UNIVERSIDADE DE ÉVORA

SCHOOL OF SCIENCE AND TECHNOLOGY
DEPARTMENT OF VETERINARY MEDICINE

Small Animal Internal Medicine and Surgery

Emmanouil Chasapis

Internal Tutor: Prof. Elsa Leclerc Duarte
External Tutor: Dr. Dimitrios Anastasiou

Integrated Masters in Veterinary Medicine

Osteosarcoma in ex-racing Greyhounds
Relatório de Estágio

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Dedication

Ουδέποτε είναι εφικτό να αναπληρωθούν στιγμές από το παρελθόν.

Αφιερώνω λοιπόν, ετούτο εδώ το γραπτό, στους γιούς μου, Δημήτρη και Κωνσταντίνο,
καθώς και στον αδερφό μου Φώτη.

Για τις αμέτρητες εκείνες στιγμές, από τα παιδικά τους χρόνια, που ήμουν απών.

Νέα Υόρκη

2017

Moments of the past can never be revived.
I dedicate this work, to my sons, Dimitris and Konstantinos,
as well to my brother Fotis.
For all those moments, during their childhood, that I was absent.
New York
2017

Acknowledgements

Without the influence of both my parents, there would be no medical route in my life. I must thank them for instilling in me, the interest to learn more about how life functions and the love for nature. They understood, that from a very young age I had an urgency to be in contact with animals and gave me that opportunity whenever possible. Lambs bottle-fed in the bathroom, kittens running around the house, hamsters escaping from their cages, dogs messing everything around, leaky fish tanks and ducklings quacking insistently, was what they had to live with.

I want to thank my father, for being there to support my efforts, with enormous patience. He, simple-handedly, gave me always want I needed and is a major example for me to follow as a person and as a doctor. I also, want to thank Eleni, Lefteris and Sissy, for standing by my side, with love and affection, in all the professional and personal choices I took.

I am grateful to my mother that she was the first to believe that I can manage myself alone away from home, even if she was the person that worried the most. I will never forget the first day of school when I was five. She stood on our home's door step, and told me "Off you go, have a nice day at school!". She gave me the choice to be who I am. Thank you, mom, for believing in me.

The support and love of Vivian and Gus, is unconditional and I have to thank them for their time and assistance particularly during the past year.

I was very fortunate to choose excellent tutors that helped me go through this last step of my academic career. Professor, Elsa Leclerc Duarte, was always ready to address all the issues I encountered and gave me valuable advice with patience and understanding. I am fully appreciative of all her assistance and guidance.

I express my deep gratitude to Dr. Dimitrios Anastasiou as well as the Staff of "A and A Veterinary Hospital" for receiving me. From the first day, I felt embraced. All the veterinarians provided an excellent learning experience that helped me integrate, in New York, as a veterinarian and as a person. Dr. Anastasiou, patiently and openly, advised me in clinical as well as in personal issues. I really appreciate all his dedication and time.

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I am thankful to my uncle, Prof. Theo Katsivas, for being the first to "inject" me with the idea of a veterinary profession and for offering critical aid for the edition of this report.

Finally, and most importantly, I thank my wife. She was there for me always. Countless hours of absence from family moments and chores, did not stop her from showing me her love. She always accompanied me with compassionate and encouraging words first thing in the morning and last thing at night, especially during the edition of this report. Thank you, Irene, for all you have done.

Abstract

This report is an effort to describe most of the clinical procedures that took place during the integrated externship of the Master's degree in Veterinary Medicine at the University of Evora. The externship had a duration of sixteen weeks and was realized in the "A&A Veterinary Hospital" which has a substantial caseload of Retired Racing Greyhounds with Osteosarcoma. The first part, of the report, relates to the casuistics of Small Animal Internal Medicine and Surgery, divided per area of interest. The second part, is a current bibliographic review on canine Osteosarcoma, followed by three case-studies of Osteosarcoma in Retired Racing Greyhounds, with different survival times.

Keywords: Small animal practice, osteosarcoma, Greyhounds, chemotherapy, amputation

Resumo

Medicina Interna e Cirurgia em Pequenos Animais Osteosarcoma em cães de raça Greyhound, ex-corredores

O presente relatório é uma descrição da maioria dos procedimentos clínicos, que se realizaram durante o estágio curricular do Mestrado Integrado em Medicina Veterinária da Universidade de Évora. O estágio, teve a duração de dezasseis semanas e realizou-se em "A&A Veterinary Hospital" que apresenta na sua casuística muitos cães de raça Greyhound, Ex- corredores, com Osteosarcoma. A primeira secção relata a casuística, em Medicina Interna e Cirurgia de Pequenos Animais, dividida por área de interesse. A classificação dos casos foi realizada com base no sistema somático afetado ou no motivo da visita. A segunda secção é uma revisão bibliográfica sobre Osteosarcoma canino, acompanhada por três casos de Osteosarcoma em cães ex-corredores, de raça *Greyhound,* com diferentes tempos de sobrevivência.

Palavras chave: Clinica de Pequenos Animais, osteosarcoma, Greyhounds, quimioterapia, amputação

Contents

Abbreviations

A&A	A&A Veterinary Hospital
AAFP	American Association of Feline Practitioners
AAHA	American Animal Hospital Association
ACA	Aminocaproic Acid
ACTH	Adrenocorticotropic Hormone
ACTHST	Adrenocorticotropic Hormone Stimulation Test
AD	Atopic Dermatitis
AKC	American Kennel Club
ALP	Alkaline Phosphatase
AST	Antibiotic Susceptibility Test
AST	Alanine Aminotransferase
BALP	Bone-specific Alkaline Phosphatase
Bb	*Bordetella bronchiseptica*
BCG	acillus Calmette-Guérin
Bcl-2	*B-cell lymphoma 2*
BG	Blood Glucose
BID	*Bis in die*
BLS	Basic Life Support
CAFR	Cutaneous Adverse Food Reaction
CAV-2	Canine Adenovirus type-2
CBC	Complete Blood Count
CCL	Cranial Cruciate Ligament
CDK	*Cyclin Dependent Kinase*
CDKN2AB	*CDK Inhibitor 2A* and *2B*
CHTH	Chemotherapy
CIRD	Canine Infectious Respiratory Disea
CIV	Canine Influenza Virus
CKD	Chronic Kidney Disease
COX-2	Cyclooxygenase-2
CPIV	Canine Parainfluenza Virus
CPR	Cardiopulmonary
CPV-2	Canine Parvovirus- 2
CTr	Collapsing Trachea
CT	Computed Tomography
DFI	Disease Free Interval
DIC	Disseminated intravascular coagulation
DM	Diabetes Mellitus
DOI	Duration of Immunity
DVM	Veterinarian
E2F	*E2F* family of DNA-binding transcription factor
ECG	Electrocardiograms
EU	University of Évora
FAD	Flea Allergy Dermatitis
FCV	Feline Calicivirus
FeLV	Feline Leukemia Virus
FHT	Feline Hyperthyroidism

FHV-1	Feline Herpesvirus-1
FIV	Feline Immunodeficiency Virus
FPV	Feline Parvovirus
GE	Gastroenterology
GH	Growth hormone
GI	Gastrointestinal
HAC	Hyperadrenocorticism
HARD	Heartworm Associated Respiratory Disease
Hct	Hematocrit
HER2/neu	Epidermal Growth factor receptors construct
HSA	Hemangiosarcoma
HW	Heartworm
HWD	Heartworm Disease
HWI	Heartworm Infection
IBD	Inflammatory Bowel Disease
IGF-1	Insulin-like *Growth Factor-1*
IGS	Inslect Growth Inhibitors
IL-2	Interleukin-2
IM	Intramuscular
IOP	Intraocular Pressure
IORT	Intraoperative Radiation Therapy
IV	Intravenous
JAK	Janus Kinases
LMN	Lower Motor Neuron
L-MTP-PE	Liposomal Muramyl Tripeptide Phosphatidylethanolamine
LPE	Lymphocytic-Plasmacytic Enteritis
MET/HGR	*Hepatocyte growth factor receptor*
miRNA, miR	micro RNA
MLV	Modified Live Vaccine
MMPs	Matrix Metalloproteinases
MOA	Months of Age
MRI	Magnetic Resonance Imaging
MRSP	Methicilline Resistant Staph. pseudointermedious
MST	Median Survival Time
mTOR	*mammalian Target of rapamycin*
NBPs	Aminobiphosphonates
NSAID	Nonsteroidal Anti-inflammatory
NSGE	Nonspecific Gastroenteritis
OA	Osteoarthritis
OE	Otitis externa
OS	Osteosarcoma
OTIS	Otitis Index Score
OVH	Ovariohysterectomy
PCR	Polymerase Chain Reaction
PD	Polydipsia
PM	Preventive Medicine
PO	*Per os*
PSL	Pancreatic Sensitive Lipase
PTE	Positron Emission Tomography
PTEN	Phosphatase and tensin homolog
PU	Polyuria
qRT-PCR	Real-Time Quantitative Reverse Transcription
RANK	Receptor Activator of Nuclear Factor Kappa
RANKL	RANK-ligand
RB	*Retinoblastoma*
***r*CDV**	Canine Distemper Virus
RGs	Racing Greyhounds
RON	*Recepteur d'origin nantaise*
RRG	Retired Racing Greyhound
RT	Radiation Therapy
SBP	Serum Biochemistry Profile
SC	Subcutaneous
SCC	Squamous Cell Carcinoma
SID	once a day
Sm-EDTMP	Radioisotope Samarium and a bisphosphonate
SNP	Single nucleotide polymorphism
SP	Superficial
SRS	Stereotactic Radiosurgery
SS	Skin Scraping
TPLO	Tibial Plateau Leveling Osteotomy
TRKs	Tyrosine Kinase Inhibitors
U/A	Urinalysis
Ultra-S	Ultrasonography
UMN	Upper Motor Neuron
US	United States
USDA	United States Department of Agriculture
UTI	Urinary Tract Infection
VEGF	Vascular Endothelial Growth Factor
WOA	Weeks of Age
X-ray	Radiographs
YOA	Year of Age

Figures

Charts

Tables

1. Prologue

As part of the obligatory curricular program, to obtain the degree of Doctor in Veterinary Medicine, from the School of Veterinary Medicine of the University of Évora, a final year student must complete a four-month-long externship in an institution/facility of his choice.

Dr. Dimitrios Anastasiou accepted the author, as an extern, at the "A&A Veterinary Hospital" ("A&A") found in 414 Franklin Avenue, Franklin Square, New York 11010, in the United States.

Dr. Anastasiou handled the student's orientation and tutorship during the clinical experience. Additionally, Dr. Anastasiou gave important scientific advice for the redaction of this report. Nevertheless, it is more exact to state that the whole hospital team welcomed the author in their working place unhesitatingly. All the clinicians as well as veterinary technicians and aiding personnel offered thoughtful guidance.

Table 1. Staff of the "A&A Veterinary Hospital"

Veterinarians	Degree	University	Interest	CL %	LVT's	CL %
Dimitrios Anastasiou	DVM, MSc.	Thessaloniki	Medicine/Surgery	40.97%	*Matthew W. Coffey*	1.17%
Marybeth Longo	DVM	Pennsylvania	Medicine/Surgery	20.39%	*Mary Healy*	-
Jessica Peterman	DVM	Ross	Medicine/Surgery	14.76%	*Margaret Gratzer*	-
Gabriel Ordas	DVM	Ross	Medicine/Surgery	11.84%	*Amy Hoose*	-
Shamli Malik	DVM	Ross	Medicine/Surgery	7.57%	*Christina*	-
Richard Blomquist	DVM	Cornell	Surgery/Head of Stuff	3.30%		-

CL%: Caseload percentage represented in this report, LVT's: Licensed Veterinary Technicians

40% of the patients were examined by the side of Dr. Anastasiou with the remaining caseload distributed among other five "A&A" veterinarians. In fact, one of the most valuable aspects of this clinical experience was that by following all attending clinicians and veterinary technicians, a panoply of knowledge in different interrogatory, diagnostic and procedural skills was accumulated by the author (Table 1). Inarguably, the work of veterinarians and veterinary technicians would be very complicated without the help of the Kennel Assistants and Receptionists.

"A&A Veterinary Hospital" was established in 1964, by doctors Altman and Apostolidis, and ever since remained open year-round until nine in the evening except in Sundays and holidays that closes at one in the afternoon. There are four examining rooms, a preparatory room, two wards that can accommodate around 40 pets at the same time and a sound proof room with regulated temperature and humidity for exotic animals. "A&A" also has an oxygen chamber, intramural laboratory analysis, an endoscope, and a fully equipped surgical room (two surgical tables) for advanced life support and

anesthesia. Other diagnostic tools available are oral and full body radiographs and ultrasound. Furthermore, there is a boarding and grooming service offered.

Officially, the clinical experience started August 8th 2016 and ended November 29th 2016. Afterwards, though, many visits were made to consult and follow Dr. Anastasiou and the rest of the staff. From day one, the author followed the clinical procedures in the Hospital along with the veterinarians and technicians. Present on the floor, were at least two veterinarians and one technician but often there were four or five doctors and two technicians working together.

Daily, early in the morning, the health care team would do rounds in the wards and discuss every case singularly. Treatment plans were evaluated and eventual changes made. Later, scheduled surgeries took place. Veterinary technicians prepared the surgical setting, induced, intubated, anesthetized the patients and executed the antiseptic preparation of the surgical field. Meanwhile, scheduled appointments started at nine a.m. During every visit, in the examining room and in the presence of the caregivers, there was a doctor, a kennel assistant, and the author. Frequently, the patients, after an initial evaluation in the examining room, were taken in the preparatory room. With the assistance of the veterinary technicians a more thorough physical exam was possible and procedures like nail clipping and ear cleaning amongst other. Frequently, the pets were more calm without the presence of the owners.

The present report is divided into two chapters. The first chapter's purpose is to guide the reader through the caseload met during the author's externship at "A&A". The second chapter is dedicated to canine appendicular osteosarcoma by a literature review and a discussion of case studies on the subject.

2. Chapter I: Casuistics

In this chapter the totality of contacts with animal patients is categorized into areas of interest ("Area") based on the somatic system treated or mostly affected by symptoms and the primary complain or procedure for which the pet was brought for a visit. By "*contacts*", the author means, every examination or procedure that happened at "A&A" during his externship and in the presence of veterinarian or veterinary technician. To differentiate between a first contact with a patient and a scheduled reevaluation (e secondary contact) the author created the "Area" *Reevaluation.* In this way, the reader knows that by exclusion of the 41 contacts of the Reevaluation "Area", the remaining 474 contacts are different individual patients. During the composition of the report though emerged that the case of one guinea pig is referred (also counted in *Exotics*) in *Oncology* and *Surgery* and another guinea pig in *Urology*. Recalculating, the different individual patients encountered were 471. Furthermore, the reader needs to clarify that the "Area" *Exotics* is the only not categorized by the somatic system affected. The total number of contacts was n= 515. (Table 2) (Charts 1 and 2)

Table 2. Caseload divided by area and species

AREAS	Dog	Cat	Guinea Pig	Psitacid	Ferret	Snake	TOTAL	% of area
dermatology	56	15	0	0	0	0	71	13.79%
preventive medicine	44	27	0	0	0	0	71	13.79%
gastroenterology	29	16	0	0	0	0	45	8.74%
oncology	27	5	1	0	0	0	33	6.41%
musculoskeletal	31	0	0	0	0	0	31	6.02%
surgery	26	4	1	0	0	0	31	6.02%
otitis externa	27	3	0	0	0	0	30	5.83%
neurology	21	3	0	0	0	0	24	4.66%
urinary	16	3	1	0	0	0	20	3.88%
endocrinology	9	7	0	0	0	0	16	3.11%
cardiology	13	0	0	0	0	0	13	2.52%
emergency	12	0	0	0	0	0	12	2.33%
respiratory	9	3	0	0	0	0	12	2.33%
anal sacs	9	0	0	0	0	0	9	1.75%
euthanasia	6	0	0	0	0	0	6	1.17%
dentistry	5	1	0	0	0	0	6	1.17%
minor wound	3	2	0	0	0	0	5	0.97%
ophthalmology	4	1	0	0	0	0	5	0.97%
decompensated	4	1	0	0	0	0	5	0.97%
behavior	4	0	0	0	0	0	4	0.78%
reproductive	3	1	0	0	0	0	4	0.78%
undetermined	1	1	0	0	0	0	2	0.39%
infectious	1	1	0	0	0	0	2	0.39%
parasitic	1	0	0	0	0	0	1	0.19%
exotics	0	0	7	5	2	2	16	3.11%
reevaluation	22	17	0	0	1	1	41	7.96%
TOTAL contacts	**383**	**111**	**10**	**5**	**3**	**3**	515	100.00%
% of species	**74.37%**	**21.55%**	**1.94%**	**0.97%**	**0.58%**	**0.58%**	100.00%	

