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Extensive En Block Resection and Adjunctive Chemotherapy of A Canine Primary Rib Osteosarcoma

Saul Chemonges Kasumbein and Panayiotis Loukopoulos
Division of Clinical Sciences (SCK) and Division of Veterinary Pathology and Anatomy (PL), School of Veterinary Science, The University of Queensland, Brisbane, Queensland 4072, Australia

Abstract: The management of a large rib osteosarcoma in a two-year-old neutered male Golden Retriever is reported. The tumour was initially misdiagnosed as a chondrosarcoma following incisional biopsy. Extensive en bloc resection and chest wall reconstruction with propylene mesh was performed, and carboplatin was administered postoperatively. Approximately 270 days after surgery there was no evidence of tumour recurrence and the patient was disease free.

Key words: Carboplatin, chest wall reconstruction, dogs, immunohistochemistry, misdiagnosis, neoplasms, osteosarcoma, surgical technique

Introduction

Bone tumours are not infrequent in the dog. (Brodey *et al.*, 1969 and Cotchin, 1953) Osteosarcoma accounts for the majority of these tumours,(Brodey *et al.*, 1959;Brodey *et al.*, 1969;Brodey *et al.*, 1963 and Liu *et al.*, 1977) is one of the most malignant and aggressive and has a predilection in large and giant breeds (Tjalma, 1966). 60 to 80% of osteosarcomas occur in the appendicular skeleton, with the remainder developing in the axial skeleton or extraskeletal sites.(Heyman *et al.*, 1992) Osteosarcoma of the axial skeleton occurs, usually in decreasing order of frequency, in the skull, spine, ribs and pelvis.(Heyman *et al.*, 1992;Howard *et al.*, 1976 and Levy *et al.*, 1997) Osteosarcoma is the most common primary tumour of the osseous thoracic wall in dogs,(Matthiesen *et al.*, 1992 and Pirkey *et al.*, 1995) followed by chondrosarcoma and haemangiosarcoma (Montegomery *et al.*, 1993). Dogs with osteosacomas of the rib have a poor prognosis, and chondroblastic osteosarcoma is particularly highly metastatic.(Hammer *et al.*, 1995 and Kuntz, 1998) Chondrosarcoma has a better prognosis than osteosarcoma, hemangiosarcoma or fibrosarcoma of the rib (Pirkey *et al.*, 1995). Most rib osteosarcomas originate at or near the costochondral junction and generally develop in young dogs;(Feeney *et al.*, 1982 and Montegomery *et al.*, 1993) the mean age of dogs with rib osteosarcoma is 4.5 to 5.4 years(Feeney *et al.*, 1982 and Heyman *et al.*, 1992) and in most,(Fenney *et al.*, 1982 and Heyman *et al.*, 1992) but not all, (Matthiesen *et al.*, 1992) studies, rib osteosarcomas occur in younger patients when compared with other axial osteosarcomas. Metastases may be present at the time of the initial physical examination, however, the patients may be asymptomatic or express vague or non-specific signs at first, progressing to the development of general malaise, decreased appetite and cachexia shortly afterwards (Straw, 1996).

There have been significant advances in many fronts with regard to osteosarcoma in recent years, including in imaging, (Waters *et al.*, 1998) surgery (Sach *et al.*, 1999) and chemotherapy, (Chun *et al.*, 2000.) following, or in parallel with, the emergence of Comparative and Veterinary Oncology as a specialised field of Veterinary Medicine and Surgery (Withrow, 1998). These advances offer an increasing availability of options to the veterinary surgeon and the dog owner in relation to the treatment of osteosarcoma. Despite the above, there is a relative lack of reports that discuss the diagnostic work up and management of canine rib osteosarcomas, and, in particular, describe the technical aspects of its surgical management in detail.

The purpose of this study was to report a case of osteosarcoma of the rib in a dog, that was initially misdiagnosed as a chondrosarcoma, and to describe in detail the surgical management of the case, that included extensive en bloc resection and chest wall reconstruction.

Materials and Methods

A two-year-old male Golden Retriever was referred to the Veterinary Teaching Hospital, School of Veterinary Science, University of Queensland, for evaluation of a large rib mass. Clinical, radiological and histopathological examination were performed in a routine fashion. Extensive en bloc resection and chest wall reconstruction with propylene mesh was performed, and carboplatin was administered postoperatively.

