour ulceration or localisation and immunoreactivity did not show any coherent results. Ilmonen and co-authors[43] further evaluated the probability of overall survival of 89,5% in patients without ezrin reactivity, compared to those patient samples that presented with weak or intense immunoreactivity. An overall survival of 77,1% and 89,3% was investigated. The analyses of disease free survival presented values of 89,5% in patients without immunoreactivity , 68,8% with weak ezrin reaction and 67,9% in patients with tumours with strong ezrin expression. These differences did not show statistical significances for disease free survival (p=0,19) and the overall survival. Therefore the study results of Ilmonen et al. highlight a correlation between the level of ezrin immunoreactivity and tumour thickness and invasion level in primary cutaneous melanoma, suggesting a correlation to tumour progression. [43]

A further study predicting survival via ezrin expression in osteosarcomas stage IIB was performed by Kim et al. in 2007.[53] The authors analysed retrospectively 64 tumour specimens with regard to the ezrin expression and compared it with the clinical outcome of the patients. All patients received surgery, pre- and postoperative chemotherapy. Out of the 64 samples 33 presented with ezrin expression. Among the ezrin positive stained samples 23 showed increased expression levels whereas 10 presented with low levels. Out of the 33 ezrin positive patients, 22 developed distant metastases.[53] Local tumour recurrence was observed in six cases, of which all were positive for ezrin expression, suggesting a correlation of these circumstances. Kim et al. found that a poor histological response to preoperative chemotherapy and ezrin-expression presented with a negative impact on metastasis free ($p<0.001$) and overall survival (p=0,001). Survival associated tumour-factors like

age, sex, tumour volume, localisation and histological features did not correlate with ezrin expression. [53]

In difference to findings of Khanna et al. [52] Kim and co-authors did not detect survival discrepancies in patients with low and high ezrin expression levels. [53] However, the study results of Kim et al. suggest ezrin as promising predictive biomarker regarding the clinical outcome in patients suffering from osteosarcoma. [53]

Also Salas et al. (2007) [91] confirmed within their analyses the suggestion of Kim et al. (2007)[53] that ezrin presents a predictive, prognostic biomarker in patients with osteosarcoma. Tumour tissue of thirty-seven patients, among them 19 were poor and 18 good responders to preoperative chemotherapy, were examined immunohistochemically before high-dose methotrexate treatment. An overriding cytoplasmic ezrin positivity was found in high- and low-grade osteosarcoma specimens (62%). After chemotherapy only two cases, out of 13 ezrin positive poor responders, presented with preserved immunoreaction. Thirteen patients developed metastases during the course of disease. Tumour tissue of 11 patients showed an expression of ezrin. In seven patients, from whom biopsy and metastases samples were available, four presented with ezrin expression of primary and metastatic tumour tissue. The statistical evaluation of Salas et al. did not achieve significant correlations for ezrin and the genesis of metastases (p=0,183) though ezrin expression rose during tumour progression, which was in line with the results of Khanna et al. [52, 91]

Also a correlation between ezrin expression and response to preoperative chemotherapy did not present with statistical significance (p=0,57). But in Cox analysis Salas and co-authors found ezrin as an independent, significant prognostic factor regarding event free survival (p<0,001) and the overall survival (p=0,003),

confirming the role of ezrin as essential part in signalling pathways for tumour spreading in osteosarcomas. The detection of ezrin's activity in this correlation might present a further onset in the treatment of advanced cancer disease. [91]

In 2008 Ferrari et al.[32] performed an analysis determining 95 patients with primary non-metastatic osteosarcoma. Eighty percent (n=76) showed an ezrin immunostaining. Out of these 37 tumours presented with an exclusive reactivity of the cytoplasm, further 39 showed an immunostaining of both the cytoplasm and the cellular membrane. Ferrari et al. did not find a significant correlation between ezrin immunoreactivity and gender, tumour localisation, SAP (serum level of alkaline phosphatase), LDH (lactic dehydrogenase) and the histological response towards primary chemotherapy. But the authors could identify the expression of ezrin as independent prognostic factor for disease free survival (p=0,009). Further Ferrari et al. evaluated a disease free survival after a period of 3 years of 80% for patients with exclusive cytoplasmic ezrin expression, and 54% for those with both cytoplasmic and membranous immunoreaction (p<0,02). Although the authors' observations did not show an ezrin dependent signalling pathway concerning control of the metastatic behaviour of osteosarcoma cells, the results highlight that an exclusive cytoplasmic immunoreactivity of ezrin may detect patients with better survival chances. [32]

Hypothesis

For the purpose that the role of ezrin, similar to survivin, in chordomas still is unknown, the aim of the study, based on the aforementioned analysis, is to examine,

if ezrin is expressed in chordomas and also can act as prognostic marker concerning the survival rate in patients.