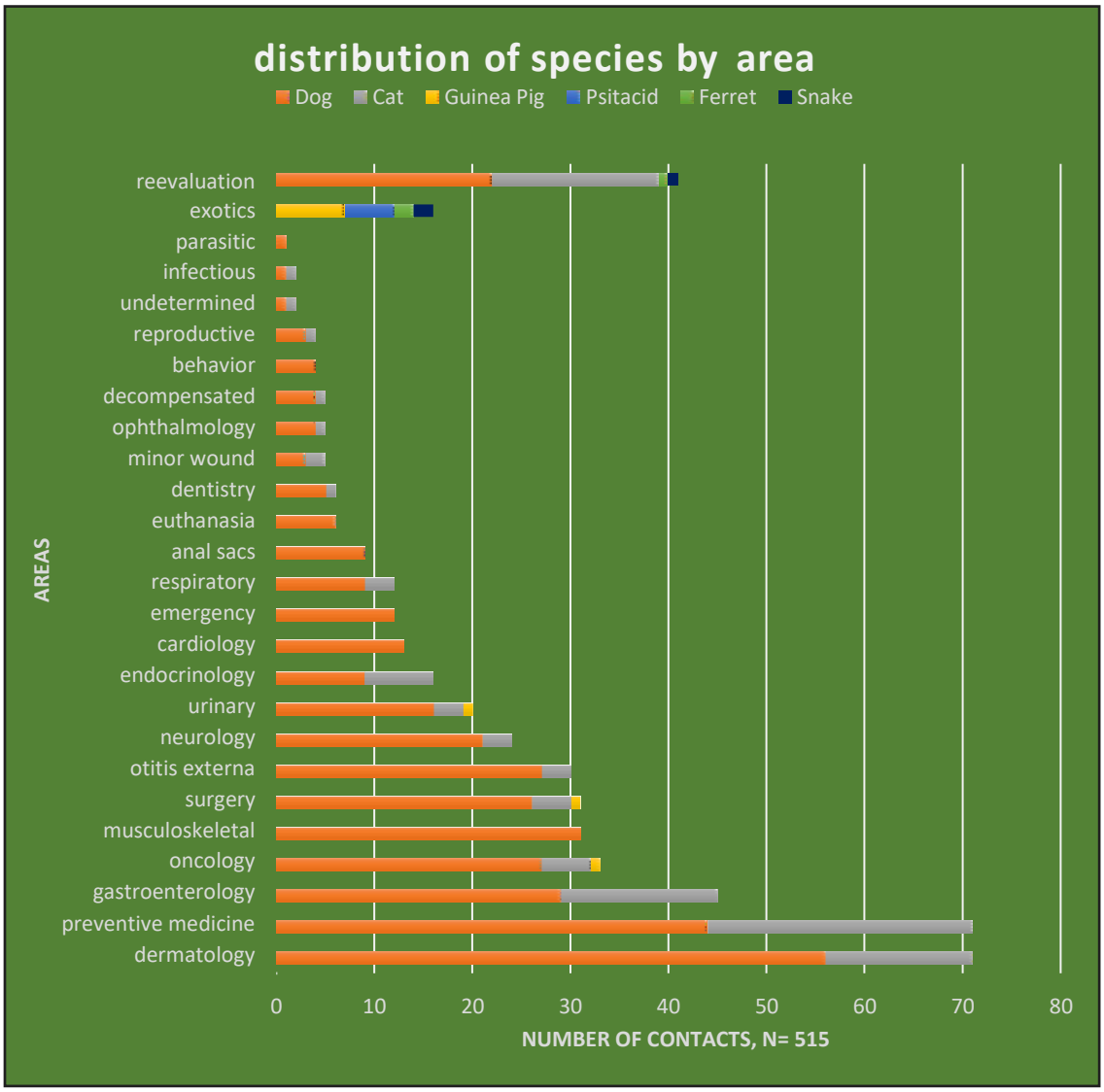

Chart 1 . Distribution of species by area

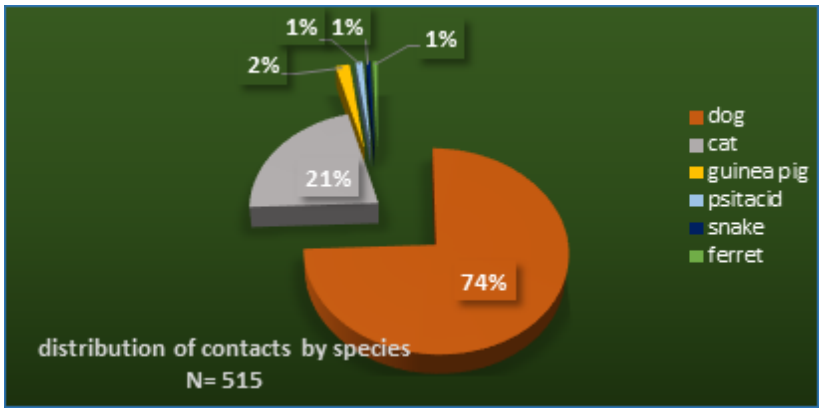

Chart 2. Total contacts with patients by species

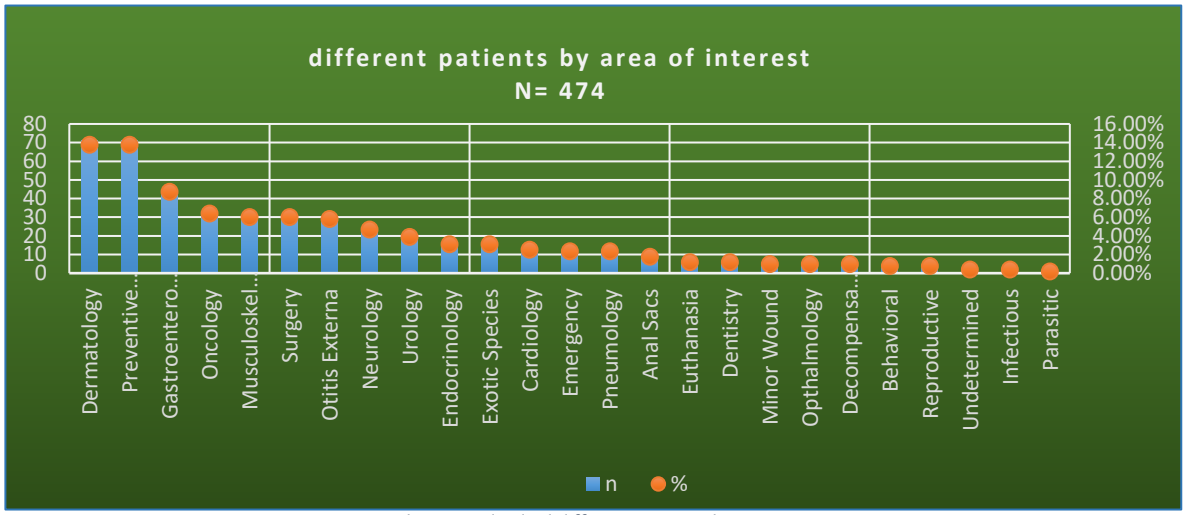

Chart 3. Individual different patients by Area

Withal, the author chose to use the absolute number of different individual patients examined as a statistical reference, exempting the area *Reevaluation.* Consequently, the total number of different cases appearing in the casuistics will be 474 (n=474). Any "Area" percentage will be related to this number even if there is a small deviation because of the three extra times two guinea pigs appear in the caseload (Chart 3). Information given will be proportionate to the weight of areas in percentage.

2.1. DERMATOLOGY

Skin is the foremost exposed organ for pet owners to see and touch. Therefore, is often the reason for which animals are brought for a visit at a veterinary office.

Table 3. Dermatology Cases

Diagnosis	n	%
Ectoparasitic infestation	10	14.08%
Canine atopic dermatitis	10	14.08%
Cyst	8	11.27%
Pyoderma	7	9.86%
Hot spot	6	8.45%
Fungal	5	7.04%
Paw pad Corns	5	7.04%
Abscess	4	5.63%
Pododermatitis	3	4.23%
Immune-mediated	2	2.82%
Blue dog dermatitis,	1 of each	1.41%
Miliary Dermatitis, Sebaceous adenoma,		each
Neoplastic Ulcer, Due claw cyst, Nail		
bed disease, Eosinophilic granuloma,		
Subcutaneous emphysema, Papilloma,		
Wound, Embedded nail		
Total	**71**	**100.00%**

In a lot of circumstances non- complicated cysts, lumps and papules are the main point of focus during a physical exam by indication of the caregivers. Meanwhile, some of the most frustrating conditions, for patients and owners, have cutaneous manifestations. Examples are immune-mediated, fungal, bacterial and viral diseases, as well as allergies, endocrinopathies and neoplasia. Hence, the skin can be considered as a sensor for Internal Medicine (IM) disorders [1]. Seventy-two (n=71) cases were classified as predominately dermatological which corresponds to 14.98% of the total (n=474) (Table 3).

2.1.1. Ectoparasitic infestations

Ectoparasitic dermatological conditions were encountered in patients, particularly during October 2016, due to higher incidence of flea infestation. Only ten of those cases though, where considered as exclusively parasitic. Six out of ten cases where diagnosed with flea infestation, four of which on cats and two on dogs. Fleas of cats, *Ctenocephalides felis felis,* are the common cause of Flea Allergy Dermatitis (FAD) for both cats and dogs, while with four stages (egg, larva, pupa and adult), life cycle can extent from 12 to 174 days depending on humidity, temperature and host availability [2]. Diagnosis was based on clinical evaluation and anamnesis. Patients, most of times, presented with pruritus, adult fleas and/or "*flea dirt*", which is a term used to describe flea feces on host. Approaching treatment of a non-FAD infestation, involved the use of oral nitenpyram (*Capstar®)* along with an insecticide shampoo Mycodex® (pyrethrin, piperonyl butoxide) and then prescription of *K9 Advantix II*® (imidacloprid, permethrin and pyriproxyfen) for dogs and *Revolution*® (selamectin) for cats, both for topical application. In one case, a cat, presented flea dirt, pruritus, mild miliary crusts and alopecia on the dorsal lumbosacral region, all associated with FAD [2]. In conjunction with products, mentioned above, for flea elimination, was prescribed oral prednisone, in an immunosuppressive protocol *bis in die (*BID), for five days and then a reduced dose for another five days. Finally, every client, that presented a pet affected by fleas, returned home with the instruction to wash all surfaces that the patient frequents, vacuum frequently carpets, under furniture and all dark corners as larvae are negatively phototactic and positively geotactic and pupae can disguise as regular dirt [2]. The use of Insect Growth Inhibitors (IGS) for environmental use was regularly indicated as necessary to control adult fleas in the environment [1].

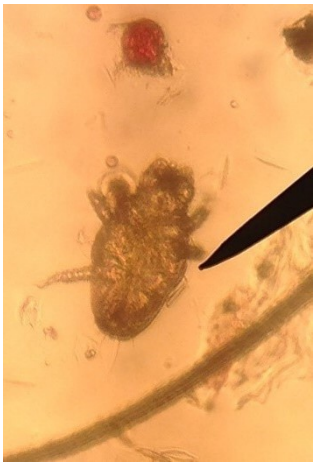

Figure 1. Cheyletiella spp. recovered by superficial skin scraping (by the author)

Three out of the four remaining diagnosed ectoparasitic infestations were due to the presence of *Demodex spp.,* all on dogs. One of the cases, an 8 months old mixed breed puppy, manifested a small alopecic patch on the head and multifocal alopecia at the periocular and perilabial areas and

mild erythema. Resulting Skin Scraping (SS) positive, on his second visit, instructions for continuing treatment with ivermectin were given to the owner. An adult, neutered male, American Pitbull terrier, presented generalized demodicosis in his third visit after starting treatment with ivermectin. Lesions were expanding all over the patient's body with multifocal and patchy alopecia along with erythema and hyperpigmentation. Per the attending clinician and owners, treatment was successful as the patient previously was pruritic, presented secondary bacterial infection, crusts, skin erosion and lichenification. Nevertheless, SS resulted still positive, although with less mites present, consequently therapy was continued, as it is recommended to do so, for a month after two consecutive negative scrapings [4]. Lastly, one more adult onset demodicosis case, resulted in the first negative SS since therapy implementation, thus ivermectin was continued until next scheduled visit.

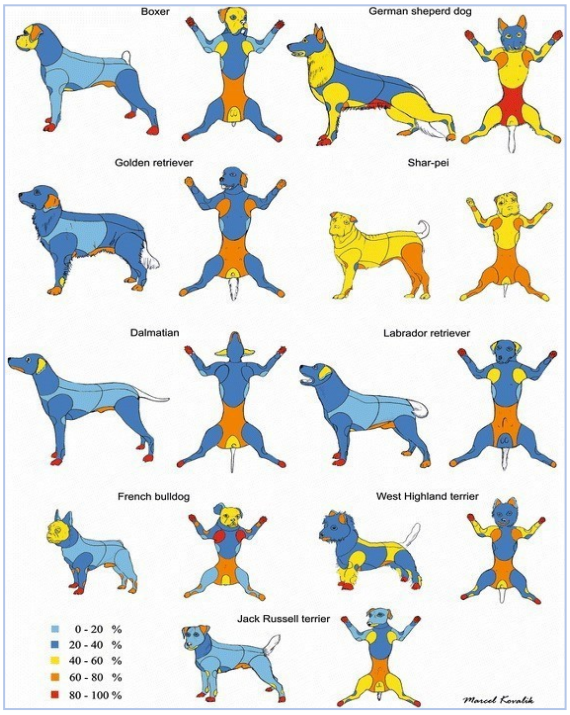

Figure 2. Silhouettes of atopic dogs of nine breeds. Each color corresponds to the percentage of affected animals (adapted from Veterinary Dermatology, International Committee for Allergic Diseases in Animals, 4-4-2017)

Finally, one puppy with cheyletiellosis presented pruritus and dorsal scaling (Figure 1). Diagnosis was achieved by both superficial SS and adhesive tape which is remarkable considering that failure to recover mites in clinical trials reached 15% in dogs and 58% in cats [5]. Treatment included three applications of topical selamectin (Revolution®) Humans can be transient hosts, but owners had no symptoms. Nevertheless, they were instructed to wash al fabrics in high temperature and use environmental acaricides afterwards.

2.1.2. Canine Atopic Dermatitis

Canine Atopic Dermatitis (AD) has been defined as a genetically predisposed inflammatory and pruritic allergic skin disease with characteristic clinical features. It is associated most commonly with IgE antibodies to environmental allergens[6]. Pathogenesis of AD combines intrinsic and extrinsic factors. Intrinsic factors include genetic and/or breed predisposition, the production of IgE, the role of many cells, numerous mediators of inflammation, and an alteration of stratum corneum intercellular lipids. Extrinsic factors include environmental seasonal allergens (grass, weed, tree pollens) and non-seasonal allergens (house dust mites, storage mites, molds, fabrics or insects) [7].Ten cases were classified as AD and in all instances, except one, the Favrot's criteria used to evaluate the probability of correct diagnosis were fulfilled [8]in[6]. Everyone presented an age of onset before three years and

initial “non-lesional” pruritus in various manifestations. Characteristically, head shaking, face rubbing or paw licking were the most difficult symptoms to classify as pruritic on behalf of the care givers. Also, all animals lived mostly indoors and had affected, in some extent, the front feet and/or the interior of the ear pinnae. Finally, lesions, when present, were mostly on the inguinal area, axillae, ventrum and distal extremities while in one German Shepherd alopecia was evident on the neck and a West Highland Terrier had his lumbar area affected (Figure 2). In four cases the breed was not documented and including the two mentioned previously the author witnessed a Beagle, a Cocker Spaniel, an American Pitbull terrier and a Dogo Argentino. This last patient was the only that did not meet all five of Favrot’s criteria because erythema and alopecia affected his pinnae margins, in contrast to all other pets. Although anamnesis evidenced a seasonal reappearance of symptoms the aggressiveness of the animal did not allow full physical examination that could aid on diagnosing. A symptomatic approach, with a difficult administration of methylprednisolone acetate injectable, on the lower lumbar area, was the only possible action. Ruling out ectoparasitic infestations by a SS, as well as FAD by the use of a flea comb, is crucial for determining whether AD is the diagnosis, so in the absence of, the author classified the Dogo Argentino case as AD on basis of anamnesis, clinical aspect and of course the attending clinician’s opinion [6]. *Staphylococcus pseudintermedius* infection and *Malassezia pachydermatis* overgrowth are common, usually secondary to AD through traumatic scratching due to intense pruritus. Nevertheless, *Malassezia* overgrowth must be ruled out when suspected as it can onset pruritus also [7]. As owners become familiar with their pet’s condition, recognition of early symptoms of pruritus gets easier, particularly when recurrence is seasonal, thus five patients presented only mild interdigital/inguinal erythema and head shaking. Exclusion of Cutaneous Adverse Food Reaction (CAFR) is mandatory for proceeding to a successful AD treatment [6]. None of the cases seen were in their first visit and CAFR has been previously discussed with all owners. At least in one, non-seasonal AD, case an exclusion diet has been implemented before reaching AD diagnosis. Allergen-specific intradermal testing and/or IgE serologies are helpful to identify hypersensitivity to environmental allergens in dogs with AD, but a positive intradermal skin or in vitro test does not necessarily imply that the dermatologic problem is due to atopic disease. There is currently no standardization in the performance of serum allergen-specific IgE assays for environmental allergens, and there is evidence that the results of IgE serological tests can vary substantially between laboratories [9]. Treatment for canine AD can be specific or symptomatic. Eviction of environmental allergens is one option when feasible although copious normally. Specific immunotherapy by hyposensitization or desensitization is another, when test results are clear. Only symptomatic therapy was applied in the author’s presence. Oral prednisone was used twice daily to induce remission of clinical signs of AD in three cases. Oral oclacitinib (Apoquel®, Zoetis®) was given to five patients twice daily for 14 days and then once daily thereafter. Oclacitinib inhibits the function of a variety of pruritogenic and proinflammatory cytokines, as well as cytokines involved in allergy

that are dependent on Janus kinases (JAK1 or JAK3) enzyme activity and is considered a novel approach for autoimmune disease and malignancy, so it is not surprising that this pathway has become an attractive target for pharmaceuticals [10]. According to a study, were pruritus was induced by injection of interleukin 31, a single oral dose of oclacitinib demonstrates a faster onset of action than oral prednisolone and produces a greater suppression of pruritus compared to prednisolone or injectable dexamethasone [11]. The West Highland Terrier was treated only with Neopredef® topical powder which contains isoflupredone acetate, neomycin sulfate and tetracaine HCl. In two cases, oral antibiotics were necessary due to established pyoderma. Cefpodoxime proxetil (Simplicef®) once a day (SID) and cephalexin BID were prescribed on a German Shepherd and on an undocumented breed patient respectively. January 2017, gave rise to advancements on therapy of AD with monoclonal antibodies which target interleukin 31. Zoetis®, achieved licensing of Cytopoint™ in the USA. The product has been used at "A&A" with satisfying response according to prescribing clinicians.