The tumour was characterised immunohistochemically for p53 protein, S-100 and Proliferating Cell Nuclear Antigen (PCNA) expression. Immunohistochemistry was performed on formalin-fixed, paraffin-embedded, silane-coated

slides employing a streptavidin-biotin-peroxidase protocol as described previously,(Hsu *et al.*, 1981;Loukopoulos *et al.*, 2003 and Loukopoulos *et al.*, 2003) counterstained with haematoxylin. The antibodies used were: S-100 (diluted 1:400), Proliferating Cell Nuclear Antigen (PCNA, PC-10, diluted 1:800) (both DAKO, Carpinteria, USA)and p53 protein (CM-1 antibody, diluted 1:75, Signet Laboratories, USA). The slides were subjected to ten minutes of microwave heating (low setting) in a citrate buffer, pH 6. Positive controls were neoplastic or normal tissues known to contain the relevant epitope;(Dong *et al.*, 1997 and Webb *et al.*, 1998) the primary antibody was substituted with non-immune sera or Tris buffer in the negative controls.

Results

Case History: A two-year-old neutered male Golden Retriever was referred to the University of Queensland Veterinary Teaching Hospital. The dog had a recent history of weight loss, for the last few daysand lameness due to interdigital pyoderma in the right forelimb. The referring veterinarian also reported a mass attached to the rib, which on biopsy, was initially diagnosed as a chondrosarcoma with osseous differentiation by a private veterinary pathology laboratory. Blood biochemical analysis showed hypercalcaemia (3.19 mmol/L) and increased alkaline phosphatase levels and was otherwise unremarkable.

Clinical and Radiological Examination: On clinical examination, the dog weighed 25.2 kg, had a temperature of 39.5°C, a heart rate of 100 beats/minuteand normal capillary refill time. A large swelling in the right caudo-ventral chest wall was discernable on palpation, with a large previous incision with a single suture distinct from the incision.

On radiographical examination, the presence of an extensive tumour was evident in the right caudo-ventral chest wall, involving several ribsand compressing the right lung medially (Fig. 1). The stomach was fully distended with food, suggesting that the tumour was preventing the stomach from emptying.

The tentative diagnosis of chondrosarcoma or osteosarcoma was offered and the dog was programmed for surgery.

Anaesthesia/ Analgesia: The dog was premedicated with 5 mg, or 0.2 mg kg^{-1} body weight (bw), of methadone (Methone® Parnell Laboratories, NSW Australia), 0.5 mg (0.02 mg kg^{-1}) of acepromazine maleate (Promex® Apex Laboratories Pty, NSW, Australia) and 1 mg (0.04 mg kg^{-1}) of atropine sulphate (Atropine injection® Apex Laboratories Pty, NSW, Australia), all mixed in one syringe and administered subcutaneously (sc). An intravenous catheter was placed in the tarsal vein, flushed with normal saline and secured in place with adhesive tape. Twenty minutes after premedication, 12.6 mg (0.5 mg kg^{-1}) of metoclopramide hydrochloride (Maxalon®) were given intravenously (iv), to enhance gastric emptying and 10.08 mg (0.4 mg kg^{-1}) of carprofen (Zenecarp® Injection, UK) sc. Anaesthesia was induced with 90 mg of propofol intravenously. The dog was intubated with a 10-mm endotracheal tube and connected to a twin canister carbon dioxide circle absorber anaesthetic machine (CIG Migdet,® Australia) with an oxygen flow rate of 3.2 L min^{-1} and an inhaled isoflurane (Flothane® Abbot Australia Pty) concentration of 3.25%.

At the start of surgery, a bolus dose of 150 μg of fentanyl (Sublimaze® Janssen Cilag, Belgium) was administered iv, followed by a fentanyl infusion at the rate of 330 μg hr^{-1} and 2 mg of vercuroniun (Norcuron® Organon Teknika B.V., Boxtel, Holland) administered iv, for muscle relaxation. The dog was then ventilated. Fifteen minutes after induction, 600 mg (22mg kg^{-1}) of cefazolin sodium (Kefzol,® Eli Lilly and Co. Taiwan Inc.) were administered by slow intravenous infusion and, ten minutes later, 50 mg of lignocaine hydrochloride (Mavlab, Slacks Creek, Queensland, Australia) were infused locally over the right chest wall. Morphine sulphate (3 mg) (David Bull Laboratories, Mulgrave Victoria, Australia) was administered sc at the end of surgery to alleviate postoperative pain.