11.2.4. CD117/c-KIT

The CD117 antigen, c-KIT or KIT, is a transmembrane glycoprotein that belongs to the class III receptor tyrosine kinase (RTK) family (RTK III family=PDGFR family). Its dedicating protooncogene is localised on chromosome 4q11-q12. A further site was found on chromosome 5q23-q34 that, for example, is consistent with the location of PDGFR (Platelet Derived Growth Factor Receptor). The intra-cytoplasmic tyrosine kinase domain is a split by a long hydrophilic insert between the ATP-binding region and the phosphotransferase active site. The extracellular region consists of five immunoglobulin-like domains whereas the first three regions are thought to be involved in the ligand binding.[11, 19, 73, 117, 118] The natural ligand of CD117 has been termed as SCF (stem cell factor) or mast cell growth factor and is expressed in a soluble as well as in membrane-bound form. Via ligand binding the intracellular domains of CD117 undergo dimerization, substrate phosphorylation and autophosphorylation, activation of proteinkinases and phospholipases and transcription of different protoncogenes.[11, 19, 73, 117, 118] Further the intracellular signal transduction, achieved via the connection of ligand and receptor, forces the cell to start the proliferation cycle militating for a mitogenesis-involvement.[11, 19, 73, 115, 116]

C-kit is expressed in a lot of various cell types. For example by 1-4% of normal bone marrow cells including megakaryocytes, osteoclasts, and renal tubulus epithelial cells, Langerhans cells, melanocytes, and even cells of sweat glands. It also is expressed by mast cells and is found in the blasts of patients with AML, but is absent from most ALL blasts. Further, haematological diseases but also other malignancies

like gastrointestinal stromal tumors (GIST) [73], angiosarcomas, Hodgkin's disease, germ cell tumours, colorectal carcinomas, pancreatic tumours[117], but also soft tissue sarcomas tend to express c-Kit. In this context it has to be mentioned that cell proliferation is supported by CD117 and SCF, but also cell survival and differentiation of KIT-expressing cells. Thus c-Kit's anti-apoptotic activities are coherent. [11, 19, 117]

Cohen et al. (1994)[23] investigated neuroblastoma and neuroepthelioma cell lines in regard to the expression of CD117 and SCF. By using RT PCR Cohen et al. found a simultaneous expression of c-Kit and SCF mRNA in 14 out of 14 investigated neuroblastoma cell lines (100%) and clones, suggesting an autocrine loop of these proteins in neurobastomas. A further stimulation of KIT with exogenous SCF did not result in any reaction, because of the maximal stimulation via the internal autocrine loop. Cohen and his co-authors also detected a significant reduction of growth rate of neuroblastoma cells (cultivated in agar, measured by clonogenic assay or 3H-thymidine uptake assay), while blocking the c-Kit function via SR-1 antibody use. Based on these study results the strong assumption is existent that growth of these tumour cells is regulated by the expression of CD117 and the autocrine loop with SCF ligand. This fact may therefore present a future therapy target. [23]

Even in chordomas the expression of KIT receptors, but also PDGFRA and –B were analysed by Tamborini et al. (2004)[102]. They investigated 31 chordoma specimens immunohistochemically and also by immunoprecipitation and Western blotting and RT-PCR. By biochemical examinations they found, that expression levels for PDGFRB were higher in chordomas than in synovial sarcomas used as positive control, but expression levels for PDGFRA and CD117 were lower than in the used positive control (GIST). In comparison the immunohistochemical analyses resulted in intense cytoplasmic reactivities for PDGFRA and –B, while a c-Kit staining only

showed a feathery immunoreaction in one out of 18 samples. Also the quantitative RT-PCR analyses detected only higher values for PDGFRB mRNA. A ligand expression was found for all factors in 31 cases. Finally the findings of Tamborini et al. support the study hypothesis of Cohen et al.[23], that tumour growth/development may be regulated via the action mechanism of the autocrine loop of the RKT family. [102]

Kim et al. (2004)[54] detected the mutations of c-Kit encoding regions on chromosome 4 which lead to an activation of the transmembrane receptor, though SCF is absent. Moreover they found these mutations more often associated with tumour recurrence and poor patients' outcome. Therefore the authors analysed genomic DNA from 86 tumours of patients treated for localised GIST and found an overall frequency of CD117 mutations of 74%. The histological results showed an increased cell concentration with a high count of mitotic figures. Results of the statistical analyses presented a recurrence free survival of 21% after 5 years in patients with mutations in the chromosomal region encoding for CD117, compared to those cases without mutations that resulted in a recurrence free survival of 60% after 5 years. [54]

In addition studies exist examining the role of Imatinib mesylate (Gleevec; Novartis Pharmaceuticals, Basel, Switzerland), a recently known tyrosine kinase inhibitor. It selectively blocks tyrosine phosporylation of CD117 but also PDGFRA and –B and BCR-ABL.[36]