2.1.3. Cysts, Abscesses, Pyoderma, Pyotraumatic Dermatitis and Pododermatitis

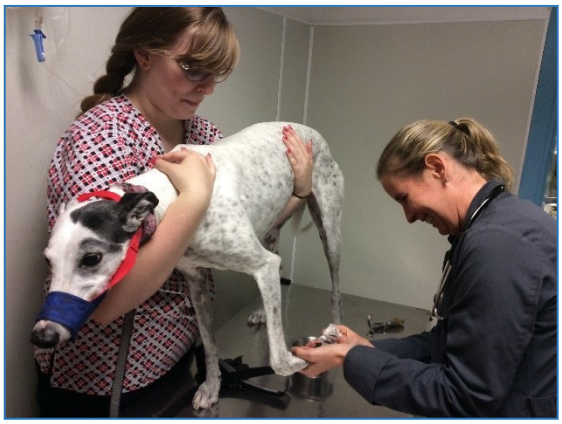

Figure 3. Corn removal from a Greyhound.

Cyst is considered an epithelium-lined cavity with fluid or semisolid matter often just beneath the epidermis [12]. Content can be grayish keratinous or serous, such as apocrine cysts, which appear fluctuant, bluish, and well circumscribed [13]. Clinical aspect of cystic formations varied relatively to size, anatomical location and importantly, pruritic or painful manifestation that conducted to trauma and infection eventually. Infection was the common cause that alarmed care givers to consult a doctor and it is clear from the treatments established for four out of eight cases (all in dogs). Four patients received an antibiotic agent which in two occasions was oral cefpodoxime (Simplicef®) and in two enrofloxacin (Baytril®) intramuscular (IM). Only in one case the singular use of a Nonsteroidal Anti-inflammatory Drug (NSAID) was elected as necessary for treatment (deracoxib/Deramax®). Prescription of Neopredef® powder for soothing local irritation and oral Temaril-P® (trimeprazine tartrate and prednisolone) along with cefpodoxime is commonly used at "A&A" for skin infections. Two dogs were not subjected to any treatment, still owners were instructed to observe the lesion daily and return, whether no regression occurred through a week, or if pruritus, pain or infection were evidenced. Lastly one dog presented an erupted cyst on the front dorsal metatarsus that was disinfected with chlorhexidine solution. A common site for the presence of cysts was the interdigital area with three patients affected. The tail, neck, thigh and torso were other sites.

The result of the collection of degenerated neutrophils and necrotic tissue cells when an infectious agent has penetrated the skin, is considered an abscess. Usually a peripheral membrane forms from necrotic tissue and fibrin [13]. Pain, along with localized edema is the common clinical aspect of an abscess. Three dogs presented abscesses two of which on the dorsal aspect of the thorax between the scapulae. The cause of the loss of dermal continuity was unknown in both cases and treatment was identical. Oral Clavamox® (amoxicillin trihydrate/clavulanate potassium) BID, for 10 days and deracoxib, BID, for seven days were prescribed. One Basset Hound showed signs of AD along with bilateral otitis externa. An abscess has formed in the proximity of the left ear under the long pinnae due to scratching. Oral oclacitinib and cefpodoxime proxetil were chosen as the basis of treatment. Drainage and disinfection was performed in all patients.

Table 4. Treatment of Pyotraumatic Dermatitis

Breed	*Lesion site*	*Treatment*
Shiba Inu	Dorso	Neopredef®, cefpodoxime
German Shepherd	Over tail	Neopredef®, cephalexin
Undetermined	Dorso	Temaril-P®, cefpodoxime, Hill's z/d diet™
Undetermined	Axillae unilateral	Neopredef®, Temaril-P, cephalexin

A dermatological manifestation of a bacterial infection can be named pyoderma and its pleomorphism can induce to erroneous diagnosis. Superficial (SP), deep and surface pyoderma are the existing classifications. SP is the common presentation in dogs. Impetigo and superficial folliculitis are the main symptoms of SP and may be diagnostically challenging because pustules rupture readily, giving rise to considerably less diagnostic crusted papules. Mostly dogs are affected with pyoderma, while in cats is rare and when present, usually the deep form is diagnosed [14]. Numerous patients were diagnosed with pyoderma along with other pathological processes but in this report, only a few were classified as predominantly pyoderma cases. Seven dogs were received, with the complain of a skin infection and the physical exam evidenced pyoderma. Two of those were returning patients that after Antibiotic Susceptibility Testing (AST), evidenced Methicillin-resistant *Staphylococcus pseudintermedius (MRSP) infection* while affected by superficial spreading pyoderma and superficial folliculitis. AST suggested the use of chloramphenicol which was the elected treatment "*per os"* (PO) for both MRSP infections. The zoonotic potential for MRSP in humans is substantially less than that for MRSA (*S. aureus*), because *S. pseudintermedius* has low pathogenicity for humans and opportunistic infection by MRSP is rare. Yet handling of chloramphenicol needs use of gloves due to possibility of aplastic anemia development and gastrointestinal (GI) side effects [14]. Owners were instructed to do so. Appearance of SP should always alarm for any underlying cause such as demodicosis, FAD or AD. One of the cases had recurrent SP and this time presented otitis externa also. Enrofloxacin and an antiseptic shampoo were prescribed besides Tri-Otic® (clotrimazole, gentamicin sulfate, betamethasone valerate) ear drops, as the care giver was reluctant in investigating for further diagnosis of the underlying cause.

Another kind of treatment pursued was cephalexin and oclacitinib in two SP cases. Unfortunately, treatment election was not documented in the remaining two cases.

Hot spots are considered surface pyodermas and are also denominated as pyotraumatic dermatitis. Superficial hot spots can be differentiated from pyotraumatic folliculitis only by histopathological means. Presence of a topical inflammation and exudative erosion was evidenced in all six patients witnessed. Empirically animals are treated for a skin infection as well, usually with good outcomes. In two visits treatment was not documented while in one pet CAFR or food intolerance was suspected and specific diet was prescribed. Generally, treatment focused on possible bacterial infection, soothing of pruritus and probable underlying cause (Table 4). Hair clipping was performed in all patients.

Pododermatitis was the only skin condition that, during the externship, affected mostly felines represented with two individuals, one young and one senior, presenting an acute and a chronic manifestation respectively. The first was covered with enrofloxacin and the later with orbifloxacin, yet both received prednisone. One dog was treated also with Baytril® and prednisone.

Paw pad corns were common in Greyhounds and were removed frequently but would also relapsed easily (Figure 3).

2.1.4. Dermatophytosis

Microsporum canis (Ringworm) in 90% of the cases is the cause of dermatophytosis in cats although in dogs combined infections with *M. gypseum* and *Trichophyton spp.* are common [15]. Studies of dermatophyte infections in Europe have indicated that there has been a shift from *Microsporum audouinii, Epidermophyton floccosum,* and *T. rubrum* to *M. canis* as the most common dermatophytes causing infections in humans[15]. The infective part of the organism is the arthrospore, formed from the segmentation and fragmentation of fungal hyphae. Definitive diagnosis of dermatophytosis is made by culture, although it is neither perfectly sensitive nor always specific for the diagnosis [15]. The Woods, ultraviolet light, lamp was also used to determine presence of fluorescent fungal material on hair shafts, although it is described as a non-sensitive test [15]. Results were positive three out of five times. Only in two out of five pets, treated for dermatophytosis there was a positive culture result for *M. canis* and these were the only dogs affected. The owner of one dog was diagnosed firstly by a dermatologist with Ringworm. She was an animal worker so this was an interesting case of a probable animal-human-animal zoonotic transmission. This German Shepherd puppy manifested pruritus, unilateral axillary alopecia and was treated by Malasseb® (miconazole nitrate, chlorhexidine gluconate) shampooing. Another canine

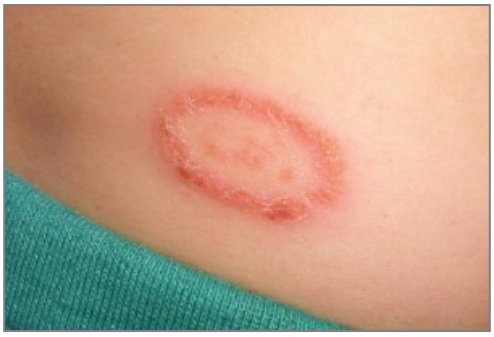

Figure 4. Ringworm lesion, very similar to the infant's at "A&A" (by http://www.moretohealthy.com/how-to-get-rid-of-ringworms/)

with established pyoderma over a dermatophytic infection was subscribed also with Malasseb® shampoo and antibiotics (cefpodoxime proxetil). One cat with pruritus was brought because the care giver's one year old infant was diagnosed with Ringworm (confirmed by culture) by her pediatrician. The author witnessed one characteristic lesion on the neck of the baby (Figure 4). Wood's lamp test was positive and oral terbinafine was the elected antifungal drug. Two more cats, previously diagnosed, showed signs of pruritus and alopecia and continued shampooing and miconazole PO.

2.1.5. Immune mediated and other diseases

Only two canines were found to have an immune mediated skin condition and they were both German Shepherds. Both cases had lesions on the nasal planum but with different presentations. One evidenced hypopigmentation and erythema while the other pustular erosions, crusts and hyperkeratosis. The first patient was diagnosed with discoid lupus erythematosus and the later with pemphigus foliaceus. Pemphigus foliaceus was diagnosed in 2014 with mild symptoms but current aspect obviously signaled a flare-up with painful lesions. Dexamethasone Sodium Phosphate IM, doxycycline and Vitamin E, PO were elected for treatment. The dog came back for checkup the following week with signs of recovery.

Between all other skin conditions with singular representation (Table 3), one case of a "Blue" dog dermatitis and one Greyhound with extended subcutaneous emphysema were the most clinically interesting. The French Bulldog in the case presented recurrent pyoderma subsequent to color dilution alopecia.

2.2. PREVENTIVE MEDICINE

One of the most important responsibilities in practicing veterinary medicine is to help patients with prevention of the occurrence of disease. Preventive Medicine (PM) not only provides to the pet population a healthy future but also protects wildlife and of course all humans from zoonotic epidemics respectively.

Table 5. Preventive medicine cases

Vaccine	51	**71.83%**
Senior Profile	4	**5.63%**
Vaccine & Bloodwork	4	**5.63%**
Vaccine & Heartworm	4	**5.63%**
Vaccine & Senior Profile	3	**4.23%**
Checkup	2	**2.82%**
Thyroid profile	1	**1.41%**
Heartworm	1	**1.41%**
Bloodwork & Heartworm	1	**1.41%**
total	**71**	**100.00%**

During a 16-week period, a total of 474 (n= 474) cases where observed, and within, 71 cases categorized by the author as predominantly in PM area, corresponding to 14.98% of the total (Table 5). This data does not indicate the totality of vaccinations or other procedures (e.g. heartworm test) related to preventive medicine that the author witnessed. In many instances, PM procedures took place but did not correspond to exclusively or predominantly PM visits so there where not considered for this section.

In the authors opinion, frequently is neglected the importance of the general practitioner's advice in any given regular visit as a detrimental tool for good PM practice. Exemplifying we can mention the very first visit of a puppy or kitten to the veterinarian (DVM). The usefulness of directions to the care givers on behavior, nutrition, hygiene, general husbandry, contact with other pets and people is immense. Other examples to name can be apparently healthy geriatric patients that come to the doctor's office for a checkup or exotic pets, such as reptiles, with inexperienced owners that need meticulous guidance through husbandry and finally pets from breeds that are genetically predisposed for alterations in physiological functions or with an anatomical conformation that prompts to specific syndromes [16-18]. All the cases mentioned above demand for a well informed and theoretically solid DVM that can assist with the decision-making process of the care givers through the life of their best animal friends and even before the acquisition or adoption of a knew pet. At "A&A", that aspect of PM, is of great importance as it was put in practice in many occasions in the presence of the author. Numerous times, during regular visits, many unwanted situations where prevented, with simple husbandry recommendations to the owners, such as to increase the cat litter boxes present in a multi-cat home, to reduce cross-contamination and inappropriate elimination or even just to wash hands before and after contacting with reptiles for prevention of salmonellosis [19-22].

2.2.1 Canine vaccination protocol

Currently in New York State there is only one disease which is mandatory by law that all dogs should be immunized for and that is rabies. Rabies is a fatal viral zoonosis and serious public health problem as all mammals are believed to be susceptible to the disease. The disease is a progressive encephalitis, presenting a furious or a paralytic form, caused by Lyssavirus genotype 1*[23].* Every dog, cat and domesticated ferret must be actively immunized with an initial vaccination no later than four months of age (MOA) and get a second vaccination within one year of the first. Furthermore, terms of subsequent vaccinations and Duration of Immunity (DOI) must follow United States Department of Agriculture (USDA) licenses of vaccines used. The above may not apply to animals:

(a) transported through the state and remain 15 days or less; (b) confined to the premises of an incorporated society devoted to the care of lost, stray or homeless animals; (c) for which vaccination against rabies would adversely affect the animal's health, as determined by a licensed veterinarian; or (d) confined for the purposes of research to the premises of a college or other educational or research institution. Veterinarians providing treatment are obligated to verify that every dog, cat or domesticated ferret is actively immunized or that is exempt under the four cases referred above. Also, it is expected that a veterinarian shall provide the owner or any public health official with a certificate of immunization. In like manner, a certified statement that an animal is exempt of vaccination due to health issues should be released if that is the case. Owners of animals that are not vaccinated against rabies can face a fine not to exceed two hundred dollars for each offense [24].

These directives where followed also in “A&A” with the first rabies vaccination administered to puppies at around three to four MOA and a second vaccination (booster) at one year of age (YOA). Adult animals would get a booster rabies vaccine either every year or every three depending on each case’s epidemiologic risk assessment. As known, maternally derived antibodies (MDA), whenever are present, can cover (“mask”) antigenic epitopes and so reduce the immunization effect. Reasonably it is recommended that initial vaccination should be given after 12 weeks of age (WOA) regarding noninfectious vaccines, such as what is usually used for rabies and thus there is a four-week time frame to vaccinate a puppy in lawful manner. Booster vaccinations in adults (older than 1 year) are shown to be necessary only after a three-year period because of a highly immunogenic antigen (glycoprotein G) and adjuvants that prolong DOI to three years when using a three-year rabies vaccine [25, 26].

Core and noncore vaccines are defined by the canine vaccination Task Force of the American Animal Hospital Association (AAHA) through guidelines that although not intended to dictate an exclusive protocol, do meet accepted standards of professional practice [25]. While core vaccines are recommended for administration to all dogs and all cats, it is the clinician who must ultimately make the decision as to which vaccines will actually be administered and when [27].

Vaccinations that are recommended as core include a Modified Live Vaccine (MLV) or *recombinant* Canine Distemper Virus (*r*CDV) immunizing both against Canine Distemper Virus (CDV), Canine Parvovirus type-2 (CPV-2) as MLV, Canine Adenovirus type-2 (CAV-2) as MLV and Rabies one-year or three-years as noninfectious. In the case of CDV using either MLV or *r*CDV puppies should be vaccinated every three to four weeks between ages of six and sixteen WOA with the last booster administered between 14 and 16 WOA, with regard to minimize the risk of MDA interference with vaccination. Dogs completing the initial vaccination series by 16 WOA should receive a single booster no later than one year after completion of the initial series and ultimately be revaccinated every three years thereafter. When the initial vaccination is administered after 16 WOA, then, one dose, is considered protective and revaccination is recommended every three years. The same recommendations are given for the MLV of CPV-2 and CAV-2. Vaccination with a CPV-2 (MLV) is expected to give immunity from disease by any field variant recognized (CPV-2a, -2b, 2c). It is essential to vaccinated for CPV enteritis because CPV is contagious and is very stable in the environment. This emphasizes that more than 90% to 95% of dogs in a given population may have to be successfully immunized to prevent spread of infection. Exposure to the virus is probable early in life, and as soon as pups are susceptible, they can become infected. Meanwhile vaccination against Infectious Canine Hepatitis (ICH) has dramatically reduced occurrence of this once widespread and potentially fatal illness [26]. Administering a CAV-2 vaccine (MLV) induces protection, not only, against canine hepatitis virus (CAV-1), but also against CAV-2 which is associated with Canine Infectious Respiratory Disease (CIRD) also known as kennel cough or tracheobronchitis [25]. Of the various

viruses known to be associated with CIRD, Canine Parainfluenza Virus (CPIV) is considered a principal respiratory pathogen. The most important bacterial pathogen in CIRD is known to be *Bordetella bronchiseptica* (Bb).