Surgery: The dog was placed in left lateral recumbency. A skin incision was made running dorso-ventrally along the full length of the thoracic wall over the top of the mass at the ninth rib, encircling and avoiding the biopsy site for nearly the full height of the chest. The subcutaneous tissue was dissected and a flap of the *latissimus dorsi* muscle elevatedand the attachment retained cranially. The *serratus ventralis* and *dorsalis* muscles were dissected and elevated in approach to the intercostal spaces 5 to 6 and 11 to 12. The *iliocostalis* and *longissimus* muscles were retracted dorsally, while the external abdominal oblique muscle was transected ventrally. A thoracotomy incision was made in the intercostal space 5 to 6 and 11 to 12. The sixth to the eleventh rib inclusive were transected at an angle dorsally at the rib neck and ventrally at the costo-chondral junction of each rib to be resected. The chest wall section (including ribs 6 to 11) was removed (Fig. 2).

Haemostasis was achieved with electrocautery. The larger blood vessels of the thorax were ligated with 2-O

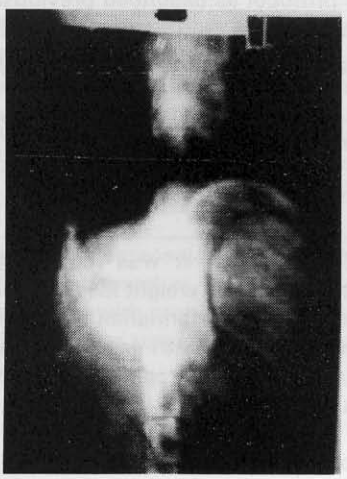

Fig. 1: On radiographical exmination of the chest, the presence of an extensive tumour is evident in the right caudo-ventral chest wall, involving serveral ribs, and compressing the right lung medially

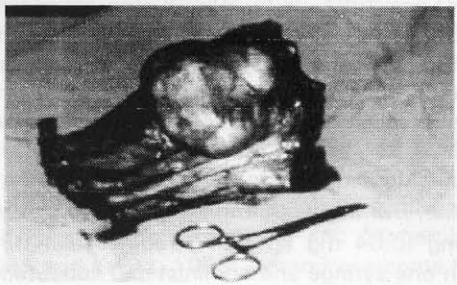

Fig. 2: The excised tumour mass and chest wall section including ribs 6 to 11

Fig. 3: Chondroblastic osteosarcoma of the rib. Although the quality and quantity of the matrix produced varied greatly within the tumour, the predominant matrix produced was cartilage or chondroid, for which reason the osteosarcoma was classified as chondroblastic. Large areas of abundant, disordered and, in places, clacified cartilage production are shown. H & E x 100.

Fig. 4: Chondroblastic osteosarcoma of the rib. A focal area of chondroid production (center) surrounded and produced by an otherwise dense chondroblast-like cell population. In places (not shown here), osteoid and/or bone was clearly prduced by malignant osteoblasts designating this tumour as an osteosarcoma

polyglyconate (Maxon®) sutures. Before closure of the wound, a chest drain (Mallinckrodt,® Mallinckrodt Laboratories) was placed exiting dorsally through the intercostal space 12 to13.
A polypropylene mesh (Prolen Mesh) was placed over the defect in the chest wall and sutured to the edges of the defect using a noose with a simple interrupted suture pattern, first to the intercostal muscles cranially, caudally and ventrally, then the *iliocostalis* and *longissimus* muscles dorsally. The mesh was placed medially to the edges of the defect. The *latissimus dorsi* muscle flap was rotated ventrally and sutured over the defect and mesh using 2-O polyglyconate sutures with a simple interrupted pattern.
The subcutaneous tissues and *cutaneous trunci* muscles were closed individually using 2-O polyglyconate sutures with a simple continuous pattern. The chest drain was aspirated to remove air and approximately 20 ml of serosanguinous fluid. The skin was closed with simple interrupted 2-O Nylon sutures. The chest drain was secured in place with a Chinese finger suture.
The surgery lasted 3¼ hours. Recovery from anaesthesia was uneventful and the dog did not express severe postoperative discomfort. The excised tissue was submitted for histopathological examination at the University of Queensland Veterinary Pathology Division.