For example Gonzalez et al. (2004) [36] investigated in vitro the effect of imatinib mesylate on Ewing sarcoma cell lines in regard to CD117 blocking. In four out of four cells lines c-Kit expression was detected via RT-PCR. A further analysis of DNA did not present mutations of CD117 though a ligand induced phosporylation of the receptor was detected in all Ewing cell lines. In addition, 110 biopsies of Ewing's

sarcoma were investigated by immunohistochemistry. Fourty-nine specimens (44,5%) presented with an intense diffuse cytoplasmic staining, while 41 only showed a weak nuclear immunohistochemical reactivity. Thereafter, respective cell lines were treated with imatinib mesylate and resulted in an increased down-regulation of CD117 phosphorylation leading to a blockage of cell proliferation. To examine the impact of imatinib mesylate in combination with the use of standard drugs in Ewing's sarcoma treatment, Gonzalez and co-authors applied doxorubicin and vincristine and found a stronger boost of the apoptotic rate. Also the study results of Gonzales et al. suggest a positive impact of imatinib mesylate in regard to the anti-proliferative activity in Ewing's sarcomas which can be increased by the use of standard drugs. [36]

Interesting study results were achieved in chordomas regarding the impact of imatinib mesylate.[21] Out of the knowledge that no drugs exist decreasing the growth of chordomas Casali and co-authors (2004)[21]of the Instituto Nazionale per lo Studio e la Cure dei Tumori in Milan, Italy, tested a sample of a chordoma-patient for the expression and activation of PDGFRB. Their investigation of the protein's expression and phosporylation supported the hypothesis of autocrine loop activation of the growth factor receptor. Therefore, the patient was treated with Imatinib mesylate, and after a few months imaging showed an anti-tumorous activity like decrease in contrast enhancement and decrease in glucose uptake. Even a liquefaction of the tumour tissue was recorded. Further five patients suffering from chordoma, underwent an imatinib therapy (daily dose of 800mg imatinib mesylate) at advanced tumour stage. After a treatment interval of 12 months the authors found in all but two patients PDGFR-expression and -activation via phophorylation with RT-PCR. A patient's benefit was observed in the early phase of treatment only. Most patients showed a slight increase of chordoma mass in the early phase of treatment, but decreased glucose uptake on PET scan and decreased contrast enhancement on

MRI. Two patients developed metastases in the lung and one patient presented with liver metastases. One of the patients, who presented with lung metastases died after a few months of treatment, while the other two patients remained asymptomatic and continued the therapy at the date of the last follow-up. One case presented with liquefaction of a part of the chordoma and in a further case also a decrease of tumour density was observed radiologically. [21]

In conclusion the study results of Casali et al. might suggest an anti-tumorous activity of imatinib in chordoma patients in late phases of treatment. To that date other activation mechanisms of the drug can not be excluded. Tumour response evolved slowly what still demonstrates a problem in this advanced stage of disease. [21]

In comparison, Mireskandari et al. (2006)[73] performed a study with 38 paraffin embedded blocks of gastric adenocarcinomas and analysed the expression of CD117 (wild type) and its association to regulatory pathways of cell proliferation. The study did not present any reactivity for KIT, leading to the assumption that there exists no correlation of this wild type glycoprotein and the development of gastric carcinoma. Maybe further investigations with other c-Kit formation will present other results. However, mutations of the CD117 encoding chromosomal area, probably playing a role in the development of gastric adenocarcinomas, could not be excluded by Mireskandari et al. [73]

Orzan et al. (2007)[78] presented an analysis of 14 examined skull base chordomas (13 primary tumours, one recurrence) by RT-PCR and immunohistochemistry in regard to the expression of PDGFRA and –B and KIT and their dedicating ligands.

For immunohistochemistry 12 cases with adequate histological material could be included in the study. The authors found a focal expression of PDGFRA and –B in most neoplastic cells. CD117 was expressed in one specimen only.

PCR examination resulted in positivity for all three examined transcripts in 13 out of 14 cases. Further, SCF was found to be expressed in one sample only, compared to an expression of both, PDGFA and –B mRNAs in 8 chordomas. Only one sample showed an expression of PDGFB mRNA exclusively. [78]

In conclusion Orzan's et al. study results also suggest an activation of RTK III family via an autocrine loop, whereas KIT and the ligand SCF are excluded from this assumption. The findings also suggest PDGFs and receptors as main targets for further therapy options in chordoma treatment. [78]

Hypothesis

Based on the aforementioned studies, the aim of the immunohistochemical analyses is to examine the expression-pattern of CD117 using a monoclonal mouse antibody our chordoma series.