The choice to administer noncore vaccinations is related to a specific regional and individual epidemiologic risk assessment. In New York State, diseases that are important to include in a prevention program and a vaccination protocol are, CIRD or kennel cough, Canine Influenza Virus (CIV) infection, Leptospirosis and Lyme Borreliosis. At "A&A" along with the core vaccinations, which are administered as mentioned in the previous paragraph, many dogs, if not all, are vaccinated against Leptospirosis, kennel cough and CIV. Although vaccination for Lyme disease is guarded mainly for outdoor animals, New York is considered a high incidence state so vaccination against Borreliosis is very common [28]. The canine vaccination protocol at "A&A" usually includes: a) administration of MLV for CDV, CAV-2, CPIV and CPV-2 (Duramune® Max 5) in three initial doses and yearly from there on, b) vaccination against Bb by intranasal administration of live nonvirulent bacteria with a singular initial dose at around four WOA and yearly thereafter, c) protection from Leptospirosis with a four serovar bacterial extract (LCAN-LGRIP-LICT-LPOM) in two initial doses (three weeks apart) after 12 WOA and yearly consequently, d) immunization from CIV (H3N2-H3N8) with two initial (three weeks apart) doses, not earlier than six WOA and annually thenceforth, e) protection from rabies.

2.2.2. Feline vaccination protocol

The patient's environment is crucial for determining a vaccination protocol for cats, as it occurs also with the canine population. Cats though are regularly found to live in multi-pet environments, as well to roam free outdoors. These environments can be shelters, breeding catteries or boarding facilities and regular habitations. Feline specialists around the globe distinguish these groups when designing vaccination protocols. In general veterinary practice though, clinicians, encounter mostly, with household cats that either live indoors, outdoors or both [29].

All household pet cats should be vaccinated against Feline Parvovirus (FPV), herpesvirus-1 (FHV-1), feline calicivirus (FCV), and rabies. The American Association of Feline Practitioners (AAFP) Feline Vaccine Advisory Panel recommends beginning as early as six WOA and every three to four weeks until 16 WOA. Implementing a three-dose protocol, like that recommended in puppies, is a reasonable inoculation schedule wherein one combination vaccine is administered at 2, 3, and 4 months of age [27, 29]. Feline panleukopenia is a highly infectious disease caused by FPV often proving high mortality. Fecal-oral is the transmission route and the virus is very resistant in the environment. Lethargy, anorexia, vomiting, diarrhea, fever but mainly, profound leucopenia are signs included. Through vertical transmission, abortion and mummified litters can occur although queens may not show signs. Kittens can be born with neurological symptoms. Vaccination is considered very effective [30]. Feline infectious respiratory disease is triggered by FHV-1 and FCV, although Bb and other

infectious agents are suggested as contributive. Signs of FHV-1 infection include depression, sneezing, anorexia, and pyrexia, followed by serous ocular and nasal discharges. Conjunctivitis and, in some cases, ulcerative keratitis is evidenced [31]. Oral ulceration is the most characteristic feature of FCV infection and may be the only clinical sign present. Ulceration is usually on the tongue but can occur elsewhere in the mouth, on the lips, and on the nose [31]. Feline leukemia virus (FeLV), when triggering a progressive infection, is a serious threat to cats as it can generate lymphoma, leukemia, thrombocytopenia, anemia, lymphopenia and local lymphoid tumors amongst other pathologic processes[32]. It is transmitted vertically and horizontally, through contact. As the virus is very unstable in the environment, indoors only cats are not in risk. Multi-cat settings with access outdoors are high risk groups and particularly kittens, under 16 WOA, when within[32]. Diagnosis is intriguing because free blood antigen can be detected only in the progressive form during productive viremia while viral deoxyribonucleic acid (DNA) is detectable in regressive infections also. In abortive and focal antigen is not discoverable by any of those methods. Regular prevention through vaccination and isolation of infected cats decreased prevalence lately[32]. Naïve cats can be protected either by isolation or by vaccination. At “A&A” cats roaming outdoors, from multi-cat households or living with FeLV positive individual were tested and when naïve vaccinated with Leukocell®2 (inactivated virus). The core protocol for all cats included also a killed virus, three-way administration against FHV-1, FCV and FPV. Primo-vaccination was scheduled around six WOA with a booster every three weeks until 16 WOA and annually thereafter. In the author’s presence, only one vaccination against Feline Immunodeficiency Virus (FIV) occurred.

2.2.3. Heartworm Infection testing and prevention

Dirofilaria immitis is transmitted by over 60 species of mosquitoes, although important mosquito vectors probably number fewer than 12 [33]. It is also known as Heartworm (HW), primarily affecting animals from the family of *Canidae***.** When Heartworm Infection (HWI) is severe or prolonged, it may result in the pathologic process called Heartworm Disease (HWD) which vary from asymptomatic with radiographic lesions only, to severe, life-threatening, chronic pulmonary artery, lung, and cardiac disease. In chronic HWI, glomerulonephritis, anemia, and thrombocytopenia may also be recognized. Severe dirofilariasis may produce acute multisystemic presentations, such as caval syndrome and disseminated intravascular coagulation (DIC) [34]. Dirofilariasis is a common parasitic disease, diagnosed around the globe. It is considered endemic in many United States (US) counties where expansion of urban areas creates “heat islands” which provide excellent microenvironment for the development of larvae in mosquito vectors during cold months [35] [36]. Several mosquito vectors live and breed for two to three months and even if in cold season, larvae evolution may cease, when vectors survive winter, the development resumes with subsequent warming [33]. Vector mosquitos collected in endemic areas showed presence of heartworm infection that was ranging from 2% to

19%, until up to 74% in kennel facilities that housed heartworm positive dogs. Due to all the reasons mentioned, it is imperative to protect pets from infection with year-round prevention, accurate testing and diagnosis [33].

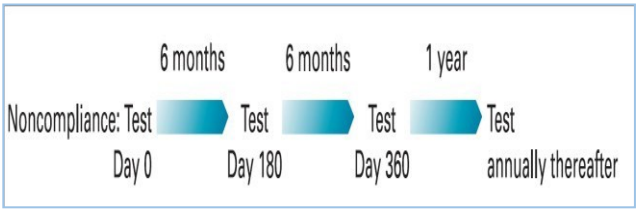

Figure 5. Noncompliance testing protocol (adapted from the American Heartworm Society)

Prevention from HWI is highly effective by using marketed macrocyclic lactones (ivermectin, milbemycin oxime, moxidectin, and selamectin) that demonstrate microfilaricidal, larvicidal and frequently adulticidal response [33, 34]. Satisfying reduction on prevalence and limited lack of efficacy are related with consistent oral (ivermectin and milbemycin oxime) or topical (moxidectin and selamectin), monthly administrations, as well as with, a slow release, parenteral moxidectin, given every six months [33]. Compliance with prevention protocols is of extreme importance. However, ectoparasitic chemoprophylaxis, should be also implemented year-round, to diminish transmission, especially in endemic areas. Vectors are very active during dusk and dawn, thus care givers should be instructed to keep their pets indoors during high risk hours [33, 34].

The American Heartworm Society recommends annual testing for achieving and maintaining prophylaxis. Whether screening asymptomatic dogs or seeking verification of a suspected heartworm infection, antigen testing is the most sensitive diagnostic method [33, 34]. It is now recommended, however, that microfilaria testing should be done along with the antigen testing to all dogs, especially, if there is a high degree of suspicion or if prevention history is unknown [33, 34]. Enzyme-linked immunosorbent assay (ELISA) and immunochromatographic tests are used for detecting circulating heartworm antigen. The earliest that heartworm antigen and microfilariae can be detected is about 5 and 6 months post infection, respectively [33, 34]. A pre-detection period should be added to the approximate date on which infection may have occurred, so a reasonable interval is 7 months. Thus, there is no justification for testing a dog for antigen and microfilariae prior to 7 months of age [33, 34]. The modified Knott test is the preferred method for detecting microfilaremia, observing morphology and measuring body dimensions to differentiate *D immitis* from non-pathogenic filarial species, such as *Acanthocheilonema reconditum [33].* In cases of noncompliance a six-month testing schedule should be implemented (Figure 5). Radiographic imaging offers an important tool to diagnosis and staging of the severity of the disease, if present, by nearly pathognomonic signs of enlarged and tortuous, inter- and intra-lobar pulmonary arteries in the diaphragmatic lobes. Additionally, echocardiography can provide definitive evidence of heartworm infection and functional consequences of the disease [33].

Domestic cats can be infected with *D immitis* and present HWD even as an atypical host. Although prevalence (9.5 % of HW exposure in Long Island) is lower in comparison to dogs, this species, often exhibits a more severe pulmonary arterial response to adult worms [34]. There is a distinction between

adult (mature) HWI and aborted infections, where adults are not present. Uniquely clinical signs can develop, even after resistance, delivering Heartworm Associated Respiratory Disease (HARD) which is related with microfilaria precardiac movement [37]. HWI in cats is more elusive and a combination of antigen, antibody and radiographic and echocardiographic testing may be used for diagnosis or before establishing a prevention protocol [34, 37]. Since microfilaremia in cats is uncommon, transient, and below concentration levels that might trigger an adverse reaction to microfilaricidal chemoprophylactic drugs, pretesting for microfilariae is unnecessary [37]. Due to frequent false-negative antigen tests, an antigen test should be described as "no antigen detected" when not positive [37]. A positive antigen test is evidence of a mature HWI while a "no antigen detected", paired with a positive antibody result, informs that a cat was exposed but that a HWI is unlikely. In any case, preventives, can be started. Heartworm chemoprophylaxis can be achieved in cats with monthly doses of either ivermectin or milbemycin oxime orally, or topical moxidectin or selamectin. Preventives should be started in kittens at 8 weeks of age and given to all cats in heartworm endemic areas, at least, during the heartworm transmission season [34, 37].

During the externship at "A&A" blood was collected for heartworm testing many times during scheduled annual visits or to exclude dirofilariasis as a differential diagnosis. None of the pets tested resulted as positive in the authors presence although positive tests occurred in the hospital at the same time. Heartworm antigen testing for dogs as well as feline antibody was performed by Antech Diagnostics®. Canine heartworm antigen test was executed alone or in combination with antigen detecting tests for Lyme disease, anaplasmosis, and ehrlichiosis in the form of Accuplex4®

2.2.4. Hematologic analysis and senior profile

There is no better instrument for early diagnosis of occult pathologic processes than a Complete Blood Count (CBC) combined with a Serum Biochemistry Profile (SBP) that in clinical terminology are together referred as "*bloodwork" (Table 3)*. As care givers gradually set up strong sentimental bonds with their pets, more often are seen owners with healthy animals soliciting health testing as it is common for humans. Senior dogs and cats are more prone to chronic diseases, therefore are tested with the incentive of the DVM. At "A&A", frequently, was suggested a minimum database screening ("*senior profile*"), to senior animal owners, that provisioned CBC, SBP and Urinalysis (U/A) occasionally combined with a thyroid screening and/or Accuplex4®. The age of the patient indicative to suggest a senior profile was relative to the species, breed and size.

2.2.5. Endoparasite prevention

Even if not documented, prevention for endoparasites was part of the clinician's daily routine substantially with puppies, kittens and newly adopted pets in general. Deworming is of critical importance for the pet's and care giver's health. At "A&A", the first deworming was usually scheduled at three weeks of age and the second at three MOA with a subsequent fecal screening.

2.3 GASTROENTEROLOGY, HEPATOBILIARY AND EXOCRINE PANCEATIC DISORDERS

Third in caseload, ranked gastroenterology (GE) in which the author included hepatobiliary and exocrine pancreatic disorders. The reader is reminded that classification of cases by area was achieved mainly by considering treatment's target or by the main complain for which the patient was admitted in the hospital or seen by a doctor. Altogether 45 pets classified as gastrointestinal (GI) patients, number that corresponds to 9.49% of the total 474 different cases witnessed (Table 6).

2.3.1. Nonspecific Gastroenteritis

Nonspecific gastroenteritis (NSGE) was the most frequent gastrointestinal disorder standing for 16 cases. Gastroenteritis is defined as the inflammation of the lining of the stomach and the diarrhea and vomiting present or suspected in all, examined by the author, patients suffering from this disorder. Diarrhea afflicted 13 pets, in four cases combined with vomiting, which overall affected seven patients. All nine animals with just diarrhea, revealed some degree of nausea in the exam room, or from anamnesis, while two (out of three) vomiting only patients revealed NSGE on radiographs (X-ray) and the owner of the third was not sure about the presence of diarrhea or not. The canine patients were 13 while only three cats were treated for NSGE.

Table 6. Gastroenterology Caseload

Diagnosis	*n*	*%*
Nonspecific Gastroenteritis	16	**35.56%**
Foreign Body	4	**8.89%**
Inflammatory Bowel Disease or Lymphoma	4	**8.89%**
Gastritis	4	**8.89%**
Enteritis	3	**6.67%**
Pancreatitis	2	**4.44%**
Undetermined	2	**4.44%**
IBD, Gallbladder Mucocele, Post-gastrotomy recheck, Giardiasis, Neoplasm/Foreign Body, Stomatitis, Constipation, Hepatic disorder, Flatulence, Antibiotic Responsive Diarrhea	1 of each	**2.22% each**
Total	**45**	**100.00%**

Excess of fecal water that may result from decreased intestinal absorption and/or increased intestinal secretion is the definition of diarrhea [39]. In small bowel diarrhea increased fecal volume is common and weight loss or vomiting may be present while flatulence and steatorrhea could evidence malassimilation. Large bowel diarrhea shows marked increase in defecation frequency along with urgency and tenesmus while hematochezia and mucus are often present [40]. Small intestinal disease causes diarrhea only if the material exiting the ileum exceeds the absorptive capacity of the colon or causes colonic secretion of water, thus, diarrhea means there is intestinal disease [41]. All pets affected by diarrhea presented with a mild acute form. Although many nonspecific pathological processes such as atypical hypoadrenocorticism, can also cause acute diarrhea, commonly diet, parasites and infectious diseases are responsible.

Vomiting is a defensive mechanism that overlooks to the expulsion of toxins or noxious substances from the gastrointestinal tract. Vomiting can be divided in three stages: nausea, retching and

expulsion of gastric contents. Nausea precedes vomiting and may include depression, shivering, hiding yawning and lip licking. Also, increased salivation and swallowing mark the lubrification of esophagus while retching is the forceful contraction of the abdominal muscles and diaphragm producing negative intrathoracic and positive intrabdominal pressure [42].

None of the patients had either a vomiting or diarrheic episode in the exam room. The major task of the clinician was to decipher the nature of symptoms from anamnesis. The author considers that communication of fecal consistency and defecation frequency was effortless on behalf of the owners. On the contrary evidencing nausea and distinguishing retching from gagging and coughing seemed challenging. Diagnostic procedure commenced with detailed anamnesis and physical exam, which included rectal palpation on patients with diarrhea, continuing with a radiographic approach when needed. Care givers signaled hemorrhagic diarrhea in three canines, which was confirmed during rectal palpation. Unfortunately, fecal consistency, existence of tenesmus and frequency of defecation were not documented in all cases, as follows, a discussion on the intestinal origin of disease is not possible. Dehydration, habitual finding in GI patients, involved seven dogs in a mild form. Minimum database screening (CBC, SBP, UR) was requested only for three dogs and resulted unremarkable. One dog and a cat presenting acute repeated vomiting and dysphoria were screened with abdominal X-ray revealing both thickening of the gastric and enteric mucosae, sign of possible gastroenteritis. Coprological analysis, which is part of the recommended diagnostic pathway, was performed in two young adult dogs resulting normal. Small breeds' prevalence was higher with Yorkshire Terrier being the most common (four patients).

Symptomatic treatment approach was elected for 14 patients while one dog and a cat were sent home with indication to return in case of recurrent diarrhea. Seven dehydrated patients received fluid therapy, intravenous (IV) in three cases and subcutaneous (SC) in four. Solely those three pets receiving IV fluids were hospitalized for 24 hours. Famotidine and maropitant were prescribed six times each, combined in four out of seven vomiting patients. The presence of vomit or suspicion of nausea drove the choice of both drugs. Furthermore, maropitant was used once with prednisone and once with fluid therapy while famotidine/metronidazole and famotidine/fluid therapy were other two types of treatment. Metronidazole, which covers protozoal and anaerobic bacterial infections, was prescribed six times while ampicillin just once. Probiotic supplements were indicated to a dog that had six vomiting episodes along with Hill's I/D ® low fat diet. Hill's I/D ® for dietary indiscretion was used once in combination with metronidazole. A 24 to 48-hour food withdrawal was recommended, only, to very nauseous pets with reintroduction of small and frequent amounts of easily digestible food after that. Water consumption was advised to be also in small and frequent amounts. Cottage cheese or boiled chicken with potato were suggested. When the only symptom was diarrhea the same diet was given in small amounts.

2.3.2. Gastritis and Enteritis

Hallmark of distinction between NSGE and gastritis or enteritis was the appearance of diarrhea and vomiting/nausea respectively. Four dogs presented with vomiting following food ingestion and no signs of diarrhea whatsoever, a clinical picture suggestive of gastritis. Three of the patients received antacid (famotidine) and antiemetic therapy. Maropitant was elected in two cases and metoclopramide in one. The last dog was prescribed with metoclopramide and probiotic supplements.

Enteritis was considered in three diarrheic dogs that transmitted no indication of nausea or vomit. All cases were prescribed with metronidazole combined once with probiotics, once with deracoxib and once with the suggestion of Hill's I/D® diet. Dietary indications for gastritis and enteritis were the same as outlined for NSGE.