Postoperative management: There was no incisional complication associated with the closure of the thoracic defect during the immediate postoperative period. The dog was re-evaluated 14 days after surgery and was doing well, the only complication being a relatively small seroma at the resection site.
Adjuvant chemotherapy with carboplatin (Carboplatin®) at a dose of 300mg m^{-2} was started on 25 days after surgery and repeated 21 days later. By 28 days after surgery, the seroma had resolved. A white cell count and differential was performed prior to each dose of chemotherapy. Blood was drawn from the jugular vein in order to preserve the cephalic vein for carboplatin administration.
The dog was re-examined and was free of disease 270 days after the first clinical examination.

Gross Pathology-Histopathology: On gross examination, the excised tumour mass was grey to white with a moderately hard cut surface.
Histological evaluation of representative samples from the excised tissue revealed an encapsulated mass that was moderately invasive locally. The surgical margins were free of neoplastic tissue. Periosteal response included a marked fibrous reaction and subperiosteal new bone and cartilage formation.
The tumour consisted of very pleomorphic cells, the nuclei of which were mostly of ovoid or spindle shape, that were arranged in various morphological patterns. In places, osteoid and bone was clearly produced by malignant osteoblasts in thin spicules, irregular islands or round foci, designating this tumour as an osteosarcoma. There were large areas of abundant cartilage production, which was disordered and, in most areas, calcified (Figs. 3 and 4); areas with solid sheets of spindle non-productive cells (Fig. 4) and similar cellular areas that produced fine extracellular matrix, mainly loose collagenand were in parts arranged in a streaming pattern. The latter areas exhibited zonal, rather than diffuse production of cartilage or osteoid. Although the quality and quantity of the matrix produced varied greatly within these areas, the predominant matrix produced was cartilage. Necrotic foci covered approximately 40% of the tumour area examined and were mainly associated with chondroblastic areas. A small amount of free blood and medium-sized vascular spaces was present.
Cellular features included ovoid, spindle-shaped or round nuclei with clear cytoplasm, stipled chromatin, small multiple or single large and prominent nucleoli, increased nuclear: cytoplasmic ratio, karyomegaly and moderate to marked anisokaryosis. Apoptotic rates were moderate to high and mitoses were frequent (average 5 per high power field) and often bizarre. Very few, if any, multinucleated cells were present.
Based on the clinicopathological findings, a diagnosis of chondroblastic osteosarcoma was offered. Based on the degree of nuclear pleomorphism, extent of necrosis and mitotic index, the tumour was classified as grade III (Straw *et al.*, 1996).

Immunohistochemistry: The majority of the tumor cells showed moderate to strong nuclear staining for PCNA. S-100 was detected immunohistochemically in 20 to 30% of tumor cells in the chondroblastic areas. Tumor cells were uniformly negative to the p53 antibody used.

Discussion

The management of a rib osteosarcoma case in a Golden Retriever is reported, with particular emphasis on the discussion of its initial misdiagnosis as a chondrosarcomaand on the description of the technical aspects of its surgical management, that included extensive en bloc resection and chest wall reconstruction. There are few, if

any, "how to do it" type reports dealing with the details of the surgical management of canine osteosarcoma, particularly for cases such as the one presented here, in which the resection of a very large number of ribs was required.

Signalment, anatomic site, weight, (Brodey and Riser, 1969 and Ru *et al.*, 1998) history and good quality, properly positioned, radiographs will accurately predict osteosarcoma in more than 90% of dogs, (Withrow, 1998) although the presence of metastases is detected preoperatively in only 10% to 15% of dogs (Withrow, 1998). In this case, the intrathoracic mass was visible radiographically, but there was no evidence of pulmonary metastatic disease. If margins of the tumour cannot be determined adequately by radiological or clinical examination, computed tomography and magnetic resonance imaging may be helpful in delineating tumour margins and detecting metastases and possible involvement of the vertebrae. (Kuntz, 1998 and Matthiesen *et al.*, 1992 and Withrow, 1998) Bone survey radiographs may also be used to detect bone metastases (Withrow, 1998). Skeletal sarcomas rarely metastasise to lymph nodes, but any draining and palpable lymph nodes should have cytology aspirates performed (Withrow, 1998). Haematological and biochemical laboratory findings are usually non-contributory and non-specific but, although they are not helpful in establishing a direct diagnosis of osteosarcoma, they may help rule out the presence of infection (such as osteomyelitis) and offer an overall evaluation of the status of the patient, which may influence treatment options. The hypercalcaemia and increased alkaline phosphatase levels of the present case were probably due to previous steroid medication and or increased osteoblastic activity.