12. Patients and Methods

12.1. Patients

Fourty-eight patients with the diagnosis of a chordoma were identified retrospectively from the database at the Medical University of Graz. They concerned 32 males and 16 females with a mean age of 50 years (range 16 to 80 years). Baseline data were obtained from the hospital database and patient charts including age, sex, date of diagnosis, tumour histology, grade, tumor stage, sites of metastases and treatment (surgery and radiotherapy). Thirty-nine patients with completeness of clinical data were included in the clinical analysis. Thirty-six patients with adequate histological material could be included into the immunohistochemical examination. In five cases biopsy was done exclusively, because the chordoma presented as inoperable, or the patient disclaimed the surgery. Eleven patients underwent radiotherapy with (n=9) or without (n=2) preceeding surgery. Survival time was recorded and calculated from the date of diagnosis until the date of death or last follow-up by using Cox proportional hazards regression/logrank test with score test criterion and Kaplan-Meier graphs. Spearman method was used to evaluate the coefficient of correlation in concern of the expression of survivin and DR4/DR5.

12.2. Immunohistochemistry

12.2.1. Survivin

The immunohistochemical staining of the samples resulted from the use of monoclonal mouse anti-human (clone 12C4, 1:200 antibody diluent; DAKO, Glostrup, Denmark). Sections were deparaffinized and rehydrated according to standard protocols. For further pretreatment HIER (heat induced epitope retrieval by microwaves, 40 min, 160 watt) was used. Incubation followed by the use of Tris HCL Urea pH 9,5 + 5% Urea/1000ml. For immunohistochemical staining a DAKO Autostainer was used. Endogenous peroxidase was blocked with peroxidase blocking solution (DAKO) for 10 minutes at room temperature. Following detection-kit was applied: CM (DAKO REAL HRP/DAB+ rab/mouse). A further treatment with chromogen DAB (diaminobenzidine) was performed. Hematoxylin was used for counterstaining. For control purposes, tissues known to contain the respective antigens were included (positive controls). Replacement of the primary antibody by antibody diluent only, always revealed negative results (negative controls).

12.2.2. DR4 and DR5

The immunohistochemical staining of the samples resulted from the use of goat polyclonal antibody against DR4 (clone C-20, 1:150 antibody diluent; Santa Cruz Biotechnology, Santa Cruz, CA) and rabbit polyclonal antibody against DR5 (1:50 antibody diluent; Merck, Nottingham, UK). Sections were deparaffinized and rehydrated according to standard protocols. For further pretreatment HIER (heat induced epitope retrieval by microwaves, 40 min, 160 watt) was used. Incubation followed by the use of Tris HCL Urea pH 9,0 (DAKO target retrieval solution pH 9,0)

for DR5 and pH 6,0 (sodium-citrate buffer pH 6,0 0,001M/1000ml) for DR4. The used immunostainer was DAKO Autostainer. Endogenous peroxidase was blocked with peroxidase blocking solution (DAKO) for 10 minutes at room temperature. Further following detectionkits were applied: Env+ (DAKO REAL EnVision peroxidise/DAB rabbit/mouse for DR5 and 743/Env+ (EUBIO/ Bethyl AB, rat-a-goat goat IgG Fc cross absorbed AB) for DR4. A further treatment with chromogen DAB (diaminobenzidine) was performed. Hematoxylin was used for counterstaining. For control purposes, tissues known to contain the respective antigens were included (positive controls). Replacement of the primary antibody by antibody diluent only, always revealed negative results (negative controls).

12.2.3. Ezrin

The immunohistochemical staining of the samples resulted from the use of monoclonal mouse antibody (clone 3C12, 1:50 antibody diluent; Santa Cruz Biotechnology). Sections were deparaffinized and rehydrated according to standard protocols. The immunohistochemical staining was performed by the use of the automated Ventana BM XT system. For pretreatment Ventana CC1 mild was applied. The following detection-kit was applied: UV (UltraView Ventana). A further treatment with chromogen DAB (diaminobenzidine) was performed. Hematoxylin was used for counterstaining. For control purposes, tissues known to contain the respective antigens were included (positive controls). Replacement of the primary antibody by antibody diluent only, always revealed negative results (negative controls).

12.2.4. CD 117

The immunohistochemical staining of the samples resulted from the use of monoclonal mouse anti-human (clone 104D2, 1:75 antibody diluent; DAKO, Glostrup, Denmark). Sections were deparaffinized and rehydrated according to standard protocols. For further pretreatment HIER (heat induced epitope retrieval by microwaves, 40 min, 160 watt) was used. Incubation followed by the use of Tris HCL Urea pH 9,0 (DAKO target retrieval solution pH 9,0) for CD117. DAKO Autostainer was the used immunostainer. Endogenous peroxidase was blocked with peroxidase blocking solution (DAKO) for 10 minutes at room temperature. The following detection-kit was applied: Env+ (DAKO REAL EnVision peroxidise/DAB rabbit/mouse). A further treatment with chromogen DAB (diaminobenzidine) was performed. Hematoxylin was used for counterstaining. For control purposes, tissues known to contain the respective antigens were included (positive controls). Replacement of the primary antibody by antibody diluent only, always revealed negative results (negative controls).