2.3.3. Foreign Body Ingestion

Comparing alimentary habits and behavior in general of dogs and cats, could clarify the reason for only canine patients appearing on this clinical section of GE. Except when kittens, felines in general are less prone into ingestion of non-comestible objects. Ingestion of bones brought a dog to X-ray screening that evidenced fragmented small pieces in the stomach and intestine that after hospitalization and IV fluids were defecated. The same outcome had an already hospitalized Greyhound that had ingested parts of his protective boot. A Pitbull with a history of rug ingestion and vomit had an unremarkable X-ray and was prescribed with maropitant and famotidine. The same dog returned the next day with seizures and it was referred to a specialty hospital where it could receive an overnight intensive care. Finally, a patient with acute vomiting was strongly suspected to have a linear foreign body due to plication and small bubbles of gas in the intestinal lumen [43]. Nevertheless, the care giver rejected any further investigation and possibility of surgery insisting that his pet had not ingested anything. Treatment was not documented in this last case.

2.3.4. Inflammatory Bowel Disease

Inflammatory Bowel Disease (IBD) is a disorder that, even for major authors, is trivial to define and categorize although proof of mucosal inflammation and elimination of other causes for GI symptoms are commonly important. [44, 45] An inappropriate immune response to bacterial and alimentary antigens is speculated as the driving pathologic mechanism. Diagnosing through histopathology, which is considered optimal amongst other diagnostic means, is viewed as inconsistent between pathologists since histologic lesions can vary in a degree that the outcome may not justify the risk and cost of a biopsy especially when the care giver's budget is restricted. Lymphocytic-Plasmacytic Enteritis (LPE) is the most common form while eosinophilic gastroenteritis can be part of hypereosinophilic syndrome in some cats and can represent true IBD occasionally. Moderate or severe LPE can be difficult to differentiate from lymphocytic lymphoma. Additionally, symptomatic treatment with prednisolone has effect on both disorders. All pets mentioned here were felines with chronic GI clinical signs and neither of the five involved was biopsied by laparotomy, as it is suggested

for cats, nor by any form of endoscopy. As follows, after elimination of alimentary, infectious and parasitic causes, distinction between lymphoma and IBD in four cases was not obvious. Only one pet was a patient with a confirmed biopsy result of IBD (Table 4). Three cats were anorexic with varying degrees of weight loss while another was vomiting and the fifth had diarrhea. Ultrasonography (Ultra-S) showed segmental thickening (small bowel) in one case and generalized inflammation in another. One cat showed on X-ray small intestine inflammation and gas. Treatment established with budesonide was not successful on the diarrheic cat and was reverted to prednisone PO. The same treatment was established for other two cats. The only that was properly diagnosed with IBD, presented leukocytosis, ictericia, received fluids IV and was prescribed with Denosyl® (S-Adenosylmethionine), metronidazole along with prednisone. Treatment was not documented in one case.

2.3.5. Hepatobiliary and Pancreatic Disorders

Hepatic evaluation through SBP accounted for many cases during the authors externship. Nevertheless, just one male, 16 years old dog, of undetermined breed, was found to be suffering from what probably was microvascular dysplasia or acquired extrahepatic portosystemic shunts [46]. High bilirubin and liver enzymes was the primary evidence. This patient returned for a physical and bloodwork while previously was subjected to ultrasonography. The clinician prescribed Denosyl®.

Gallbladder mucocele appeared to be the cause of lethargy of a senior canine patient. First suspicion came up on X-ray and was confirmed later by Ultra-S. SBP returned high liver enzymes values, elevated bilirubin while CBC evidenced leukocytosis and monocytosis. Medical treatment with Ursodiol® and Denosyl® was the primary approach.

Canine pancreatitis has various clinical presentations ranging from subclinical to severe disease. However, both Pitbull Terriers diagnosed with pancreatitis had history of vomiting, lethargy, abdominal discomfort and mild dehydration. The two were young with nine MOA and two years. The older dog had a history of 15 days of vomiting while the puppy had only vomited three times. Differential diagnosis after physical examination was oscillating between an infectious cause, ingestion of foreign body and pancreatitis. Clear signs of pain by the characteristic posture of staring at the abdomen and on palpation were concerning and the owners were recommended to proceed with a full diagnostic plan which included CBC, SBP and X-ray. Only the care giver of the puppy agreed to all but X-rays were unremarkable and the dog was given only maropitant and SC fluids. Increased Amylase activity and mostly canine Precision Pancreatic Sensitive Lipase (PSL)™ (ANTECH®) confirmed initial suspicion of pancreatitis and as anamnesis showed no evidence of a drug, trauma or dietary indiscretion relation, a supportive approach with an ultra-low fat diet was adjunct to maropitant since the owner would not hospitalize [47, 48]. After X-ray evidenced just gastric wall thickening and as the client reconsidered in proceeding with blood analysis the same treatment

was elected for the second patient suspected with pancreatitis. However, by an internal error, blood samples were sent and analysis came back with increased PSL.

2.4. ONCOLOGY

Comparative studies and exchange of knowledge from advancements regarding human neoplasia, have helped with diagnosis and treatment of pets.

Table 7. Oncology Casuistics

Neoplasm	*n*	*%*	*Diagnosis*
Osteosarcoma	6	**18.18%**	No#
Hemangiosarcoma	3	**9.09%**	Yes
Lipoma	3	**9.09%**	Yes
Undetermined	3	**9.09%**	No
Transitional Cell carcinoma	2	**6.06%**	No
Mammary Gland Mass	2	**6.06%**	No
Squamous Cell Carcinoma	2	**6.06%**	Yes
Lymphoma	2	**6.06%**	Yes
Sarcoma	1	**3.03%**	Yes
Pancreatic Carcinoma	1	**3.03%**	No*
Epulis	1	**3.03%**	No**
Thoracic Mass	1	**3.03%**	No
Thymoma	1	**3.03%**	No
Endometrial Hyperplastic Fibroadenoma & Ovarian Cystadenoma	1	**3.03%**	Yes
Thyroid Carcinoma	1	**3.03%**	Yes
Anal Sac Adenocarcinoma	1	**3.03%**	No
Actinic Keratosis	1	**3.03%**	No
Oral Squamous Cell Carcinoma	1	**3.03%**	No
Total	**33**	**100.00%**	

Yes= Histopathologic confirmation (diagnosed by ultrasound, ** clinical diagnosis, # Only one case with cytologic diagnosis of a metastatic subcutaneous nodule)*

Vice versa, studies in pets have given assistance in evaluating epidemiologic risk and evidencing common cancerous pathophysiologic processes, in humans that coexist in the same environment. The genetic basis of cancer is now beyond question. It is estimated that at least five to seven mutational events are required for malignant transformation, and genomic instability seems to be necessary to establish a self-renewing population of cells that expand to cause clinical disease [49]. Hanahan and Weinberg in 2000, synthesized six essential, acquired characteristics necessary for cellular transformation known as “Hallmarks of cancer”. These are: (a) self-sufficiency in growth signals, (b) insensitivity to antigrowth signals, (c) the ability to evade apoptosis, (d) limitless replicative potential, (e) sustained angiogenesis, and (f) the capacity to invade tissues and metastasize. In 2011 the same authors added other four, equally important “Hallmarks”:

(a) genome instability, (b) tumor promoting inflammation, (c) deregulation of cellular energetics and

(d) avoidance of immune destruction [50, 51]. The importance of evidencing these characteristics is clear when considering the latest cancer therapies that they have helped shape.

Oncologic casuistics in this section are organized by the somatic system that the neoplastic processes have affected. Furthermore, the author will mention which cases reached a definitive histopathologic diagnosis (Table 7). In total 33 patients were classified as predominantly oncologic. Six of those cases where skeletal tumors and they will be discussed in the second chapter of this report along with a detailed bibliographic review. Cases without a definitive diagnosis were classified based on clinical observation and indicated in accordance to the most common neoplastic processes of the affected region. In such manner, the cases where histopathology was not performed were classified clinically and radiologically (or ecographically).

2.4.1. Cancer of the Skin and Subcutaneous Tissues

Asymptomatic lipomas were the most common subcutaneous masses palpated during physical exams by the author. Although the frequency of the finding was not documented, for it is of benign nature, it was surprisingly high at dogs in “A&A” in comparison to former clinical experience that the author gained in University of Évora (UE) [52]. Lipomas usually were spherical, the size of a coin or bigger, found in various anatomic regions and of fatty consistency. Loose from the surrounding and underlying tissue, these benign tumors did not cause pruritus or pain. Usually patients presented more than one. All three cases mentioned here were dogs with growths that impeded movement (or could impede in the future) in some extent. The first patient was a Labrador Retriever with an oval shaped mass on the left flank with the size of a small football. Physical exam and X-ray were non- remarkable while following a lumpectomy, biopsy resulted lipoma. Two months later the patient returned with a smaller growth five centimeters from the first incision. That, was also removed, resulting in a liposarcoma this time. Clinicians would advise the care givers to remove big, growing lipomas, situated near joints (stifle, axillae, elbow). The next two cases went through lumpectomies for removing a mass in the medial axillae and medial elbow that histopathology later reported as lipomas. No infiltrative, intermuscular or parosteal lipomas were found.

Squamous Cell Carcinoma (SCC) was diagnosed in one pet and strongly suspected in another. The first case was a dog that presented a lesion, five centimeters in diameter, on the dorsal aspect of the right front carpus. During this returning visit, euthanasia was performed due to histopathological confirmation of SCC. A white/grey cat with a lesion on the margin of the ear pinna presented with some pruritus. The clinician was suspecting of actinic keratosis, a SCC related do ultraviolet radiation exposure, due to the animal’s color and sparse hair on the pinna [52]. The owners would not pursue further diagnostic investigation.

2.4.2. Cancer of the Gastrointestinal Tract

Oral SCC was diagnosed in one pet and strongly suspected in another. SCC is the most common oral tumor in cats and the second most common in dogs [53]. The first patient was a Golden Retriever with a two centimeters (diameter) lesion on the rostral aspect of the mandible, infiltrating the gingival

mucosa. The lesion was hemorrhagic and ulcerated. X-ray confirmed suspicion that there was some deep tissue and bone involvement (Figure 6). The lesion was biopsied and the pathologist reported SCC. Bilateral rostral mandibulectomy or radiotherapy were not options for the client and eventually the patient was euthanized before the end of the externship. Although an experienced clinician had strong suspicion of hypoglossal SCC in a cat with chronic ulcerative stomatitis, the cat's owner would not proceed with diagnostics. Another owner brought his asymptomatic dog, worrying for a tumor on the animal's gingiva, but he was relieved to hear that

Figure 6. Intraoral radiograph of the mandibular incisors of a Golden Retriever with oral SCC. The bone "pulls away" from the advancing tumor, leaving a decalcified, soft—tissue-filled space.

most probably was a benign peripheral odontogenic fibroma (epulis) [53].

Pancreatic carcinoma was diagnosed in a cat with hyperthyroidism that presented anorexic. Ultrasound is considered a good diagnostic tool for pancreatic neoplasia in cats. Singular masses bigger than two centimeters usually are not hyperplastic nodules[54] . The ultrasound report confirmed ascites and lymphadenopathy with a singular pancreatic mass, hepatic nodules and thickened mesenteries. Pancreatic carcinoma with carcinomatosis and hepatic metastasis was the imagiologist's diagnosis.

Finally, there was a suspicion of perianal adenoma or anal sac apocrine adenocarcinoma on a female Pitbull that had lumpectomy history of a perianal benign mass and tenesmus with scooting. On rectal palpation, an almond-sized mass was found on four o'clock on the right anal sac. Since anal sac adenocarcinoma is second after lymphoma, in creating paraneoplastic hypercalcemia, CBC and SBP were performed but no such finding was confirmed [55] .

2.4.3. Hemangiosarcoma

Hemangiosarcoma (HSA) is a malignant neoplasm of vascular-endothelial origin. HSA accounts for 2.3% to 3.6% of skin tumors in dogs and 45% to 51% of splenic malignancies while it is also considered that two thirds of all splenic masses are malignant [56]. Three very similar cases of canine hemangiosarcoma were characteristic because all of them presented pale mucous membranes, lethargy, inappetence and ascites that after abdominocentesis classified as serosanguineous. Radiographic screening was crucial to highlight loss of abdominal definition, rule out metastasis in the cardiac and pericardial tissue as well in the lungs. Thrombocytopenia was marked and anemia was marginally regenerative. The author saw one case as follow up, after laparotomy and splenectomy and one was followed through these interventions. The third case was a five-year-old Labrador that was followed from the initial visit through laparotomy which did not continue with a

splenectomy. In this case, the exploratory outcome, was the excision of several neoplasms from the peritoneum. Pathologists indicated all cases as HSA although in one case, a secondary evaluation was needed because the primary resulted in hemangioma.

2.4.4. Lymphoma

Lymphoma represents one of the most common neoplasms in dogs with origin from the lymphoreticular cells of lymph nodes, spleen and bone marrow as well from other tissues[57]. In canines, the multicentric form is seen in 84% of cases, five to seven % has the alimentary form, five % the mediastinal, while cutaneous lymphoma is rarer as well as other atypical forms[57]. With lymphoma, the goals of chemotherapy are to induce a complete durable (6 months) first remission (termed *induction*), to re-induce a remission when the patient relapses, following achievement of a remission (termed *reinduction*), and finally, to induce remissions when the cancer fails to respond to induction or

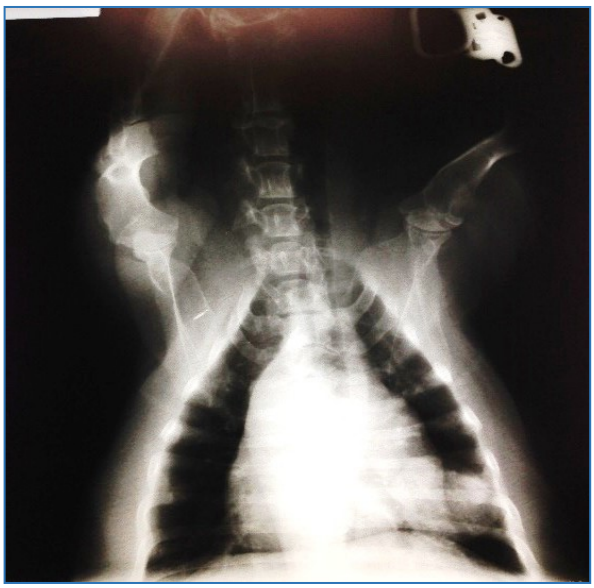

Figure 7. Thoracic radiography with a radiopacity in caudal left lobe, suggestive of a tumor.

reinduction using drugs not present in the initial protocols (termed *rescue*) [57]. Amongst two dogs that were diagnosed with multicentric lymphoma one received treatment that followed the University of Wisconsin-Madison Protocol, known as the "Wisconsin Protocol". This was a system of alternated multi-drug therapy with a series of 25 treatments, once a week, skipping on weeks five, 10, 16, 18, 20, 22 and 24. The pharmacological agents used were vincristine, prednisone, cyclophosphamide and doxorubicin. Every week, the 12-year-old female Maltese, would get an intramural CBC before treatment while the most experienced veterinary technician usually performed the procedure. At the 25th treatment instead of doxorubicin was used dacarbazine as *rescue* due to asthenia and re-established lymphadenomegalia. The patient did not achieve *induction* and was euthanized a month following the last treatment and seven months from diagnosis.

2.4.5. Other types of neoplastic disease

The only exotic patient in this section was a guinea pig with suspicion of cystitis and uroliths that underwent through exploratory laparotomy. Instead, an ovarian and uterine mass were discovered and after ovariohysterectomy (OVH) and biopsy, resulted in endometrial hyperplastic fibroadenoma and ovarian cystadenoma. A cat was subjected to thyroidectomy and biopsy of the gland. The result was a low-grade thyroid carcinoma. Two dogs with strong suspicion of urinary bladder transitional cell carcinoma (ultrasound screening), were treated with piroxicam. Mammary gland neoplasms were

suspected twice in one intact and one spayed dog. Nevertheless, diagnostics and treatment did not proceed. In another dog, a thoracic mass was evidenced in X-ray, during a pre-anesthetic checkup before a detartration (Figure 7). Definitive diagnosis was not achieved in another three cases. Considering all patients, that were strongly suspected but not diagnosed by histopathology, the number of not definitively diagnosed cases rises to twelve. The author exempts pancreatic carcinoma that was diagnosed by ultrasound and the case of epulis that has traditionally aclinical diagnosis.

2.5. MUSCULOSKELETAL DISORDERS

Osteoarthritis (OA) and other joint disorders were the most frequent musculoskeletal disorders. The cases with a predominantly musculoskeletal etiology were 31 in number and counted for 6.54% of all the caseload (Table 8). Canine and feline population could be subjectively characterized as overweight, especially when reaching older age. Excessive body weight exacerbates and accelerates degenerative joint disease and dysplastic disease [58]. Additionally, in the suburban setting, bigger dogs would not get the proper time and intensity of exercise needed to maintain physique and build up muscle endurance, especially when confined in a back yard and left

Table 8. Musculoskeletal disorders report.

