EVALUATION OF PROGNOSTIC FACTORS IN PATIENTS WITH OSTEOSARCOMA: THE HUSM EXPERIENCE

by

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OS: Overall Survival

EFS: Event-Free Survival

DFS: Disease-Free Survival

LDH: Lactate Dehydrogenase

ALP: Alkaline Phosphatase

LSS: Limb Sparing Surgery

ABSTRACT

PENILAIAN FAKTOR – FAKTOR PROGNOSIS PESAKIT – PESAKIT OSTEOSARCOMA: PENGALAMAN HUSM

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Pengenalan:

Osteosarcoma adalah kanser tulang yang amat merbahaya, maka penilaian faktor-faktor prognosis yang akan mempengaruhi kadar kemandirian adalah amat penting untuk menentukan pendekatan rawatan yang optimum terhadap pesakit-pesakit ini. Walaupun dengan kemajuan dan pengkhususan di dalam bidang kemoterapi, masih terdapat di antara 30 – 40% pesakit meninggal dunia disebabkan oleh osteosarcoma; oleh itu faktor-faktor prognosis yang boleh dipercayai adalah mustahak untuk merancang rangka perawatan yang lebih agresif kepada pesakit – pesakit yang menpunyai risiko tinggi untuk kegagalan rawatan. Di antara beberapa pembolehubah yang telah dikenalpasti, metastasis paru – paru dan kemo-nekrosis adalah komponen prognosis yang terbaik untuk mengenalpasti kelangsungan hidup. Pada ketika ini, tidak ada kata sepakat tentang kepentingan prognosis penanda biokimia iaitu serum pra-rawatan alkaline phosphatase (ALP) dan lactate dehydrogenase (LDH). Kajian ini menganalisis faktor – faktor

prognosis untuk osteosarcoma, secara khususnya ALP dan LDH, dan juga hasil kemandirian secara kesuluruhan.

Kaedah:

Data klinikal sekurang-kurangnya 5 tahun yang berkaitan dengan faktor-faktor prognosis daripada 163 pesakit osteosarcoma yang berada di bawah rawatn susulan Hospital Universiti Sains Malaysia telah dikaji semula secara retrospektif. Ini adalah satu kajian kohort retrospektif daripada semua pesakit yang dirawat di antara Januari 2005 dan Disember 2010. Sejumlah 163 pesakit, dengan usia purata sebanyak 18.9 tahun (julat 6 - 59 tahun) telah dinilai. Terdapat 109 lelaki dan 54 perempuan. Sebahagian besar pesakit adalah daripada latar belakang etnik Melayu (82.2%), berbanding dengan kaum Cina (11.66%), diikuti oleh kaum India (4.29%). 55.2% daripada pesakit kami mempunyai metastasis paru-paru. Dari segi jenis pembedahan, 66.1% daripada pesakit menjalani pembedahan *limb salvage surgery* (LSS) dan 33.9% telah menjalani amputasi.

Keputusan:

Kadar survival keseluruhan adalah 40.5%. Purata serum pra-rawatan LDH adalah 493,19 IU/L, dan 52.8 % daripada pesakit kami mempunyai nilai yang tinggi. Purata serum pra-rawatan ALP adalah 273,93 IU/L, dan 53% daripada pesakit kami mempunyai nilai yang tinggi. Kadar survival secara keseluruhan 5 tahun untuk pesakit di dalam kumpulan serum pra-rawatan LDH normal adalah 66.2%, manakala di dalam kumpulan serum pra-rawatan LDH yang tinggi kadarnya adalah 17.4%.

Kadar survival secara keseluruhan 5 tahun bagi pesakit di dalam kumpulan serum pra-rawatan ALP normal adalah 55.8%, manakala di dalam kumpulan serum pra-rawatan yang tinggi ALP kadarnya adalah 26.7%. Di dalam analisis statistik multivariat, hanya serum pra-rawatan LDH dan kehadiran metastasis mengekalkan kepentingan prognosis, manakala kedua-dua serum pra-rawatan ALP dan jenis pembedahan hilang nilai pembolehubah tidak bersandar. Berkenaan pembolehubah yang lain, 66.9% daripada pesakit adalah lelaki, dengan kadar survival 39.4%. Pesakit wanita kami juga mempunyai kadar survival yang serupa iaitu 42.6%. Metastasis paru-paru wujud di dalam 90 pesakit (55.2%) kami. Kadar survival keseluruhan pesakit-pesakit ini adalah 27.8% berbanding dengan 56.2% pada mereka yang tidak mempunyai penyakit metastatik. Bagi pesakit yang menjalani pembedahan LSS, kadar survival adalah 56.8%, manakala di dalam kumpulan amputasi kadar survival keseluruhan adalah 17.6%.

Kesimpulan:

Kadar survival keseluruhan pesakit osteosarcoma kami dipengaruhi oleh kehadiran metastasis paru-paru dan jenis pembedahan. Serum pra-rawatan LDH dan ALP juga mempengaruhi kadar survival mereka. Walaubagaimanapun, umur, jantina, bangsa, tumor utama, jenis histologi, tidak mempengaruhi kadar survival pesakit – pesakit osteosarcoma kami.