12.3. Evaluation of immunohistochemistry

All immunohistochemically stained sections were microscopically examined by three independent oberservers (Elke Fröhlich, Dr. Koppany Bodo and Prof. Dr. Alfred Beham) in a blinded manner without the knowledge of clinicopathologic data and patients outcome.

For survivin Kawasaki's et al. (1998)[50] scoring method was applied. The intensity of nuclear and cytoplasmic staining and the percentage of positive tumour cells were evaluated in at least five areas at ×400; according to the percentage of positive tumour cells, cases were given a score of 0-4 as follows: 0, <5%; 1, 5-25%; 2, 25-50%; 3, 50-75%; and 4, >75%. The intensity of immunoreactivity was scored as follows: 1+ (weak); 2+ (moderate); and 3+ (intense). In tumours with heterogeneous immunostaining, the predominant pattern was considered for scoring.

Regarding DR4 and DR5 percentage of positive tumour cells the scoring method by Spierings et al. (2003)[97] were used: ≤10% positive nuclear and cytoplasmic staining was regarded as negative, >10% positively stained cells were regarded as positive. The intensity of immunoreactivity was scored as follows: 1+ (weak); 2+ (moderate); and 3+ (intense). In tumours with heterogeneous immunostaining, the predominant pattern was considered for scoring.

To estimate the quantity of cytoplasmically ezrin-positive stained cells, the score test by Ferrari et al. (2008)[32] was applied as follows: (1)1-25%, (2)26-50%, (3)51-75% and (4)76-100%. In addition, according to Ilmonen et al. (2005)[43] the intensity of immunoreactivity (IR) was defined by the following score criteria: 0 (negative IR), 1 (weak IR), 2 (intermediate IR) and 3 (strong IR).

13. Immunohistochemical study results

13.1. Survivin

Survivin was expressed in 27 out of 36 samples and continuously presented with cytoplasmic staining. 28% of the positively stained specimens (n=10) showed a staining of 5-25% of the cells. 42% of the samples (n=15) presented a weak immunoreactivity only. Details are given in Table 10 and immunohistochemistry is illustrated by Fig. 18 and 19.

Positive stained samples			**Immunoreactivity (IR)**		
staining	*number*	*%*	*IR*	*number*	*%*
0	9	25	0	9	25
1	10	28	1	15	42
2	8	22	2	9	25
3	4	11	3	3	8
4	5	14			
total	36	100	total	36	100

Tab.10: Immunohistochemical results-Survivin

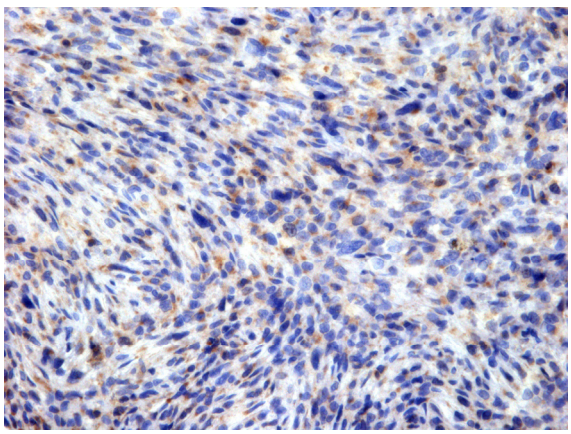


Fig.18: Demonstration of survivin-immunostaining in the majority of tumour cells. x 400.

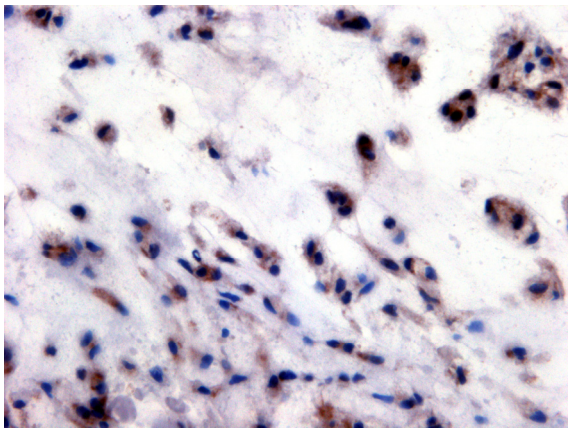


Fig.19: Typical cytoplasmic immunostaining for survivin. x 600.

The survival analyses did not present significant results. The correlation to the immunoreactivity resulted in a p-value of 0,15. The correlation of the positively stained sample-area of each specimen resulted in a p-value of 0,87.