Diagnosis	*n*	*%*
Primary Osteoarthritis	5	**16.13%**
Muscular Injury	4	**12.90%**
Articular Injury (Sprain)	4	**12.90%**
Cranial Cruciate Ligament Rupture	4	**12.90%**
Hip dysplasia	3	**9.68%**
CCL or Muscular Injury	2	**6.45%**
Luxating Patella	2	**6.45%**
Metatarsal Fracture, Panosteitis, Immune Mediated Polyarthritis, Arthritis & Myasthenia, CCL & Hip dysplasia, Osteophytes, Muscle Atrophy	1 of each	**3.23% for each**
Total	**31**	**100.00%**

unattended. Although many are the households with a dog, only few dogs are seen exercising outdoors daily. While this is another subjective observation, deserves to be mentioned. Another valuable remark is that, in New York, strict laws encircle pet ownership and animal rights, which in combination with spaying and adoption programs, lessen the circumstances of dogs running free outdoors or the existence of free-ranging dogs. Reflecting on that remark, could give reason for which, in this section, there was just one case of a stress fracture (non-surgical) that regarded an incident of a Greyhound running around in a dog park. However, outdoor cat population was well represented in the hospital.

2.5.1 Osteoarthritis

Primary OA is a disorder in which cartilage degeneration occurs and often follows aging. Roughening of the articular surface due to cartilage fibrillation occurs first and subsequent fissures can extend to subchondral bone while free cartilage fragments initiate an inflammatory response [59]. Collagen breakdown is induced by interleukin-1, tumor necrosis factor and by destructive enzymes released from chondrocytes, synoviocytes and inflammatory cells. Finally a vicious cycle of

inflammation and cartilage destruction results in pain and loss of articular function [59]. Patient history assessment in OA was based on the identification of painful movements by asking the owners about their pet's mobility. Unwillingness to play, go for a walk or get to the door and greet the caregiver seemed to be the most alarming signs that eventually impelled patients to the hospital. Short steps, constant weight displacement and an agitated behavior when standing, were clinical signs seen in the exam room. Caregivers also evidenced other signs of chronic pain such as difficulty on lying down and rising, climbing stairs and hoping on a couch. Often running or even walking where affected while movement, after a long rest (mornings) or after a major exercise ("weekend warrior syndrome"), was difficult or impeded. All five patients with primary OA were dogs and returning patients. All patients were submitted to a full physical exam with palpation and verification of the range of motion which was often reduced especially on the coxo-femoral joint. Management of OA and other arthritic conditions in general depended on several principles. Keeping ideal body weight was promptly appointed to owners as crucial and occasionally appointments were scheduled only for weight control. Nutritional supplementation was instituted with Omega-3 fatty acids, glucosamine and chondroitin sulfate to ease inflammation, pain and provide a chondroprotective effect[58]. During maintenance of pets with OA, owners were told to keep moderate exercise (walking on a leash) in the daily routine, perform simple warming up exercises (extension-flexion) before setting out for a walk and trying to avoid intense, sudden exercise. Polysulfated glycosaminoglycan (Adequan®) IM protocol was executed as follows: twice a week for four weeks then once a week for a month, skip a week and finally two injections a week apart for a total of 14 injections. Adequan® has anabolic effects on cartilage metabolism and caregivers seemed to acknowledge some benefits [59]. Only one dog was examined after a flare up with acute pain and lameness and it was medicated with deracoxib which amongst other NSAIDs are the basis for antinflammatory and pain treatment in OA. Resting and confinement for three days was stressed to the owner. In the acute phase of joint inflammation and pain, cryotherapy on joints with cold packs was recommended. In chronic OA, physical rehabilitation was advised to strengthen musculature and keep range of motion which was done at home most of times. Massage, passive range of motion exercises, standing-balancing exercises and swimming were the easiest tasks to perform by caregivers. Other non-severe articular disorders like hip dysplasia, cranial cruciate ligament (CCL) rupture and luxating patella, were approached in the same way with OA regarding medical treatment and long term management.

2.5.2. Cranial Cruciate Ligament Rupture

Stifle injuries in cats are uncommon and all cases with a diagnosed CCL rupture or with strong suspicion off at least a partial rupture were dogs. As CCL, can result from degenerative and traumatic causes, dogs presented with a progressive or an acute lameness which varied from continuous and non-weight-baring to intermittent or prolonged weight-baring. Diagnostic procedure involved palpation and a sense for a positive cranial drawer test or positive tibial compression[60]. One

Labrador with hip dysplasia suffered a CCL rupture and was followed with monthly Adequan® injection. For other two patients with unilateral acute weight-baring lameness but without signs of joint pain or a positive CCL test, differential diagnosis was a muscular injury or CCL and were medicated also with deracoxib. Amongst four dogs diagnosed with CCL rupture, two had already been operated on and came for an Adequan® injection while in good status. One of the remnant cases was a Shitzu with positive drawer test which was submitted to X-ray. Findings gave sign of a distention of the caudal joint capsule, compression of the infrapatellar fat pad and slight alterations in radiopacity on the distal pole of the patella (Figure 8). During surgery, spectated by the author, suspicion was verified, menisci were intact and an imbrication technique, by partial excision and closure of the *fascia lata*, was performed.

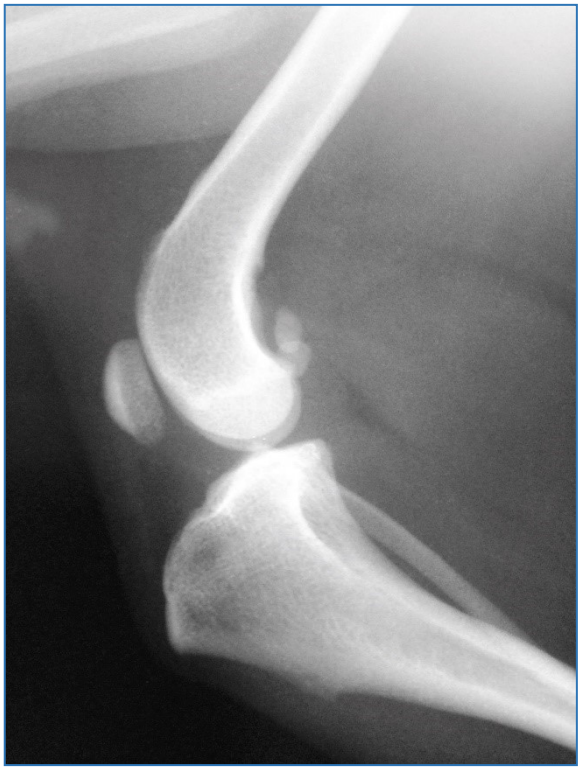

Figure 8. Left stifle of a Shitzu with Cranial Cruciate Ligament rupture.

2.5.3. Hip Dysplasia and Luxating Patella.

Coxo-femoral joints when affected by degeneration or trauma are cause of pain which forces patients to alter their gait by shifting their weight forward. This results from flexing the thoracic spine, shoulders and elbows and from widening the stance of their forelimbs. Crepitus and decrease in range of motion were characteristic on two dogs already diagnosed that came in for an Adequan® injection while a Labrador that presented lame, taken X-ray showed femoral head and acetabular degeneration.

Diagnosing a luxating patella can be very rewarding for a veterinarian as the caregivers get very impressed when their non-weight-baring, lame dog, gets to walk normally in a matter of minutes. Both patients were found with a medial patellar luxation and they were small dogs. One was a young adult with a non-weight-baring lameness and the other, with intermittent weight-baring lameness, was nine MOA. During physical examination *grade I luxation* was suspected on the puppy because it was possible to elicit medial luxation but not a permanent one [61]. *Grade II luxation* must have been the case for the young adult whose patella reduced only after full stifle extension. Both patients were medicated with deracoxib and they were rescheduled for a recheck while a radiographic screening was advised.

2.5.4. Other Musculoskeletal Disorders

An interesting case of panosteitis in a German Shepherd, six MOA puppy, with acute lameness and pain near the joints was treated with deracoxib and signs resolved. A Dalmatian with arthritis (receiving Adequan®) was suspected of acquired myasthenia gravis but without megaesophagus. Appendicular muscle weakness after exercise was signaled by the owner and the patient was falling on one, or all the limbs, when standing or walking in the exam room. Neurological exam was non-remarkable and the attending clinician was considering a scheduled edrophonium chloride test [62].

2.6. OTITIS EXTERNA

Otitis externa (OE) was the most frequent disorder met during the authors externship. As there was no other Otorhinolaryngologic disorder encountered, OE, was classified on its own. In total, 30 animals (27 dogs and three cats) visited the hospital for a painful, itchy, inflamed or "*dirty*" ear. Caregivers described that their pets were head shaking, ear scratching with rear limbs or rubbing against surfaces. Also, painful ears gave away wails when touched and dogs were apprehensive during physical examinations. The owners were queued with questions that surfaced onset of symptoms and initial aspect of the condition. Further questions would investigate lifestyle and incidents that could have influenced. Indoor pets would be less probable to have foreign bodies or ectoparasites and living with other pets would raise issues of contagious agents. Immunological status and age would affect differential diagnostic and individual husbandry (method of ear cleaning) could give indications on etiology. Caregivers would often clean ears with cotton swabs or use inappropriate products. Furthermore, questioning on chronicity showed seasonal recurrence that suggested an atopic underlying issue. OE is mostly a secondary process affected by predisposing, primary and secondary factors. Conformation of the ear, humidity and hair in canal are factors predisposing. Ectoparasites, allergic dermatitis, keratinization disorders, pyoderma, autoimmune dermatosis, foreign bodies and tumors can be primary factors. Finally perpetuating or secondary factors are yeasts, bacteria, epidermal hyperplasia and ulcerations.

A complete physical and dermatological examination was performed before going ahead to the ears. Clinicians were looking on paws and dermis for signs of inflammation or infection and the pinnae were thoroughly examined. Inflammation, erythema, presence of cerumen and stenotic canals were highlighted upon visual inspection. The otoscope was used first in the apparently healthy ear to avoid contamination. Otoscopic evaluation was focused on the condition of the tympanum, type of secretions, presence of ulcerations, edema and erythema. Grossly, in one out of three cases an ear swab was examined under the microscope and in a handful of patients a cytologic examination, stained by Diff-Quick was performed. No mites were seen on direct swab examinations. *Cocci* were present on slides from infected ears that suggested the presence of commensal *Staphylococcus pseudintermedius*. A pilot study, attempting to establish a clinical score for otitis (Otitis Index Score - OTIS) in scales from zero to three (OTIS3) and zero to five (OTIS5), achieved satisfactory results in

distinguishing affected from healthy ears or ears in remission [63]. Four clinical parameters were evaluated: exudate, edema, erythema and erosion/ulceration for a total score from zero to 12. The OTIS3 scale was found more reliable and easy to use while the parameter erosion/ulceration that related mostly to supurative otitis and was absent in erythroceruminous otitis was the least well correlated with ear canal scores. The authors suggest that further validation among larger cohorts of cases by more clinicians could give result to a good tool for clinical use.

Table 9. Topical otic solutions used for otitis externa

Product	*Antibiotic*	*Antiyeast*	*Antinflammatory*
Panolog®	Neomycin + Thiostrepton	Nystatin	Triamcinolone acetonide
Tri-Otic®	Gentamicin	Clotrimazole	Betamethasone valerate
Mometamax®	Gentamicin	Clotrimazole	Mometasone
Osurnia®	Florfenicol	Terbinafine	Betamethasone acetate
Synotic®	-	-	Fluocinolone acetonide + Dimethyl sulfoxide (DMSO)

Treating otitis externa involved in all cases ear cleaning and topical medication. Only in two cases was used deracoxib and in four cases were used systemic antibiotics. One methicillin resistant *cocci* infection was cultured and was treated with neomycin. Prednisone PO, in an antinflammatory dose, was prescribed once. Used topical otic solutions included an antibiotic, an antifungal and an antinflammatory agent while dimethyl sulfoxide was utilized for treating stenotic ear canals with hyperplastic conditions (Table 9). Most of pets were reevaluated a week later and showing improvement although supurative and stenotic otitis were more copious to treat.

2.7. NEUROLOGY

Because neurological lesions can alter a variety of body functions, the beginning of a physical exam, involved assessing the mental state, posture and gait, while owners would inform on anamnesis. Severe presentations like paresis or paralysis were pointed by the owners but more subtle signs like a slight hypermetric movement or wide circling were overlooked. Anamnesis offered input on onset of signs, progression and bodily functions such as control of micturition and defecation, vision and audition. Postural reactions, muscle tone and size, spinal reflexes, sensory and pain evaluation as well cranial nerves function were all

Table 10. Predominantly neurological cases

Diagnosis	n	%
Thoracolumbar Lesion	8	**33.33%**
Lumbosacral Lesion	3	**12.50%**
Cervical Lesion	3	**12.50%**
Facial Hemiparalysis	2	**8.33%**
Vestibular Disease	2	**8.33%**
Lumbosacral Stenosis	2	**8.33%**
Intracranial Disorder	2	**8.33%**
Seizure	1	**4.17%**
Peripheral Neuropathy	1	**4.17%**
Total	**24**	**100.00%**

assessed on the examining table. Neurological cases accounted for 5.06% (n=24) of the caseload (Table 10).

2.7.1. Spinal Lesions [64-66]

Cervical disk disease was signaled in three dogs that all presented with "*root signature"*. Unilateral front lameness and signs of pain, during neck manipulation, were common in all cases but none of the three dogs gave signs of Upper Motor Neuron (UMN) paresis or Lower Motor Neuron (LMN) weakness, so spinal cord compression was unlikely. One dog was a Chihuahua, that gave signs of intervertebral disk disease (C2-C4) on the X-ray, taken under anesthesia. All three pets were covered with dexamethasone and deracoxib.

Three dogs were affected by lumbosacral pain with LMN paresis and depressed pelvic limp withdrawal while for one of them, LMN signs were more severe. That patient, was a 10-year-old paraplegic female with decreased anal sphincter reflex and incontinence. Patellar reflexes were normal for all three dogs and evaluation of cutaneous trunci reflexes, along with all other signs, gave indication that two of the patients had a lesion between L6 and S1 while the third between S1 and S3. Interestingly that last dog, after medical treatment, seemed to recuperate but relapsed in the following week and on reevaluation showed signs of a probable ascending-descending myelomalacia, with complete loss of anal sphincter control and bilaterally absent patellar reflex, so unfortunately, it had to be euthanized promptly. Pain control was attempted with tramadol, in this last case. For all three pets, remaining treatment was as described on cervical lesions.

Thoracolumbar disk disease was diagnosed in eight pets, all dogs. The major complain of caregivers was that their animals would not climb up stairs or hop on the couch. Yelping was a common sign of pain in various movements (e.g. sitting) and mainly on palpation of the thoracolumbar junction. Neurological signs were evident in four out of eight patients. Four were just painful with an abnormal arched truncal posture but with normal neurological exam. Normal reflexes and non-ambulatory paraparesis, evident only in one case, guided to a diagnosis of a severe UMN lesion. The other three dogs were ambulatory, paraparetic with proprioceptive ataxia on the hind limbs. Summarizing, eight dogs were considered to have a T3-L3 lesion that was probably localized between T11 and L3. Four of those were less severe with just pain on spinal palpation, three were ambulatory but paraparetic and lastly, one was non-ambulatory but with no urine retention. That last dog was hospitalized and treated with fluids, methylprednisolone, famotidine and ampicillin. Butorphanol and dexamethasone was used in two cases. Deracoxib only, was prescribed for the four non-neurologic dogs and one dog was treated with dexamethasone alone.

Magnetic Resonance Imaging (MRI) was recommended to all caregivers, especially for dogs that had severe neurologic signs. Resting in confinement for six weeks was stressed as very important to owners, however, not all could comply to this measure. Avoiding jumping, running and any sudden moves was a more feasible objective.

Lumbosacral stenosis was diagnosed for two middle aged, male Greyhounds with reluctance to sit, do stairs and a stiff posture. Pain was present by lifting the tail and on palpation of the sacral area. A slight proprioceptive deficit detected during prior neurological screenings was not worsening. Both were returning patients under treatment. One was receiving oral prednisone and the other was treated with bilateral IM injections in the lumbosacral spaces.

2.7.2. Vestibular Disease and Intracranial Disorders[67] [68]

One young male cat and one 12-year-old Boston Terrier were diagnosed with idiopathic vestibular disease. Acute onset of symptoms and head tilt was common for both pets, showing a unilateral lesion. The Boston Terrier had milder symptoms with mild erratic circling, a wide stance and slight loss of balance towards the side of the lesion. There were no signs of otitis or a bulging tympanum on otoscopy. The patient presented a horizontal, jerk nystagmus with the slow phase towards the side of the head tilt and that was not changing direction in different head positioning. The cat also presented the same symptoms except that the nystagmus was rotary so both pets were considered to have peripheral idiopathic vestibular disease. Although a proper neurological exam was not easy for the cat, no proprioceptive or cognitive deficits were clear. Both were medicated with meclizine and the cat received also the antiemetic maropitant. Effectively, two days later the cat was perfectly fine. The progression of symptoms of the dog was not documented.[67]

Two cats were diagnosed with intracranial disorders. Severe hypermetria, ataxia and head tremor, were the symptoms of a cat with congenital cerebellar hypoplasia. Additionally, this feline presented confused and constipated. A recumbent, cachectic and dehydrated cat was found to have hypotonic muscles, depressed spinal reflexes, unresponsive mydriasis and decreased menace response. Along with a depressive level of consciousness, the patient, would score under eight, using the modified Glasgow coma scale [68]. Differential diagnosis for this cat were neoplasia, trauma, a vascular accident or other.