Supervisor: Professor Wan Faisham Nu'man Wan Ismail

ABSTRACT

EVALUATION OF PROGNOSTIC FACTORS IN PATIENTS WITH OSTEOSARCOMA: THE HUSM EXPERIENCE

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Introduction:

Osteosarcoma is a highly malignant primary bone tumour thus the evaluation of prognostic factors influencing the survival rates is extremely essential for defining the approach to the management of these patients. Despite the recent advancement in chemotherapy and improved prognosis, 30 - 40% of patients still succumb to the disease; therefore reliable prognostic factors would be essential to plan a more aggressive treatment in patients at a higher risk of failure of treatment.

Among multiple variables evaluated in literatures, it is known that pulmonary metastases and chemo-necrosis are the best prognostic components on the survival. At present, there is no consensus on the prognostic significance of simple and cheap biochemical markers of pre-treatment serum alkaline phosphate (ALP) and lactate dehydrogenase (LDH).

This study analyzed the prognostic factors of particularly ALP and LDH and other general variable for prognosis and overall oncological outcome.

Method:

Clinical data of at least 5 years related to prognostic factors of 163 patients with osteosarcoma who were under follow-up under Hospital Universiti Sains Malaysia were retrospectively reviewed. This was a retrospective cohort study of all patients treated between January 2005 and December 2010. A total of 163 patients with the mean age of 18.9 years (range 6 – 59 years old) were evaluated. There were 109 males and 54 females. Majority of the patients were of Malay ethnic background (82.2%), compared to the Chinese (11.66%), followed by Indian ethnicity (4.29%). 55.2% of our patients presented with pulmonary metastases. In terms of type of surgery, 66.1% of our patients underwent limb salvage surgery and 33.9% underwent amputation.

Results:

The overall survival was 40.5%. The mean pre-treatment serum LDH level was 493.19 IU/L, and 52.8% of our patients had high values. The mean level of pre-treatment ALP was 273.93 IU/L, and 53% of our patients had high values. The 5-year overall survival rate of patients in the normal pre-treatment serum LDH group is 66.2%, whereas in the high pre-treatment serum LDH group is 17.4%.

The 5-year overall survival rate of patients in the normal pre-treatment serum ALP group is 55.8 %, whereas in the high pre-treatment serum ALP group is 26.7%. In the multivariate statistical analysis, only the pre-treatment serum LDH and presence of metastasis maintained its prognostic significance as both the pre-treatment serum ALP and the type of surgery loses its independent predictive value.

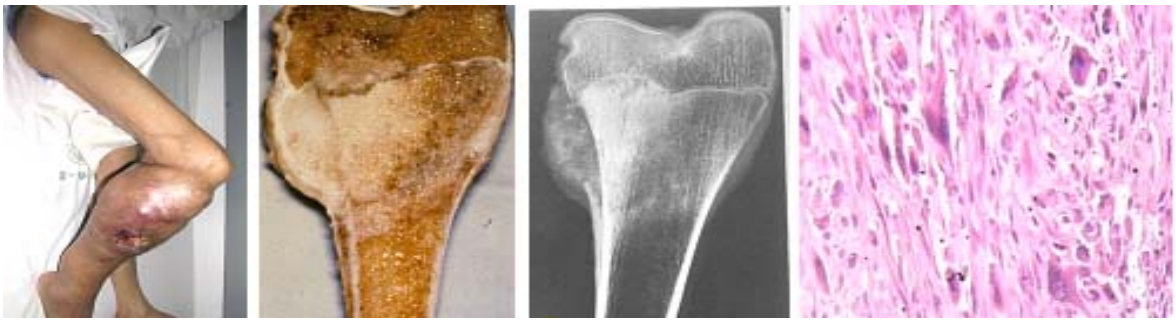
In regards to the other variables, 66.9% of our patients were male, with the overall survival of 39.4%. Our female patients had a similar overall survival of 42.6%. Pulmonary metastases presented in 90 patients (55.2%). The overall survival of these patients was 27.8% compared to 56.2% in those without metastatic disease. For patients who underwent limb-sparing surgery, their overall survival was 56.8%, whereas in the amputation group the overall survival was 17.6%.

Conclusion:

The overall survival of our osteosarcoma patients was influenced by the presence of pulmonary metastases and type of surgery. Pre-treatment serum LDH and ALP were of significant influence on the final survival. However age, gender, race, primary site of tumour, histological sub-type, were not of significant influence on the survival of our patients.

Supervisor: Professor Wan Faisham Nu'man Wan Ismail

CHAPTER 1



INTRODUCTION

1.1 Problem Statement

Osteosarcoma is a primary malignant tumour derived from primitive bone forming mesenchymal tissue, which is characterized by production of osteoid or immature bone, by malignant proliferating spindle cells. It most commonly affects the adolescent and childhood age group, and has a strong predilection for around the knee region.