13.2. DR4

Death Receptor 4 was expressed in 32 (89%) out of 36 samples, with cytoplasmic expression in 83%. 47% (n=17) presented with a weak immunoreactivity. Out of the 32 positively stained samples 89% showed a staining of more than 10% of the tumour cells. Details are given in Table 11 and immunohistochemistry is illustrated by Fig. 20 and 21.

Cytoplasmic(C)/Nuclear(N)			**Positive stained samples**			**Immunoreactivity (IR)**		
expression pattern	*number*	*%*	*staining*	*number*	*%*	*IR*	*number*	*%*
negative	4	11	negative	4	11	0	4	11
C	30	83	positive	32	89	1	17	47
C/N	2	6				2	9	25
						3	6	17
total	36	100	total	36	100	total	36	100

Tab.11: Immunohistochemical results-DR4

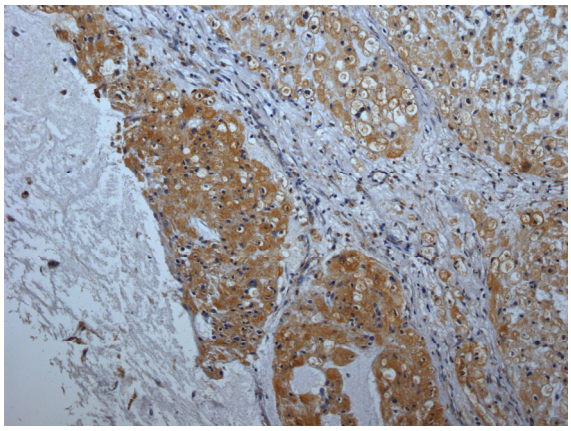


Fig.20: Demonstration of DR4-immunostaining. x 200.

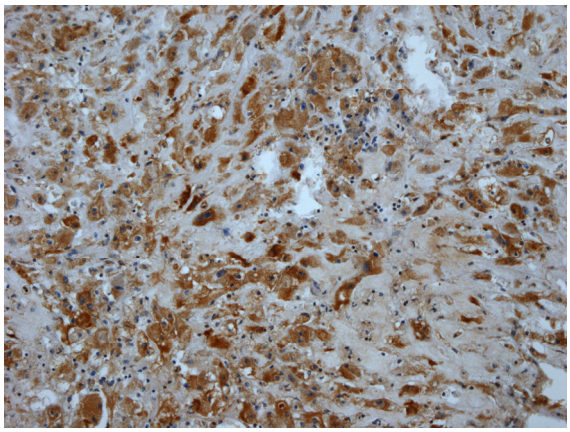


Fig.21: Typical cytoplasmic immunostaining for DR4. x 400.

The survival analyses did not present significant results. The correlation to the immunoreactivity resulted in a p-value of 0,27. The correlation to the positively stained sample-area of each specimen resulted in a p-value of 0,93.

13.3. DR5

Death Receptor 5 also was expressed in 32 (83%) out of 36 samples, with cytoplasmic expression in 75%. In all, two sections could not be judged because of technical reasons. 83% (n=30) of the positively stained samples showed a staining of

more than 10% of the tumour cells. 39% (n=14) presented with a weak immunoreactivity. Details are given in Table 12 and immunohistochemistry is illustrated by Fig. 22 and 23.

Cytoplasmic(C)/Nuclear(N)			**Positive stained samples**			**Immunoreactivity (IR)**		
expression pattern	*number*	*%*	*staining*	*number*	*%*	*IR*	*number*	*%*
negative	4	11	negative	4	11	0	4	11
C	30	83	positive	32	89	1	17	47
C/N	2	6				2	9	25
						3	6	17
not judgeable	2	6	not judgeable	2	6	not judgeable	2	6
total	36	100	total	36	100	total	36	100

Tab.12: Immunohistochemical results-DR4

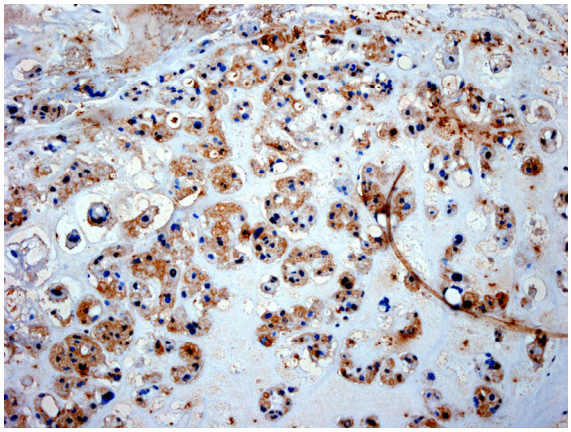


Fig.22: Demonstration of immunostaining for DR5, x 200.