2.8. UROLOGY

Amongst 20 cases that classified as predominantly urologic the author witnessed 11 dogs with Urinary Tract Infection (UTI) and two cats. Chronic Kidney Disease (CKD) affected one cat and three dogs. Urolithiasis was diagnosed on a guinea pig as well as on a dog. Canine was also the patient affected by Polypoid Inflammatory Cystitis (Table 11).

Table 11. Urology caseload.

Diagnosis	*n*	%
Urinary Tract Infection	13	65.00%
Chronic Kidney Disease	4	20.00%
Urolithiasis	2	10.00%
Polypoid Inflammatory Cystitis	1	5.00%
Total	20	100.00%

2.8.1. Lower Urinary Tract Infection [69]

Two old female spayed cats were diagnosed with UTI. Both had pollakiuria, polyuria (PU) and polydipsia (PD). Slight hypocalcemia and neutrophilia were seen on bloodwork and U/A showed proteinuria, hematuria and pyuria on the oldest cat (17 years old). Apparently, these were first time infections. A first line antibiotic therapy (amoxicillin) was the choice of treatment for both cats but as infections in cats can underline a systemic disease, these patients were not considered uncomplicated UTI's and owners were adverted that maybe further diagnostics would be necessary. One intact, five spayed females and five male dogs (three neutered, two intact), were diagnosed also with UTI. All patients were older than eight years. Two female geriatric patients (17 and 13-year-old) had complicated UTI's. Lately, the 17-year-old, had relapsing infections and reinfections that were due to immunologic decadence. The obese, 13-year-old, had a recessed vulva and an episioplasty was suggested. Radiographic screening was used to exclude urolithiasis. Cystocentesis was always the first choice for urine collection but when not possible, catheterization was executed. Echo-guided cystocentesis was performed on a male Basset Hound. Urine culture was performed when a second UTI would appear. First line antibiotics used were amoxicillin and sulfonamide trimethoprim. A frequent choice for second line antibiotic was enrofloxacin. Non-complicated UTI's were treated for seven to 15 days while relapsing cases were medicated for at least four weeks. Antibiotic medication was adjusted by augmentation of dosage in persistent infections or by substitution of the drug, following culture results. All pets were followed up after five to seven days. Adequan® as well as glucosamine and chondroitin supplements were occasionally used for cats with signs of cystitis.

2.8.2. Chronic Kidney Disease [70]

Four patients were found to suffer from kidney disease. Chronic kidney disease, is irreversible because of permanent loss of functioning nephrons. Poor coat and body condition was a common finding as well as PU and PD. Three of the patients with CKD were being treated. Unfortunately, the caregiver of an old lethargic and dehydrated Shitzu could not afford proper treatment, therefore, only SC fluids were administrated every three days. Urine specific gravity of 1.013, evidenced hyposthenouria on a 12-year-old cat, named Nabisco. Hyperphosphatemia (serum PO_4: 21.9 mg/dL), high creatinine (13.9 mg/dL) and high blood urea nitrogen (262 mg/dL) showed deterioration in preexisting CKD. The patient was signaled in 2014 having "International Renal Interest Society" stage III CKD, with creatinine at 3.0 mg/dL. In October of 2016, Nabisco, was proteinuric and hematuric but with no signs of anemia on CBC and was euthanized due to anorexia and weight loss. Adjustments in hypotensive therapy with enalapril were set up, for a 13-year-old dog with CKD. Finally, minimum database screening confirmed that another dog was in non-progressive CKD. Regularly scheduled follow ups with kidney patients were pursued extensively at "A&A". Although these four patients were the only that the author examined during an appointment, renal cases were often hospitalized for rehydration through IV fluids. Dietary therapy was a common concern. Renal diets are designed with

reduction in protein (but with high biological value), restriction in phosphorus and sodium, increased caloric density, supplementation in B vitamins and Ω-3 fatty acids, soluble fiber added while with a neutral effect on acid-base balance.

2.9 ENDOCRINOLOGY

Diabetes mellitus (DM), canine hyperadrenocorticism (HAC) and feline hyperthyroidism (FHT) were the only endocrine disorders diagnosed. Dogs and cats were equally represented (four of each) for a total of eight exclusively diabetic patients. Additionally, one Maltese was diagnosed with both DM and HAC. Canine HAC affected four animals and FHT three. Overall, 16 cases classified as predominantly endocrinological, defining 3,38% of the total caseload (Table 12).

Table 12. Endocrinology caseload

Diagnosis	n	*%*
Diabetes Mellitus	8	**50.00%**
Canine Hyperadrenocorticism	4	**25.00%**
Feline Hyperthyroidism	3	**18.75%**
Cushing's & Diabetes Mellitus	1	**6.25%**
TOTAL	**16**	**100.00%**

2.9.1. Diabetes Mellitus and Canine Hyperadrenocorticism [71,72]

Hyperglycemia and how to control it, was the major concern for all diabetic pets. Just one three-year-old dog, was diagnosed for the first time, while all other patients were already on treatment. PU and consequently PD where the symptoms that alarmed the owner, who himself was diabetic. A quick urine strip test evidenced very high glycosuria. Suspicions were confirmed using a glucometer and action to reduce blood glucose (BG) was taken with insulin therapy.

Even if all cases of HAC and DM were interesting, one patient was diagnosed with both conditions. The 12-year-old female Maltese, named Holly, was signaled with PU/PD, muscle weakness, hair loss and was panting in March of 2015. Alanine Aminotransferase (AST) was 105 units/L, Alkaline Phosphatase (ALP) was 8185 Units/L and Cholesterol was found at 437 mg/dL. These values along with clinical condition alarmed for HAC and an Adrenocorticotropic Hormone (ACTH) Stimulation Test (ACTHST) surfaced that resting cortisol was at 19.3 mcg/dL and post-ACTH cortisol, at 46.7 mcg/dL. Trilostane administration SID was established and 3 weeks later, after another ACTHST, she still showed high cortisol values (16 mcg/dL resting, 31.9 mcg/dL post-ACTH) and adjustments were made on therapy. Subsequent ACTHST's one month and three months later confirmed success of treatment. Resting cortisol reached 6 mcg/dL and post-ACTH was at 7.9 mcg/dL, values compatible with trilostane treatment SID. Resolution of PU and PD was achieved and Holly was feeling better, until December 2015, when the same symptoms returned. This time hyperglycemia (629 mg/dL), glycosuria and ketonuria (on urine test strip) were concerning. Fasting BG of 301 mg/dL confirmed diagnosis of DM, which is not common, occurring just in five percent of dogs with HAC. Holly started with intermediate acting insulin (NPH) and was scheduled for a BG

curve. Two BG curves later, the final dosage of NPH insulin was instituted at five units per kilogram. Fructosamine, when tested, was at 495 micromol/L which per the referring laboratory shows a fair control. Meanwhile ACTHST's continued to show good control of HAC. The author encountered with the patient in November of 2016 when a routine fructosamine test resulted in 526 μmol/dL. Insulin resistance due to HAC was causing failure of DM treatment and further augmentation of insulin dosage to six units was elected. Both conditions are difficult to manage and require strong owner commitment and excellent communication with the attending veterinarian, like in Holly's case. At "A&A", special attention was given to inform and guide people through their pet's condition and treatment for HAC and, especially, DM. Printed handouts for clients, had an instructional and informational purpose. Training owners to administrate insulin SC was part of the newly diagnosed, DM patient's, appointment. Finally contacting regularly with the clients was crucial for good follow up and prognosis.

2.9.2. Feline Hyperthyroidism

Untreated hyperthyroidism can be fatal due to the physiological importance of thyroid hormones. Considering the cardiovascular system, the thyroid hormone has a positive chronotropic effect, causes shorter atrioventricular conduction times and upregulates myocardial beta-adrenergic receptors. Rarely, cats with FHT are hypertensive and a variety of renal functions are affected by the thyroid. Three cats that were diagnosed with FHT presented common clinical signs described in literature. Weight loss, hyperactivity, tachycardia, tachypnea and a second-grade systolic heart murmur were encountered. Only one of the pets was diagnosed for the first time in the author's presence while the other two cats were already on treatment with methimazole. Laboratory analysis evidenced that total thyroxine (tT4) was at 10.5 mcg/dL (reference: 0.8-4.0 mcg/dL), ALT was increased and along with monocytosis the cat was proteinuric and hematuric. No further diagnostics were procured (scintigraphy etc.) and methimazole PO was initiated. Six weeks later and five hours' post methimazole administration, tT4 was at 2.3 mcg/dL. Cats can live for a long time when properly treated with owners that are committed and engaged.

2.10. EXOTIC SPECIES

As classifying the exotic species by somatic system was complicated, all cases were grouped in this section together. The most common exotic species encountered were guinea pigs (*Cavia porcellus*) and the most exciting, was an African Rock Python (*Python sebae*) (Figure 9). In this section the reader will find the only two cases, in the whole report, that are referred twice. A guinea pig, that was diagnosed with urolithiasis, is also referred in the Urology section and another guinea pig with ovarian and uterine neoplasia is mentioned in Oncology and Surgery. Totally 16 patients were exotic species making this the only section which's classification is not based on diagnosis.

2.10.1. Mammals[73, 74]

A dyspneic guinea pig unfortunately died on the examining table within minutes, in the physical exam, while another dyspneic animal was given oxygen and was anesthetized with isoflurane for X-ray that resulted nonremarkable. One patient was cachectic, dehydrated and was administered SC fluids, medicated with enrofloxacin and supplemented with Vitamin C. Owners were told to mix Gatorade® with its food and force feed, with a syringe, in case anorexia was continued. One more case of urolithiasis (not accounted in Urology) was diagnosed radiographically on a guinea pig with stranguria that was medicated with meloxicam. Finally, a growth, probably of neoplastic origin, on the nasal planum was concerning the owners of an old female.

Ferrets (*Mustela putorius furo)* have their first recorded reference as pets (that can be assumed to refer to a ferret), around 350 BC, by Aristotle and were introduced into the United States, from Europe, by the shipping industry in the 1700s. They may have come as pets or as hunting companions. One animal presented an upper respiratory infection which could have been due to influenza. Ferrets are susceptible to various strains of human influenza virus. The clinical picture was good and just palliative care was elected. On other pet was lethargic intermittently and presented weak, especially in hind limbs. Hypoglycemia and hyperinsulinemia evidenced on laboratory analysis confirmed suspicions of possible insulinoma. Prednisone was prescribed and the owner was instructed to feed often that initially gave good results. Nevertheless, reevaluation showed no significant response to treatment.

2.10.2. Birds [75]

Three periquits (*Melopsittacus undulatus)*, a cockatiel (*Nymphicus hollandicus)* and an African grey parrot *(Psittacus erithacus*) were the avian species met. The parrot was having various seizure episodes and was hypocalcemic. Hypocalcemia and hypomagnesaemia are reported as seizure inducing in this species. Treatment was implemented with levetiracetam and supplementation in calcium, and vitamins. Two periquits were unilaterally lame, with one having an interdigital trauma that was infected while the third was just a checkup. Meloxicam only was prescribed for one while the other received enrofloxacin in addition. Finally, the only cockatiel examined, has been recently purchased and general husbandry education for the client was the purpose of the visit.

2.10.3. Reptiles [76]

A 3 meter long, female African Rock Python, with upper respiratory signs that included nasal discharge and bubble blowing was returning for treatment and was doing better because there were no signs of dyspnea.

Figure 9. African Rock Python (Python sebae) with upper respiratory infection.

Respiratory sounds were clear and treatment was established with five IM injections of amykacin spaced every three days. A water snake with an edematose growth between the nostrils was treated in the same way.

2.11. CARDIOVASCULAR DISORDERS

Merely 13 dogs suffered from a heart disorder accounting for just 2.74% of the total caseload. There were no cats with a heart related disease.Three animals with a pre-diagnosed heart murmur that were assymptomatic, returned frequently for checkups.The remaining 10 dogs were already under treatment for heart failure.(Table 13)

Table 13. Cardiovascular disorders

Disorder	*n*	*%*
Heart Failure	10	76.92%
Valvular Murmur	3	23.08%
Total	**13**	**100.00%**

2.11.1. Heart Failure [77]

A patient with inability to sustain a normal cardiac output, in consequence of an abnormality, usually demontrates clinical symptoms. Exercise intolerance, pallor, cold extremities and generalized hypotension are common with inadequate output. An animal can also show signs of congestive or backward failure when cardiac filling pressures are high. In this case, congestion in venus circulation can lead to edema and pulmonary congestion or ascites. Tachypnea, dyspnea and abdominal distention are common findings. Eventually, when clinical signs appear, signifies that compensatory mechanisms were overwhelmed. Consenquently, it can be said that the organism is in heart failure. In many cases there are more interlinked failure points in the cardiovascular system which result in a severely debilitated patient either chronically or acutelly. Functional impairment of the myocardium, the heart valves, the pericardium or increased resistance to ejection are the habitual causes of cardiac failure.

All 13 patients mentioned here, presented an acquired cardiac dysfunction. Diagnosis involved a physical exam, auscultation, X-ray, echocardiography and electrocardiogramms (ECG). Therapy for heart failure was medical and commonly used drugs were diuretics (furosemide), inotropic-venodilators (pimobendan), angiotensin-converting enzyme inhibitors like enalapril and antihypertensive vasodilators like sildenafil. All cases were under treatment with one or a combination of those medications. Due to an extesive anamnesis and particularity of each case the author elected just one for reporting purposes.

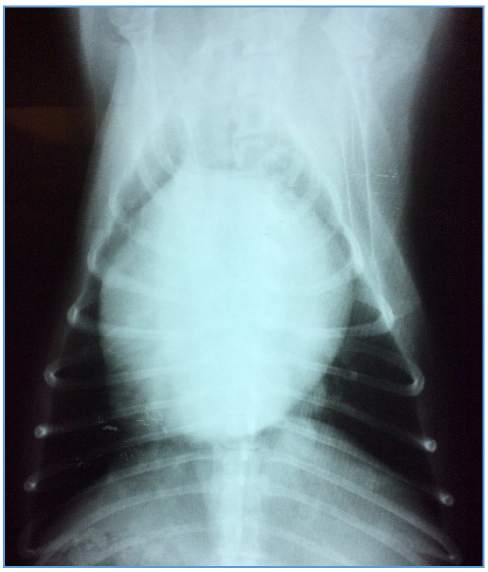

Figure 10. Thoracic X-ray of a female dog showing an enlarged globoid cardiac silhouette.

A Cavalier King Charles Spaniel with 13 years of age was admitted to the hospital while dyspneic. The spayed female, was mildly pale with a weak pulse and was panting. On auscultation a cardiac murmur was worst than in previous meetings. Thoracic X-rays showed an enlarged cardiac silhouete, which for its globoid shape, especially on a ventro-dorsal view, alarmed for a pericardial effusion (Figure 10). Enalapril was prescibed and a scheduled echocardiography was performed. The cardiologist reported a severely dilated left atrium, moderately dilated left ventricle and both right chambers mildly dilated. Normal aortic valve and cardiac walls function was contrasted by severe mitral regurgitation, due to myxomatous degeneration and consequent leaflet thickening and prolapse. Furthermore, the tricuspid valve was also affected by myxomatous degeneration, evidencing severe regurgitation. The report concluded that the patient was suffering from chronic valvular disease and mild pulmonary hypertension but had no signs of pericardial effusion. Effectively, X-rays are referenced as poorly sensitive in detecting cardiac tamponade [78]. The treatment plan combined enalapril, furosemide and pimobendan as well as another echocardiography in six months. All patients examined were screened in the same way and were diagnosed in their majority with congestive heart failure due to mitral valve degeneration.

2.12. EMERGENCIES

Urgent care was provided to 12 dogs during the author's clinical externship. Three pets had to be treated to prevent intoxication, other three had suffered a heat stroke, two presented with a minor trauma. Finally, singular cases were an upper airway obstruction, a cardiapulomonary arrest, a septicemic shock and a bite wound all encountered in dogs. Patients that classified as emergencies were unschedulled visits that needed immediate medical attention.(Table 14)

Table 14. Emergencies

Emergency	n	%
Intoxication	3	25.00%
Heat Stroke	3	25.00%
Minor Trauma	2	16.67%
Upper Airway Obstruction	1	8.33%
Cardiopulmonary Arrest	1	8.33%
Bite Wound	1	8.33%
Septicemic Schock	1	8.33%
Total	12	100.00%

Two dogs were admitted after ingestion of potentially toxic quantities of chocolate but had shown no clinical signs such as vomit or nausea. A tablet of the emetic apomorphine in the conjunctival sac effectively provoked vomiting. One of the dogs had ingested a large amount of small chocolate bars with their wraps. The third intoxication case was more concerning because with acute ingestion of ibuprofen in dogs, vomiting, diarrhea, nausea, anorexia, gastric ulceration, and abdominal pain can be seen with doses of 50-125 mg/kg; these signs in combination with renal damage can be seen at doses at or above 175 mg/kg; and at doses at or above 400 mg/kg neurological effects such as seizure, ataxia, and coma, may occur [79]. The number of ibuprofen tablets ingested was unknown and apomorphine was used along with famotidine. Provoked emesis showed no signs of the tablets and since the time of ingestion was also unknown, activated charcoal was administered.

Severe hyperthermia, also known as heat stroke, can result in organ failure and must be recognized and treated promptly [80]. All three dogs were seen in the second week of September 2016 during elevated temperature and high humidity conditions. Body temperatures upon arrival of the patients were,106.8° F (41.5°C), 104°F (40°C) and 102.2° F (39.9°C) respectively. Emergency treatment involved cooling off the body by spraying cool (not cold) water and placing cool water packs under the axillae and inguinal areas. IV fluids were given (starch and/or Ringer's lactate) and broad spectrum antibiotics were administered also. Oxygen therapy was implemented for all patients but the two that presented with higher temperature were given also glucocorticoids due to signs of inflammatory, upper airway obstruction. The patient with the higher temperature, after a seizure, entered in coma and unfortunately did not survive even after administration of diazepam. In the other two cases the outcome was good.