Treatment typically includes preoperative chemotherapy, surgical resection, and postoperative chemotherapy. Limb-salvage procedures with wide surgical margins are the mainstay of surgical intervention. Advances in chemotherapy protocols have led to a 5-year survival rate of 60% to 70%. Despite improved prognosis recently, roughly about 30-40% of patients still succumb to the disease; therefore reliable prognostic factors would be essential to plan a more aggressive treatment in patients at a higher risk of relapse.

Although it is widely reported in Western countries, but in Asian population where the incidence is not well documented, those reported prognostic factors may not have the same influence on clinical outcome. Furthermore, as we are among the few referral centres around this region of South East Asia for musculoskeletal tumour, there is a need to establish a database and to review the outcome of the patients treated here in Hospital Universiti Sains Malaysia.

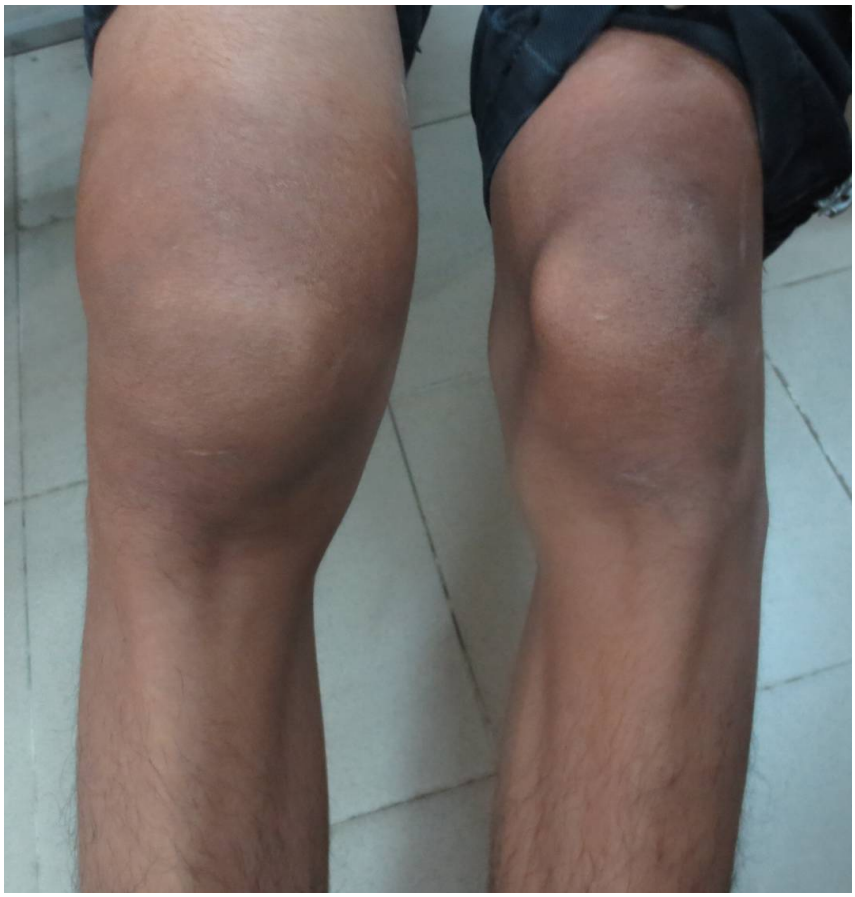
1.2 Justification of the Study

This study was designed because at the current moment there is no similar study done in our population as reference. Although the disease is widely reported in Western and European population, but in the Asian community with it is not well documented.

1.3 Benefit of the Study

What we aim from this study is to identify the possible prognostic factors in relation to our osteosarcoma patients, as there is a need to distinguish patients with high risk of having disease relapse or risk of developing metastases which may indicate chemo-resistant patients. Thus, a better or intensified treatment could be prescribed to avoid recurrence and improved survivorship, and also preventing from over-treating a patient. This may trigger a future scoring system in evaluating prognosis of the patients which may help as a guideline in delivering optimum treatment. And we hope to create a systematic method of data collection among our patients, as well as collaboration with other musculo-oncology centres here in Malaysia.