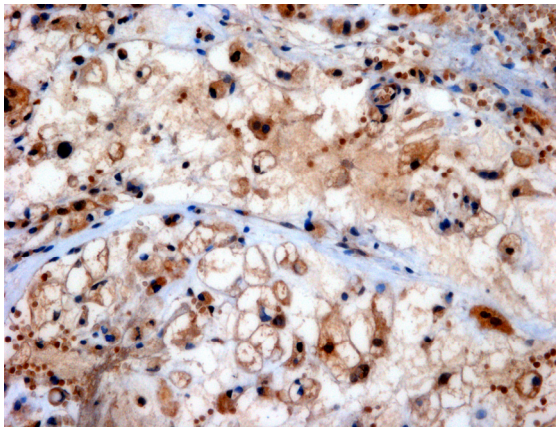


Fig.23: Typical cytoplasmic immunostaining for DR5. x 400.

The survival analyses did not present significant results. The correlation to the immunoreactivity resulted in a p-value of 0,88. The correlation to the positive stained sample-area of each specimen resulted in a p-value of 0,49.

To evaluate the correlation between the expression of survivin and the death receptors 4 and 5 the Spearman method was applied. The results do not confirm a significant correlation between the expression of survivin and the death receptors 4 and 5 (Table 13).

variable 1	*variable 2*	*coefficient of correlation (rho)*	*p-value*
survivin pos.st.	DR4 pos.st.	0,0567	6,4
survivin pos.st.	DR5 pos.st.	0,1717	2,3
survivn IR	DR4 IR	-0,0506	6,7
survivn IR	DR5 IR	0,0476	6,6

Tab 13: Coefficients of correlation and p-values

13.4. Ezrin

Out of 36 samples 29 presented with a positive immunoreactivity. Of these samples 75% (n=27) showed cytoplasmic expression patterns. In 11 sections (31%) a staining reactivity of 26-50% of the tumour cells was found. 16 slides (44%) presented a weak staining intensity. Details are given in Table 14 and immunohistochemistry is illustrated by Fig. 24 and 25.

Cytoplasmic (C)			Positive stained samples			Immunoreactivity (IR)		
expression pattern	*number*	*%*	*staining*	*number*	*%*	*IR*	*number*	*%*
negative	7	19	0	7	19	0	7	19
C	29	81	1	9	25	1	16	44
			2	11	31	2	7	19
			3	7	19	3	6	17
			4	2	6			
total	36	100	total	36	100	total	36	100

Tab.14: Immunohistochemical results-Ezrin

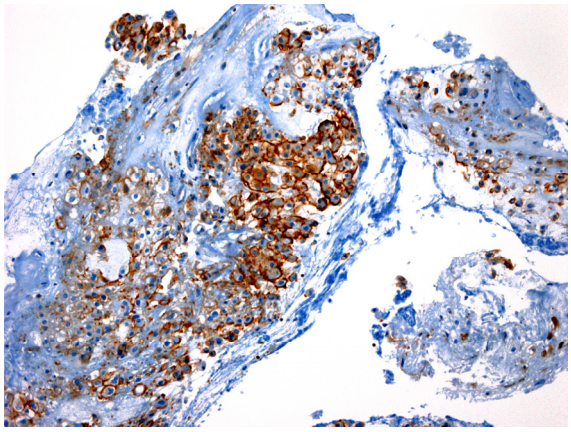


Fig.24: Demonstration of immunostaining for ezrin. x 200.

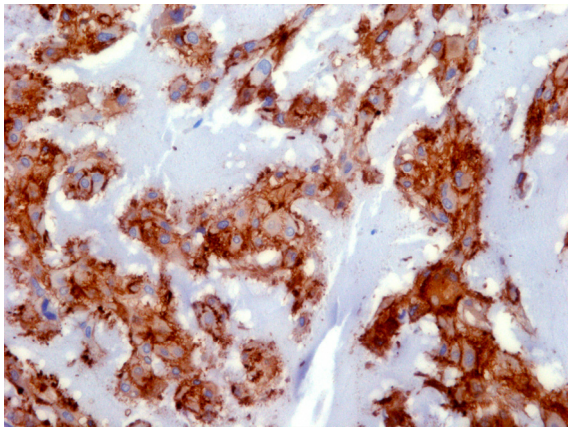


Fig.25: Typical cytoplasmic immunostaining for ezrin. x 400.

Out of six recurrent tumours four presented with cytoplasmic expression patterns. In two samples a staining of 1-25% of the tumour cells was observed, in one case a staining of 51-76% was found. The fourth sample showed a staining of 76-100% of the tumour cells. Three samples presented a weak staining intensity, while one

section showed a strong intensity of staining. Two recurrences were not available for further immunohistochemical investigations.

Out of three cases that developed metastases one sample presented with cytoplasmic staining patterns and a staining of 26-50% of the tumour cells. A weak staining intensity was found. Two metastases were not available for further immunohistochemical investigations.