Definite diagnosis of osteosarcoma is established by histopathological examination of excisional or incisional biopsy samples (Smith and Sutton, 1988). Large volume biopsy specimens are necessary to distinguish osteosarcoma from chondrosarcoma and heamangiosarcoma. The case presented here was initially diagnosed as a chondrosarcoma with osseous differentiation, based on examination of the biopsy sample submitted. Later histopathological examination of the resected mass, in which numerous samples from various areas of the tumour were examined, resulted in a diagnosis of chondroblastic osteosarcoma. The wide pattern variation of the tumour in the case presented here, apparent even within the same microscopic slide, indicates the difficulty of establishing a correct diagnosis when sampling of rib or other tumours is inadequate or non-representative. This is especially so for cases with separate, rather than intermingling, matrix patterns; in these cases, biopsy material taken from the chondroblastic areas alone would lead to a diagnosis of chondrosarcoma. As rib osteosarcomas may tend to be more chondroblastic on the outer tumour areas, taking a biopsy sample from the outer area of a rib osteosarcoma, as is usually the case in practice, may lead to the examination of a non-representative sample and, possibly, as is the case here, misdiagnosis. In these cases, we are of the view that a statement should be included in the pathology report, emphasising that a diagnosis of osteosarcoma cannot be ruled out. Although the initial diagnosis of chondrosarcoma did not alter the decision by the veterinary surgeon to proceed with surgery, such a statement could have altered the clinician's decision regarding the timetable for the administration of chemotherapy; it could have also had an effect on the owner's decision to proceed with surgery, given the heavier prognosis for osteosarcoma.

Without any treatment, the pain experienced by the dogs because of the extensive destruction of bone and surrounding tissue, leads most owners to elect to have their animals euthanised soon after diagnosis (Straw, 1996). However, management for dogs with osteosarcoma has changed from suggesting euthanasia to a more proactive approach on both the primary and metastatic sites (Withrow, 1998). Treatment options for osteosarcoma include curative-intent therapy and palliative therapy. (Peaston and Watson, 1995) The former aims to control the tumour locally and to prevent or control the development of metastasesand may include surgery, chemotherapy, immunotherapy, or radiation therapy. The latter aims to improve quality of life by controlling pain and preventing pathological fractures and may include, or has previously included, radiation therapy, chemotherapyand administration of non-steroidal anti-inflammatory drugs, opioids, bisphosphonates, (Ehrhart, 2000 and Haburjak and Mandelker, 1998) or oestrogens. (Donaldson, 1975). The prognosis following surgical resection of thoracic wall masses depends on histologic diagnosis, the use of chemotherapy for osteosarcoma and the completeness of surgical margins. (Pirkey *et al.*, 1995) Factors such as age, weight, sex, number of ribs resected, tumor volume and total cisplatin dose seem not to influence survival nor disease-free interval (Pirkey *et al.*, 1995). Surgical excision of rib tumours alone does not improve the median survival time in dogs; (Matthiesen *et al.*, 1992) in one study, 20 dogs with rib osteosarcoma showed a median survival time of 3 months, with 20% 6 month survival time. (Matthiesen *et al.*, 1992) The completeness of surgical margins is prognostic for disease free interval and survival in dogs with rib tumours, emphasising the need for radical tumour resection and marking of surgical margins. (Kuntz, 1998) A 66.7% tumour recurrence following surgical excision of axial osteosarcomas has been reported, (Heyman *et al.*, 1992) although the study was not specific to rib osteosarcomas. If surgical margins are incomplete, a second surgery or adjuvant radiation therapy should be considered. (Kuntz, 1998)

Analgesic drugs, usually given before anaesthetic recovery for postoperative pain relief, include the narcotic analgesics, such as the commonly used pethidine and buprenorphineand the non-steroidal anti-inflammatory drugs (NSAIDs), such as flunixin and dipyrone (Watson *et al.*, 1996). A postoperative protocol specific to canine thoracotomy patients has been suggested.(Kuntz, 1998) Morphine may provide adequate analgesia for dogs after lateral thoracotomy, as in the case presented, although its effects may be short lived; local anaesthesia with bupivacaine may be equally effective. (Pascoe, 1995) For longer lasting analgesia, epidurally administered oxymorphone would be preferable in alleviating pain after thoracotomy (Poilskis *et al.*, 1991).