CHAPTER**2**



LITERATURE REVIEW

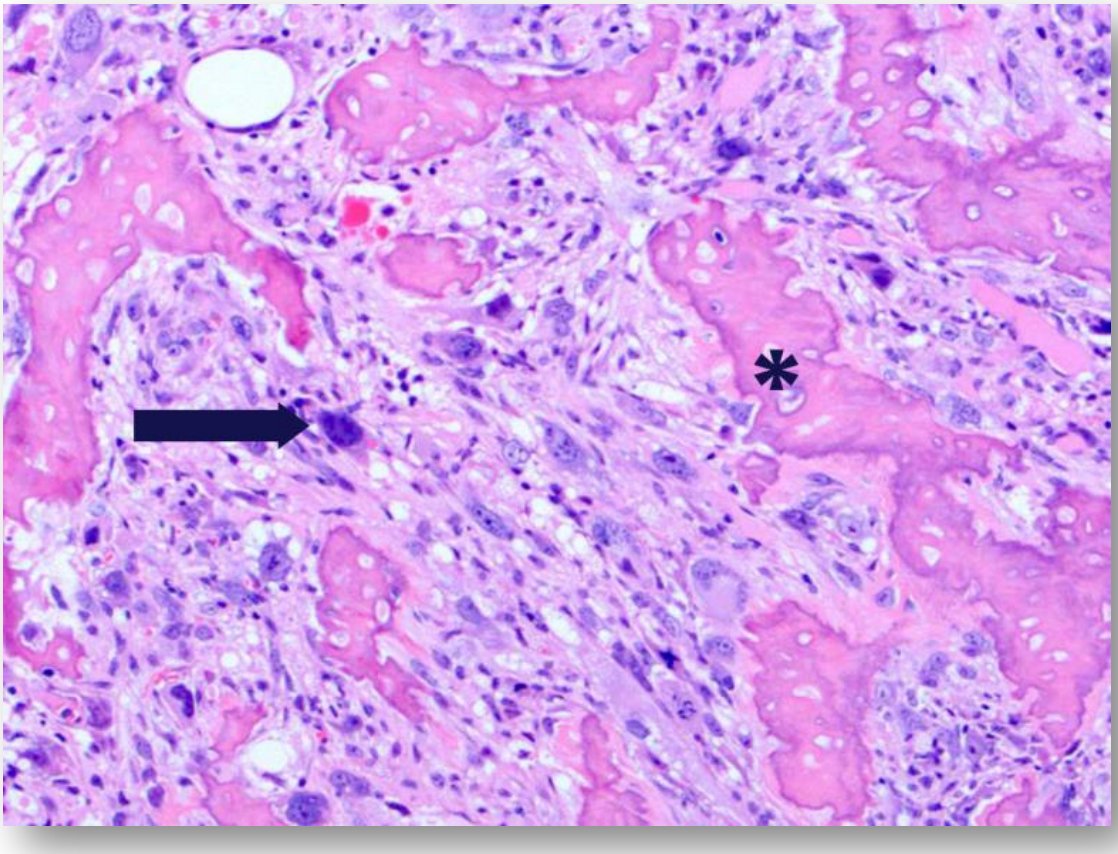
2.1 Diagnosis

Biopsy is a crucial step in the management of musculoskeletal sarcoma (Mohana, Faisham et al., 2007). The diagnosis of osteosarcoma requires tissue biopsy to provide histopathological examination of the lesion, in correlation with the clinical and also radiological findings of the patient. The biopsy site must be selected or planned with consideration for definitive tumour resection. The subsequent surgical approach for the tumour resection must include the biopsy tract along, as it must be removed en bloc with the tumour mass, as to reduce the risk of local recurrence. Biopsies that are done badly or against the principles of biopsy, poorly placed incisions and ensuing complications of biopsy procedure can considerably compromise subsequent management of the tumour. It is advisable that the biopsy be performed by the same surgeon, who will also likely perform the subsequent tumour surgery on the patient (Simon, 1982; Simon & Bierman, 1993).

The tissue diagnosis is essential before an oncologist can decide on a treatment plan for musculoskeletal tumours. It also has prognostic implications as well as therapeutic consequences. It is universally accepted that the resection of the entire biopsy tract is mandatory for surgical treatment of osteosarcoma (Mohana, Faisham et al., 2007).

The biopsy can be either with an open method, or incisional biopsy; or with a closed method or needle biopsy. Furthermore, the needle biopsy can

be a core biopsy or a fine needle core biopsy (trephine). In an established and experienced musculoskeletal oncology centres, needle biopsies can yield high positive results (Stoker et. al., 1991)



Characteristic elongated cells called spindle tumour cells (centre of image, with purple nuclei) some of which are very abnormal (arrow) have replaced the normal bone marrow, and deposited new, but disorganized bone tissue (pink areas/asterisk).

This abnormal bone deposition of cells is the hallmark of an **osteosarcoma**.

2.2 Incidence

Osteosarcoma is the most commonly diagnosed primary non-hematopoietic malignancy of the bone, particularly among children and adolescents. Osteosarcoma incidence in childhood and adolescence seems to be relatively consistent throughout the world (Mirabello et al., 2009).

In Western literature, it is reported that the disease affects approximately 560 children and adolescents each year in the United States (Horner et al., 2009). Osteosarcoma incidence in the youngest cases (age 0–24 years) was greatest in Asian or Pacific Islanders, whereas it was greatest in Blacks and Whites in the middle age group (age 25–59 years) and elderly (age 60+ years) patients, respectively (Mirabello et al., 2009).

The incidence of osteosarcoma in Malaysia, among the three separate major racial groups was reported to be between 0.11 (Malays) to 0.23 (Chinese and Indians) per 100000 population, per year (Silva et al., 1974).