The survival analyses did not present significant results. The correlation to the immunoreactivity resulted in a p-value of 0,95. The correlation to the positive stained sample-area of each specimen resulted in a p-value of 0,6.

13.5. CD117

None of the 36 samples presented with an immunostaining for c-Kit. Therefore no statistical analysis was performed.

14. Discussion of the immunohistochemical results

To the author's knowledge this is the first study examining survivin, ezrin, DR4 and DR5 in chordoma samples. All aforementioned proteins presented with a positive antibody-staining with mainly cytoplasmic expression patterns.

The cytoplasmic expression of survivin argues for a "cell protective" function of the protein and therefore is connected with a poor patient's prognosis, while the nuclear localised expression represents the "affected survivin" function leading to an increased patients survival.[47, 77, 79, 99] Even tumour recurrences present with higher levels of survivin expression and thus correlate with a poor patients outcome.[47]

Within our analyses we found that survivin mainly presented with a weak cytoplasmic staining in chordomas, but our survival analyses did not result in significant p-values. The expression of survivin in chordomas and the patients' survival did not correlate, what may be due to the low number of follow-up information that was used in the analysis (36 samples, follow-up of 26 patients).

Though survivin presents an ideal tumour-associated antigen that is expressed in various different malignancies, it is a suitable molecular target in cancer treatment. The phase I clinical study for patients suffering from advanced or recurrent breast cancer has been already finalized and presents acceptable results. However, further studies will be essential for the improvement of the immunological as well as clinical response in order to tap the full potential of this drug. [107]

Death Receptors 4 and 5 also presented with mainly cytoplasmic expression patterns in our chordoma series. This result argues for a general sensitivity towards TRAIL and its pro-apoptotic impact on chordoma cells but has to be examined in further in vitro studies.

Study results of Van Valen et al.[108], Keane et al.[51], Yoshida et al.[119], and Kischkel et al.[55] also presented an expression of DR4 and DR5 in different cell lines. The authors showed an up-regulation of these receptors by treating the cell lines with chemotherapeutics. Due to the increased concentration of DR4 and DR5 the sensitivity towards TRAIL was observed, ending in lower cell survival. Mirandola et al.[72] not only found an increasing TRAIL sensitivity but also a decrease of survivin mRNA, what leads to the assumption that the aforementioned proteins (survivin, DR4, DR5) may use opposite pathways.

Via Spearman-method we evaluated a correlation between the expression of death receptors and survivin in chordomas. In fact the evaluation did not show significant correlations between the expression of the death receptors and survivin.

Like survivin, also the pathways of death receptor 4 and 5 present suitable molecular targets in cancer treatment. Phase I and II studies with mapatumumab in patients with advanced cancer disease have been already finalized. Mapatumumab, directed to DR 4, presents an agonist acting antibody that interacts with the activated external domains of the transmembrane receptor and enhances the induction of apoptosis. Mapatumumab, a drug well tolerated by patients, also delivered acceptable results. However, future studies in combination with standard chemotherapeutics will be essential to improve the impact on cancer cell apoptosis in order to tap the full potential of this drug. [37, 105]

The majority of samples examined for ezrin presented with a cytoplasmic expression pattern. All examined recurrent tumour samples and samples of primary chordomas that led to metastases showed a positive immunoreaction for ezrin. Unfortunately, the small number of examined tumours does not allow any argumentation yet, why during the next year further studies in cooperation with the National Centre For Spinal Disorders, Budapest, Hungary, will be carried out to increase the number of chordoma samples.

According to Ferrari et al.[32] an immunoreaction for ezrin would lead to poor patients' outcome. In fact the statistical evaluation of our results did not show a significant correlation between the expression of ezrin and the patients' outcome, what may be associated with the low number of follow-up information (36 samples, follow-up of 26 patients).

Study results of Casali et al.[21] present a potential „antitumour activity" of imatinib mesylate in chordomas. Out of the reason that the drug acts as tyrosine kinase inhibitor we investigated the expression of CD117 in our chordoma series.

In none of the 36 samples we found a positive staining for c-Kit suggesting pathways of imatinib via PDGFRs and their dedicating ligands. Our study results therefore indirectly support the findings of Tamborini et al. [102] who found high expression levels of PDGFRA and –B and just a slightly expression of CD117 in one out of 17 chordomas. Due to the circumstance that the role of the tyrosine kinase receptor family is not yet fully understood further investigations in this area are essential to use the pathways as molecular target in cancer treatment.

Thus during the following year further immunohistochemical studies with chordomas will be performed, especially investigating the expression of PDGFR-A and PDGFR-B, but also steroid hormone receptors (ERa, ERb, AR), EGFR (epidermal growth

factor receptor) and cox2. In line with this project we already achieved further paraffin embedded chordoma blocks from the “National Centre For Spinal Disorders”, Budapest, Hungary, to increase the number of samples in the study, what might have a positive bearing on the statistical results.

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