Postoperative complications associated with rib resection are relatively uncommon. Infection, however, can be a problem associated with the implantation of foreign material, such as polypropylene mesh, (Matthiesen *et al.*, 1992) but was not observed in our case. It should be noted that the use of synthetic meshes is rarely required for reconstruction of defects involving a small number of ribs, as the surrounding soft tissues, including the *latissimus dorsi* and diaphragm are frequently sufficient. (Orton, 1995)

Because, in many cases, clinically non-detectable metastases are present at the time of diagnosis, rib resection alone should be considered a mainly palliative procedure and is minimally effective in prolonging survival times of dogs with primary rib osteosarcoma. (Matthiesen *et al.*, 1992) Canine appendicular osteosarcoma patients treated with surgery plus cisplatin have significantly increased median survival time (240 days), compared with patients treated with surgery alone (90 days). (Kuntz, 1998 and Mauldin *et al.*, 1988) (Bergman, *et al.*, 1996). Similar findings have been made for surgery plus carboplatin, (Bergman, *et al.*, 1996) while chemotherapy including cisplatin with or without doxorubicin has also been shown to significantly improve survival in dogs with rib tumours (Pirkey *et al.*, 1995). The behaviour of rib osteosarcoma appears to be same as for appendicular osteosarcoma,(Kuntz, 1998) which is consistent with the above-median survival time observed in the case presented here. Carboplatin is commonly used in the treatment of osteosarcoma and is a well-tolerated chemotherapeutic agent that may be given safely every 21 days at a dose of 300 mgm^{-2}, neutropenia being the dose-limiting toxicity. (Bergman, *et al.*, 1996) Carboplatin is usually given in a first-stick peripheral vein catheter over 15 minutes for 4 doses, diluted in sterile water. (Bergman, *et al.*, 1996) The guidelines of chemotherapy administration concerning blood counts and safety must be adhered to strictly. (Bergman, *et al.*, 1996)

p53 was not detected immmunohistochemically in the case presented here. heng Although immunohistochemical detection of p53 protein demonstrates alterations in the p53 gene or product, (Liang *et al.*, 1999) which normally acts as a tumour suppressor gene, (5) lack of p53 expression does not rule out the presence of p53 gene alterations. S-100 is a protein that serves as a marker for bone tumors originating in the cartilage, the notochord and T-zone histiocytes and is also involved in the calcification of normal and neoplastic cartilage. (Chano *et al.*, 1996 and Okjima *et al.*, 1988) The fact that the present case was S-100 positive in the chondroblastic areas supports the notion that chondroblastic osteosarcomas are comprised of mixed chondroblast and osteoblast cell populations, or are derived from multipotential cells of the osteoblastic lineage, that differentiate depending on the varying microenvironment conditions. Proliferating Cell Nuclear Antigen (PCNA) acts as an auxiliary protein for DNA-polymerase delta and is increased in proliferating cells as opposed to mitotically quiescent cells (Wolf and Dittrich, 1992). It serves as a proliferation marker (Hall *et al.*, 1990) and has been shown to be of prognostic value for a number of tumor types. (Roels *et al.*, 1999 and Vesalainen *et al.*, 1994) The majority of tumor cells in our case showed strong nuclear staining for PCNA. Interestingly, intratumoral variation in PCNA immunoreactivity was minimal, indicating that the various tumor components proliferate at more or less similar rates.

The veterinary surgeon should be aware of the possibly increased risk of misdiagnosis, when interpreting a pathology report from a rib tumour case. A diagnosis of chondrosarcoma based on the examination of a small biopsy sample should not rule out chondroblastic osteosarcoma. Satisfactory clinical results may be achieved in canine rib osteosarcoma cases, involving large size tumours, with en bloc resection of even extensive parts of the chest wall, reconstruction with synthetic meshand carboplatin administration.

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