According to Mirabello in 2009, using the Cancer Incidence in Five Continents, International Agency for Cancer Research database, they found out that osteosarcoma incidence rates among individuals who were less than 24 years were generally consistent worldwide, with peaks around time of puberty. Most incidence rates ranged from 3 to 5 per million in men (average 4.3) and 2 to 4 per million in women (average 3.4). The disease was more common in men than women in most countries. The overall world male-to-female ratio of osteosarcoma in ages 0 to 24 years was 1.43:1. The incidence

peaked in men at the age 15 to 19 years, while in women they peaked at 10 to 14 years of age. Osteosarcoma was more common in men than in women in most countries, with a male-to-female ratio of 1.28:1.

2.3 Prognosis

Prior to the introduction of chemotherapy, when amputation was the mainstay of treatment for patients with osteosarcoma, the predicted long-term survival rate was about 10% to 20% (Dahlin et. al.,1967; Gaffney et al., 2006; Longhi et al., 2005).

These miserable survival rates were presumably attributable to pulmonary metastatic disease. Since then survival rates dramatically increased during the 1970s and 1980s with the evolution of chemotherapy. In one study, adjuvant therapy, in conjunction with surgical resection, resulted in improved long-term survival rates of 60% to 65% (Link et al., 1986).

Based on western literatures, at the moment the long-term survival rates are about 60% to 78% for patients with localized osteosarcoma (Bacci et al., 2000; Bielack et al., 2002).

Despite the use of chemotherapy, the expected 10-year survival rate declines significantly to 20% to 30% in patients with clinically detectable metastasis (Bielack et al., 2002; Kager et al., 2003). Most of the patients that do not survive ultimately die because of respiratory failure caused by the metastatic complications (Meyers & Gorlick, 1997).

Poor prognostic factors for patients with osteosarcoma include metastasis on presentation, primary tumour located in the axial skeleton, large tumour volume, increased alkaline phosphatase or lactate dehydrogenase levels, poor response to preoperative chemotherapy, discontinuous tumour of bone, and any presence of lymph node involvement (Bielack et al., 2002; Kager et al., 2003; Meyers & Gorlick, 1997).

2.4 Treatment

A multidisciplinary approach is needed in the treatment of patients with osteosarcoma, including surgical and oncologic specialists.

As mentioned before, prior to 1970s, amputation was the only surgical treatment available for osteosarcoma, and 80% of patients died of metastatic disease, mainly involving the lungs.

Thus currently it universally accepted that the treatment strategy of managing osteosarcoma patients, is giving preoperative chemotherapy, or neo-adjuvant chemotherapy, followed by surgery and adjuvant therapy. This approach has greatly improved the survival rates of patients with the disease.

In our clinical setting, the chemotherapy regime that was used to treat paediatric patients in follows the Memorial Sloan Kettering T10 protocol (Cho et al., 2011; Rosen et al., 1982), albeit with some modifications.

The total duration for this regime is about 12 months. This includes 3 phases. Phase One is given to the patients pre-operatively, which consists of Vincristine at week 1, then high dose Methotrexate at week 2 and 3, which is followed by Vincristine after each dose of the above-mentioned Methotrexate. After that at week 4, Vincristine is given again.

At week 5, surgical resection of the tumour is done, followed by Phase Two of the chemotherapy regime. This includes giving Bleomycin, Cisplatin and Dactinomycin at week 5, subsequently after the surgery. 3 weeks after that, high dose Methotrexate is given at week 9 and 10, with each dose followed by Vincristine. At week 11, Adriamycin is given to this group of patients. Then after 3 weeks, which is approximately at week 14 and 15, another 2 high doses Methotrexate is given, and again each dose is followed by a dose of Vincristine.

At week 20, Phase Three is commenced with maintenance chemotherapy which includes either the Arm T10A or the Arm T10B protocol at week 20. In Arm T10 A protocol, treatment is given to patients who are poor responders to chemotherapy. This includes giving Adriamycin and Cisplatin which is repeated after 2 weeks. Then after 3 weeks, Bleomycin, Cisplatin and Dactinomycin is given for a duration of about 6 weeks.

Meanwhile in the Arm T10 B protocol, treatment is given to patients with good response to chemotherapy .This includes giving Bleomycin, Cisplatin and Dactinomycin at week 20, as previously mentioned. After 3

weeks, high dose Methotrexate, followed by Vincristine is given twice, at weekly intervals. This is followed by Adriamycin. Another 3 weeks after that, a further high dose of Methotrexate is given, and again followed by Vincristine, given twice at weekly intervals.

In adolescents or adult patients, the chemotherapy regime used in our hospital setup as a first line of treatment is based on the European Osteosarcoma Intergroup (Craft, 2009) protocol. This protocol is utilized prior to the limb salvage surgery, and after a thorough work up of the patient. The work up includes 24 hours urine creatinine clearance estimation, full blood counts, renal function tests and liver function tests.

After 3 cycles of chemotherapy, the patients were evaluated for the limb salvage surgery. The post-operative histopathological examination report is reviewed for the continuation and or modification of the chemotherapy. If the tumour necrosis factor is more than 95%, then the same pre-operative regime is continued post-operatively. If the necrosis is less than 95%, then the patient will be considered for the second line chemotherapy. In addition, the second line of regime is also given to patients who developed metastasis during the treatment period.

First line chemotherapy includes, injections of Cisplatin at 100mg/m^2 divided in 3 days is given 6 hourly each day; injections of Adriamycin at 25mg/m^2 in 24 hours infusion for 3 days, along with anti-emetic cover with H2 receptor antagonists, to minimize the side effects of nausea and vomiting.

These schedule will be repeated every 3 weeks for a total of 6 cycles; 3 cycles preoperatively, after which followed by 3 cycles after the tumour surgery.

As for second line of chemotherapy, injections of Ifosfamide 2gm/m^2 is given for 2 hours infusion, for 4 days with injections of Mesna 20% of the Ifosfamide dose given at 0, 4 and 8 hours after the mentioned Ifosfamide injections, to help protect the bladder from the urotoxic metabolites of the Ifosfamide. Next are the injections of Etoposide at 100 mg/m^2, given for an hour infusion for 4 days. This is repeated every 4 weeks, for a total of 4 cycles.

2.5 Prognostic Factors

Biological Prognostic Factors:

More than 2 decades ago there was a review article about prognostic factors in non-metastatic osteosarcoma of the extremities, which was based on the analysis of eight reports published between 1973 and1992, which concluded that the most important prognostic variable for patients with osteosarcoma of the extremities was the rate of tumour necrosis induced by pre-operative chemotherapy, however there was no consensus on the prognostic significance of patient gender or age and tumour size and location. In that same report, no mention was made of the role of biochemical markers such as serum alkaline phosphatase (ALP) and lactate dehydrogenase(LDH) (Davis et al., 1994)

The available literature on the prognostic value of the alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) serum levels remains controversial till today. Several authors describe a correlation of pre-treatment levels of the two above-mentioned serological markers within the normal range with a better outcome (Bacci et al., 1996); whereas there are other authors that could find such an association only for serum LDH, but not for serum ALP (Pochanugool et al., 1997).

According to COSS, the interdisciplinary Cooperative German-Austrian-Swiss Osteosarcoma Study Group, which was founded in 1977 and has since registered more than 3,000 osteosarcoma patients from more than over 200 institutions; demonstrated in their series a correlation of a high

serum ALP serum values with a worse outcome. Other serum factors like erythrocyte sedimentation rate, lymphocyte count, and serum albumin were found to be of no prognostic value (COSS-80, COSS-86).

2.5.1 Lactate Dehydrogenase (LDH)

The glycolytic enzyme lactate dehydrogenase (LDH)is a biological marker for cytosol in various tissues, and its serum levels are high in much pathology. In bone tumours, serum LDH has been found helpful as a prognostic factor and to evaluate the response to treatment in patients with Ewing's sarcoma (Bacci et al., 1999). Previously, little is known about the significance of serum LDH levels in patients with osteosarcoma. The observation of LDH production by human osteosarcoma transplanted into nude suggested that LDH could also be a useful biological marker in this tumour (Nakamura & Kitagawa, 1985). Nevertheless, the possible correlation between serum LDH levels and prognosis has been investigated in a small number of clinical studies (Bacci et al; 1994; Link et al, 1993; Meyers et al., 1993).

In a paper published by the esteemed group from Rizzoli Institute, in a study involving 656 patients, they reported serum LDH level to have a clear prognostic value in patients with osteosarcoma of the extremity (Bacci et at., 1994). They reported that pre-treatment serum LDH levels were a significantly important prognostic factor. Taking into consideration of patients with localized disease at presentation, the 5-year disease-free survival was 72% in

those with normal LDH values and 54% in those with high pre-treatment serum LDH levels.

Subsequently 10 years later, a follow-up study involving a larger series of 1421 patients, of which their pre-treatment serum LDH were analysed, and they concluded that the pre-treatment serum LDH had prognostic value, and it should be considered in evaluating the results of therapeutic trials of chemotherapy, as well as defining a category of patients at high-risk of relapse to be treated with a more aggressive protocols (Bacci et al., 2004).

In this study the activity of serum LDH was estimated by the standard method by the German Society of Clinical Chemistry; of which serum levels lower than 240 IU/L were considered normal, whereas values greater than 240 IU/L were considered pathologically high. They observed that the percentage of patients with elevated pre-treatment serum LDH levels in the group of patients with metastatic disease was 2-fold to that of the group of patients with localized disease, which was statistically significant.

In regards to pre-treatment serum ALP levels, they observed that pre-treatment serum LDH was significantly higher in patients who also had increased levels of serum ALP, in comparison with those patients with normal values of the serological marker, which was also statistically significant.

Another observation they made is the association of pre-treatment serum LDH with the stage of the disease; they reported that the specificity of

high pre-treatment serum LDH levels in detecting metastatic patients was high (0.81), but its sensitivity was low (0.38). More so, only 36% of patients with metastatic tumour at presentation had high pre-treatment serum LDH levels. Consequently due to the fact that their patients with metastatic disease differed for number and sites of secondary lesions, also and due to their non-standardized treatment, the correlation between pre-treatment serum LDH and outcome was not evaluated.

Furthermore, correlation between pre-treatment serum LDH and outcome in patients with localized was also evaluated, the 5-year survival rate was 60% for patients with normal pre-treatment serum LDH values, and 39.5% for patients with elevated pre-treatment serum LDH levels. They reported that the correlation between pre-treatment serum LDH and the survival outcome was highly specific but with a low sensitivity. Adding to that, they also considered the association between pre-treatment serum LDH with the time of death in those patients who succumbed to the disease. The noted that for patients who died of the tumour, the time of death (calculated from the beginning of treatment), was significantly longer in patients with normal pre-treatment serum LDH levels than those with high values.

However, they reported that prognostic significance of pre-treatment serum LDH disappeared when the variable of histological response was included in the multivariate analysis, and they concluded that in spite of this consideration, on the basis of their study, they believed that pre-treatment serum LDH values should be reported in papers concerning the combined

treatment of this osteosarcoma. This to make comparison of the results achieved with different treatment protocols, in different trials or in single institution studies more reliable.

They further added that pre-treatment serum LDH levels should also be considered in planning new randomized clinical trials to compare different protocols of chemotherapy. In recent years, risk-adapted chemotherapy protocols have been designed. The prognostic factors considered to stratify patients in these protocols were tumour size and histological subtype, in addition to the histological response to pre-operative treatment; and considering the results of their latest study, they believed that pre-treatment serum LDH should be considered for this purpose.

In another relevant study, using the Multi-Institutional Osteosarcoma Study Evaluation, the authors found that an initial serum LDH greater than 400 IU/L was the best predictor of outcome in osteosarcoma patients (Link et al., 1991). They reported that pre-treatment serum LDH level to be the only prognostic factor significant by multivariate analysis. In fact, the rate of event-free survival at 8 years was 40% for the 31 patients with high serum levels and 74% for the 82 patients with normal values.

Likewise, in another study of a 10-year experience of adjuvant and neoadjuvant chemotherapy for osteosarcoma of the extremities, involving 255 patients, they demonstrated by multivariate analysis that the disease-free survival correlated with initial baseline lower pre-treatment serum LDH. The

cumulative probability of disease-free survival was 62.2% for patients with normal LDH levels and only 42% for the group of patients with high levels (Meyers et al., 1993).

A report on evaluation of the relationship between patient-related and treatment-related factors and prognosis was carried out in 300 osteosarcoma patients treated from 1986 to 1992, showed that the pre-treatment serum LDH was predictive of disease-free survival, and a significantly more favourable prognosis was found in patients with normal values (8-year DFS of 63%), compared to the group of patients with high values (8-year DFS of 51%) (Ferrari et al., 2001)

2.5.2 Alkaline Phosphatase (ALP)

Alkaline phosphatase (ALP) is a non-specific enzyme that can hydrolyze variety of phosphate in alkaline hydrolysis conditions. It is a membrane metal glycoprotein, formed by four isozymes. Many pathological conditions or diseases cause different isozyme elevation, thus increasing the total activity of the ALP. Because the enzyme is derived from multi-source, ALP increase was not specifically caused by changes in bone metabolism (Hayashi, 2004).

In comparison with the prognostic significance pre-treatment LDH, the prognostic importance of pre-treatment serum ALP levels has been more widely reported by several authors (Bacci et al., 2002; Lockshin et al., 1968; Meyers at al., 1993; McKenna et al., 1966; Thorpe et al., 1979).

Alkaline phosphatase (ALP) is an easy, cost effective method to evaluate, at any stage of the disease, and has been shown by some authors to have a predictive value for survival (Bacci et al., 1993; Ferrari et al., 2001; Stokkel et al., 2002; Thorpe et al., 1979) or chemotherapy response (Juergens et al., 1981) Others however did not find a correlation of pre-treatment serum ALP for either survival outcome nor chemotherapy response (Pochanugool et al., 1997).

Most authors report only on ALP levels before chemotherapy, or after surgery. Alkaline phosphatase (ALP) has specifically been addressed as a prognostic factor by several authors. The enzyme has been shown to be produced directly by osteosarcoma cells and its level can be raised in patients with osteosarcoma (Singh et al., 1974).

Before the era of neo-adjuvant chemotherapy, a study was published and demonstrated an association of pre-treatment serum ALP levels and prognosis, albeit in a small group of patients (Thorpe et al., 1979).More recently studies with larger patient samples established the prognostic value of pre-treatment serum ALP. It is reported that pre-treatment ALP levels to have a predictive value for survival, but not for chemotherapy response (Bacci et al., 1993; Ferrari et al., 2001; Stokkel et al., 2002). However, none of these studies however looked at the ALP levels after chemotherapy and before surgery.

There was a report in 2005 that evaluated on both the pre-treatment and post-treatment serum ALP; of which the authors concluded that ALP levels before chemotherapy, after chemotherapy, and the change of alkaline phosphatase after chemotherapy are possible valuable factors in predicting chemotherapy response and survival in high-grade osteosarcoma in adults (Bramer et al., 2005).

In this study of 448 patients, the alkaline phosphatase levels were assessed before chemotherapy, after chemotherapy but before surgery, and the changes in the level of alkaline phosphatase after chemotherapy were recorded. For the analysis of the normalisation of serum ALP, patients were divided in 3 groups; firstly, those where pre- treatment serum ALP did normalise; secondly, where it did not; and lastly those where pre-treatment serum ALP levels at diagnosis was not raised were called not applicable, because obviously in these patients ALP could not normalise. For clarification purposes they classified ALP as normal (if the value is less than 100% of its upper limit), high (if the value is in between 100% and 200% of its upper limit) or very high (if the value is more than 200% of the upper limit).

Furthermore they evaluated pre-treatment serum ALP and its relation to survival outcome, and they found out that normal or high pre-treatment serum ALP is associated with better survival at 10 years (64% and 70% respectively) than very high pre-treatment serum ALP (37%), which was statistically significant. In regards to post-treatment serum ALP, it was

significantly correlated with survival rates of 68%, 39% and 25% in the normal, high and very high group respectively.

They observed that a pre-treatment serum ALP above twice normal value correlated with a far worse survival. If the levels of serum ALP decreased after chemotherapy, but was still raised, survival was better, but still worse than those patients that had their serum ALP normalised. Also, they mentioned that a raised post-chemotherapy serum ALP predicts poor chemotherapy response. They conclude that serum ALP, measured before chemotherapy, after chemotherapy, and the change of serum ALP after chemotherapy are possible valuable factors in predicting chemotherapy response and survival in high-grade osteosarcoma. This method is cost-effective and reproducible easily, together with other factors, play a role in improving individual prognostication. Finally they recommended that it should therefore be determined systematically in a prospective manner in order to further evaluate its usefulness.

Of note, there was a report that illustrated an association between these 2 serological markers, the authors observed a correlation between pre-treatment serum LDH and ALP at presentation of the disease; they noted that pre-treatment serum LDH was significantly higher in patients who also had increased levels of ALP (30.0%), in comparison with those patients with normal values of the serological marker (15.0%) (Bacci et al., 2004). They also mentioned that the 5-year disease-free survival rates for pre-treatment serum ALP was at 67.3% for patients with normal values and 37.7% for those

patients with high values, which was statistically significant on a univariate analysis.

Another relevant report, a study of 255 osteosarcoma patients, they demonstrated by multivariate analysis that the disease-free survival correlated with initial baseline lower pre-treatment serum ALP levels (Meyers et al., 1993).

A study from the Rizzoli Institute, focusing on evaluation of the relationship between patient-related and treatment-related factors and prognosis was carried out in 300 osteosarcoma patients, demonstrated that the pre-treatment serum ALP was a statistically significant predictive of disease-free survival, a higher 8-year DFS of 63%, was found in patients with normal values, as compared to patients with high values with the DFS rate of 55% (Ferrari et al., 2001).

Other Possible Prognostic Factors:

2.5.3 Age

Age at diagnosis is a well known prognostic factor in many different malignancies; its significance for patients with osteosarcoma is however controversial. Osteosarcoma in younger children may have a different prognosis compared to those in preadolescent age group. The fact that their physical and physiological status is dissimilar from that of adolescents, the pathophysiology of osteosarcoma development in this group might be different from that in adolescents. However, data on the clinical features and survival rates among preadolescent patients have shown conflicting results; some reports suggest a poorer prognosis in the older age group of patients (French Bone Tumour Study Group 1988; Scranton et al., 1975; Winkler et al., 1984), whereas other studies show no difference (Bacci et al., 2005; Cho et al., 2006; Rytting et al., 2000).

Age was then identified as a possible parameter of prognostic significance.

2.5.4 Gender

Osteosarcoma is known to be more common in males than in females. Females tend to develop it at a slightly earlier age; this is possibly because they tend to have their growth spurts earlier in their childhood.

In regards to the Western literature, according to the findings by the U.S. Cancer Statistics Working Group (2004), the incidence rates and 95% confidence intervals of childhood and adolescent steosarcoma are 5.0 (4.4–5.8) per million persons per year for males and 5.1(4.4–5.8) per million for females. Having said that, the incidence of osteosarcoma has always been considered to be higher in males than in females (Dahlin et al., 1986; Gurney et al., 1975; Mascarenhas et al., 1998).

And recently, according to the most up to date SEER (Surveillance Epidemiology and End Results) data in 2008, it was a rate of 5.4 per million persons per year in males vs. 4.0 per million in females (Linabery & Ross, 2008).A similar observation was also found in a study performed by the Scandinavian Sarcoma Group which showed that gender was linked with the outcome. They noted that female osteosarcoma patients had fewer relapses and better survival rates than their male counterparts (Saeter et al., 1997). This finding was also replicated in an analysis of prognostic factors by the Chinese group (Min et al., 2013).

To gain more insight in the prognostic role of age, we performed this retrospective study in our institute.