Other two dogs were admitted in critical condition. A middle-aged Golden Retriever was urged in the hospital in cardiopulmonary arrest that, supposedly, had happened a minute before his entrance while in the owner's car. After confirming clear airways, Cardiopulmonary Resuscitation (CPR) procedure started immediately by a three-membered team and an endotracheal tube was placed. Basic Life Support (BLS), with chest compressions and ventilation, continued while advanced life support by ECG and end tidal carbon dioxide monitoring initiated. After one cycle (two minutes), there were no complexes appearing and the ECG was a flat line, which is a non-shockable arrest rhythm, for defibrillation therapy [81]. Intratracheal epinephrine and atropine were given (while BLS was continuing) because an IV catheter was not easy to place. After a few cycles, CPR was aborted because nor a shockable or a perfusing rhythm would appear. The second patient in critical condition was a dog that presented extended ascites, was lethargic and very pale. Abdominocentesis, evidenced an hemopurolent (macroscopically) content and suddenly the pet collapsed while being transported to X-ray. CPR was not tried because the pet had a high fever and the probable cause of death was septicemic-anoxic shock.

Lastly, a young Labrador came in with the complain of sudden respiratory distress while eating. An upper respiratory obstruction was suspected. The dog was sedated, administered with glucocorticoids and placed in oxygen while X-ray evidenced a round endotracheal foreign body that thankfully was expelled (a dog croquette) during chest compressions by an experienced Veterinary Technician (something like a Heimlich maneuver).

2.13. RESPIRATORY DISORDERS

Twelve pets suffered from a respiratory related disease (Table 15). Two cats were diagnosed with asthma and the rest of the patients were dogs. The most common disorder was collapsing trachea (CT) and, naturally, dyspnea and cough were frequent clinical symptoms.

Tracheal collapse is a common respiratory disorder that affects mainly middle aged and small or toy dogs [82]. During the author's externship, various dogs were seen with this condition but only four were examined during an appointment. Dyspneic dogs with a characteristic honking cough often showed exercise intolerance and were cyanotic in severe cases. All four dogs diagnosed with CT were obese and of small breeds

Table 15. Respiratory disorders

Disorder	*n*	*%*
Collapsing Trachea	4	**33.33%**
Kennel Cough	3	**25.00%**
Feline Asthma	2	**16.67%**
Bronchitis	2	**16.67%**
Laryngotracheitis	1	**8.33%**
Total	**12**	**100.00%**

(two Beagles, one French Bulldog and one Pomeranian/mixed breed). Also, it was possible to provoke coughing by tracheal palpation. Upon anamnesis and physical examination, diagnosis was almost certain, but X-rays confirmed suspicion. There was an effort to achieve two projections during inspiration and expiration but just in one case was achieved with success. Nevertheless, a dorsoventral flattening was clear in all four patients. One Beagle with severe inspiratory dyspnea had a collapse in the cervical trachea, while the other three pets were affected in the cervicothoracic junction. Treatment, focused on ameliorating oxygenation, reducing inflammation with prednisolone and easing the cough with hydrocodone. The beagle, mentioned above, was sedated with acepromazine and butorphanol while the other dog of the same breed was hyperadrenocorticoid and could not take any corticosteroids. The necessity to lose weight was stressed as urgent to owners as well as the use of harnesses, instead of collars.

Kennel cough symptoms (infectious tracheobronchitis or CIRD) and pathophysiology were discussed in the section 2.2. on preventive medicine.

Respiratory distress in two cats that were examined had different presentations. One feline had severe dyspnea and was panting while the other was coughing and wheezing. On auscultation crepitation and increased expiratory effort was common for both animals. Both cats were obese and middle aged. Minimal manipulation, immediate IM administration of dexamethasone and oxygen therapy were the first actions. Bronchoalveolar lavage fluid or an endotracheal wash analysis are not yet considered effective to distinguish between chronic bronchitis and asthmatic cases and have never been performed on these patients [83]. Additionally, a bronchodilator was administered and in less than an hour the patients were breathing better. These were two previously diagnosed cases that now, were examined, in status asthmaticus. Fact that proves how difficult is, for owners, to recognize respiratory distress in a cat and to judge how urgent a visit to the veterinarian should be.

2.14. OTHER INFIRMITIES AND PROCEDURES

Anal sacs, in dogs, were often asked to be emptied by owners although veterinarians were trying to avoid starting this process because once done, it frequently relapses. Nine caregivers marked appointments to specifically check and empty the anal sacs due to scooting or malodor.

Witnessed euthanasia was happening at least once a day at “A&A” but for client privacy issues and for the importance of such a delicate, emotional moment, the author, was present in only six procedures. There were differences on the approach of each veterinarian. To the author’s opinion, the less stressful modus operandi, for both the patient and caregivers, was to sedate IM the patient and leave the room until the effect of the sedative kicked in. Then return and administer the lethal injection IV. In this way family members, can have a moment on their own with their pet and the transition from an alert state to death is smooth. Beuthanasia®-D Special (pentobarbital sodium/ phenytoin sodium) was used.

All dentistry procedures seen were executed by certified Veterinary Technicians. Sedation, anesthesia and radiographic screening were executed in this mentioning order. Detartrations were also happening on a daily basis. The author followed six of them from admission to post anesthetic recovery.

Five minor lacerations (Minor Wounds) that came in a scheduled appointment needed just disinfection and cleaning.

Three dogs with a superficial corneal ulcer, one with glaucoma and one with conjunctivitis formed the ophthalmological caseload. Increased lacrimation and irritation were the symptoms that brought in the dogs with a corneal ulcer. Fluorescein staining confirmed suspicions and treatment with topical triple antibiotic (neomycin, polymyxin B, bacitracin / Trioptic-P®) was established. The only patient with glaucoma had an intraocular pressure (IOP) of 35 mmHg (Normal IOP is 12–25 mmHg in dogs and 12-27 mmHg in cats) and signs of pain and loss of sight. Mannitol was being considered but instead, the patient was rushed to an ophthalmologist as an emergency in the same day.

Four dogs and one cat were suffering from various concurrent disorders that it was not possible to categorize them, so they are referred as *Decompensated* patients. The 14-year-old feline was anorexic, dyspneic and tachycardic. Presented ascites and on X-ray showed a thoracic mass. One dog had both chronic kidney disease and congestive heart failure. Another was also in heart failure, had collapsing trachea, an abdominal mass and pyoderma. A patient had signs that indicated a central nervous system disorder and keratoconjuctivitis. Finally, another pet was an unregulated diabetic and showed symptoms of a thoracolumbar lesion (paraparesis) while an abdominal mass was also palpated.

Behavioral alterations were not an uncommon concern for caregivers but just a few times a medical approach was implemented. In the author’s presence four dogs were medicated for behavioral issues, two of them were Greyhounds. A specialized behaviorist had prescribed fluoxetine (Prozac®) to one of the retired racing Greyhounds that showed anxiety and an aggressive behavior. The other Greyhound was restless especially at night and the use of melatonin was elected. Treatment with amitriptyline (Elavil®) for two other dogs with anxiety was successful and clients were

pleased when contacted by phone. The use of melatonin also seemed to relieve the problem of the Greyhound with increased nocturnal activity.

Within all the patients examined, merely four classified as with a reproductive issue. Two dogs were diagnosed with a hyperplastic prostate and signs of prostatitis. One Bullmastiff had a, post-surgical, scrotal hematoma and finally one young cat that was brought in because of a “bloated belly” was confirmed that was gestating on X-ray which evidenced that the 45 days needed for embryo bone mineralization were reached [84].

The author classified as infectious only two cases. This can sound contradictory as within this report the involvement of an infectious agent in a pathologic process was signaled many times already. The reason behind this classification lies within the clinical presentation of the patients. As explained before, classification was organized based on clinical symptoms, diagnosis and the major complaint of the care giver. All other infectious diseases presented were classified based on the somatic system that was mostly affected. In these two cases though the difference was that the patients were in critical condition with multiple organ failure. One was a case of an unvaccinated Chinese Hairless Crested Dog with leptospirosis and the other a case of a cat with confirmed feline infectious peritonitis. Both pets were euthanized.

The only parasitic disease seen was that of a Spanish Galgo rescued, from the province of Seville in Spain that had a positive Leishmania antibody titer but was asymptomatic.

2.15. SURGERY

Treatment of a patient frequently escapes the reach of medical means. Although many could argue that is not true, surgery could be considered the most empathetic form of iatric intervention. It is one of the last resorts of science for life maintenance. If a good surgeon must be empathetic to his patients, then, a veterinary surgeon must take empathy to another level since his patients cannot express discomfort in words, before or after an intervention. Many cases are straight forward because the surgical table is the only root for treatment. The art of practicing medicine and surgery though, is based on decision making skills and the decision to “cut” or not, is one of the most important.

Table 16. Surgical procedures followed.

Procedure	*n*	*%*
Lumpectomy	6	**19.35%**
Ovariohysterectomy	5	**16.13%**
Orchiectomy	4	**12.90%**
Nodulectomy	2	**6.45%**
Otic Pinna Hematoma	2	**6.45%**
Cystotomy	2	**6.45%**
Bite Wound Repair	1	**3.23%**
Exploratory Laparotomy & Peritoneal Hemangiosarcoma Excision	1	**3.23%**
Inguinal *Hernia Repair*	1	**3.23%**
*Exotics (Cavia porcellus), Exploratory Lapa*rotomy & Ovariohysterectomy	1	**3.23%**
Laceration Repair	1	**3.23%**
Exploratory Laparotomy & *Splenectomy*	1	**3.23%**
Exploratory Laparotomy & *Enterectomy*	1	**3.23%**
Entropion Repair	1	**3.23%**
Cranial Cruciate Ligament Arthroplasty	1	**3.23%**
Femoral Osteoplasty	1	**3.23%**
TOTAL	**31**	**100.00%**

During the authors four-month externship, 31 surgical interventions were witnessed. Twenty-six dogs, four cats and one guinea pig were submitted to surgery (Table 16). As mentioned in the section of exotics, the case of the guinea pig is one of the two patients that are referred multiple times in this report (Figure 11). The CCL repair is also mentioned twice in this report.

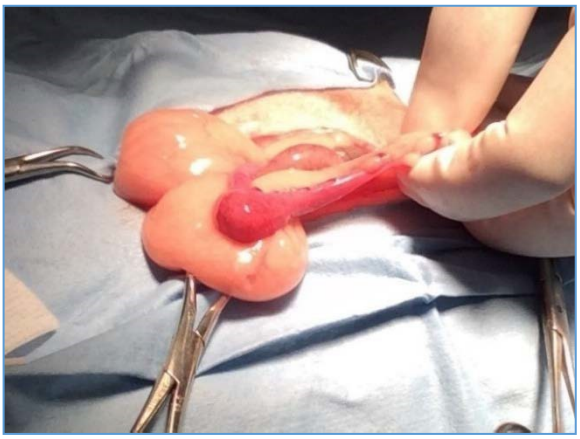

Figure 11 Exploratory laparotomy in a guinea pig. The uterus and ovaries found with growths were removed

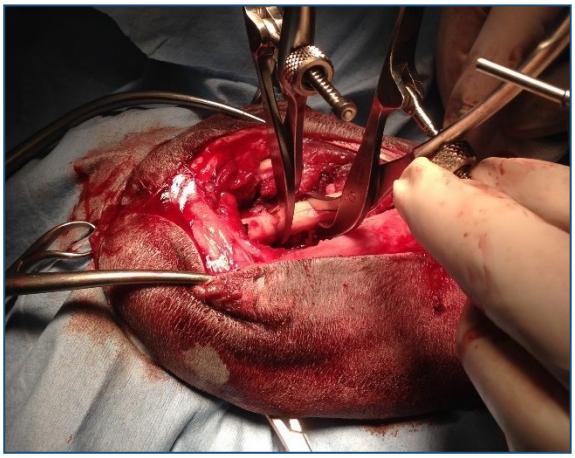

Figure 12. Osteoplasty of the right femur of a cat.

One cat underwent an exploratory laparotomy and enterectomy. A histopathology report showed intestinal lymphoma. The only osteoplastic surgery followed, was a femoral osteoplasty on an adult cat (Figure 12). The fracture was comminuted but reducible. Initially, the surgeon tried to stabilize with a plate. The angle of the fracture lines though would not permit it. Eventually, an intramedullary pin, tree cerclage wires, three proximal (two monocortical, one bicortical) and four distal bicortical screws had to be used. The other two case of felines mentioned, were elected ovariohysterectomies.

A very interesting imbrication technique for a CCL rupture repair was followed, as mentioned in the musculoskeletal disorders section. Two cystotomies were performed and various uroliths were extracted by urohydropropulsion. An inguinal hernia repair was performed on a male Jack Russel. A neck wound bite repair, needed various visits until successful closure, due to poor owner compliance with post-surgical care. Ear pinna hematomas were repaired with the use of X-ray film plates.

A meticulous pre-surgical exam was executed on an English Bulldog with entropion. Evaluation of the skin folds and determination of a plan for the intervention was imperative for a good result. Various vertical and horizontal folds were reduced on the frontal and zygomatic skin surfaces of the dog, which appeared more like a Shar-pei before the surgery (Figure 13). The outcome had a remarkably positive effect on the life quality of the pet that could finally see and ambulate freely. Ceasing corneal irritation, from the previously inverted eyelids, induced reduction in lacrimation while reduction of skin folds gave a cleaner skin with less risk for skin infections. OVH's and orchiectomies were performed daily in cats and dogs. "A&A", once a year sterilizes all retired racing Greyhounds, rescued by a local cynophile association (Grateful Greyhounds), in week-long marathon of spays and neuters.

Figure 13 Initial incision during entropion correction, (B) final incisions and (C) final aspect of the patient.

2.16. REEVALUATION APPOINTMENTS

Amongst the 514 contacts that the author had with patients, 41 of them were reevaluation appointments. In Table 17 there are listed all reevaluations divided by area of interest. In the exotics, the two animals examined were a ferret with insulinoma and a water snake with a facial inflammation. In Chart 4, there is a comparison of the areas of interest related to the species examined.

Chart 4. Reevaluations by species

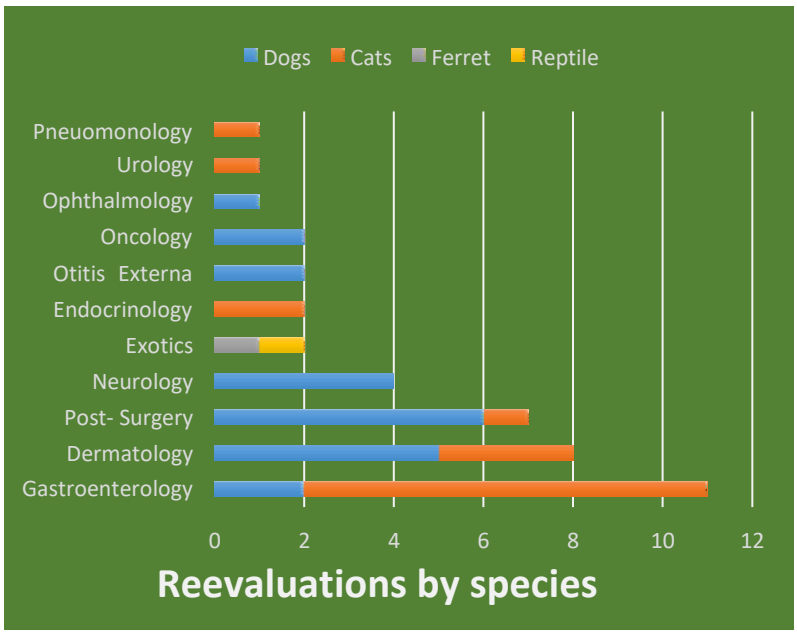

Table 17. Reevaluations by Area

Area	*n*	*%*
Gastroenterology	11	**26.83%**
Dermatology	8	**19.51%**
Post- Surgery	7	**17.07%**
Neurology	4	**9.76%**
Exotics	2	**4.88%**
Endocrinology	2	**4.88%**
Otitis Externa	2	**4.88%**
Oncology	2	**4.88%**
Ophthalmology	1	**2.44%**
Urology	1	**2.44%**
Pneumology	1	**2.44%**
Total	**41**	**100.00%**

3. Chapter II:

3. Appendicular Osteosarcoma in Retired Racing Greyhounds

3.1. CANCER MAKES NO DISTINCTION BETWEEN SPECIES

A *tumor* is any tissue mass or swelling and may be neoplastic or not, although today is a generic term used to describe any neoplasm. *Neoplasia* is the abnormal growth of a tissue into a mass that is not responsive to normal control mechanisms and may be benign or malignant. *Cancer* refers to a malignant neoplasm [85].

There is no doubt that cancer is recognized as a grave and fatal illness in all sociocultural layers in the developed and most of developing countries around the globe. Human suffering from its effects strikes almost every family or social group. In contrast, maybe, to popular belief that cancer is a disease of the modern world, there is evidence of its diagnosis in the oldest known surgery textbook. An Egyptian papyrus dating around 1600 BC (or 3000 BC) known as the Edwin Smyth Papyrus relates a breast cancer case and that had no treatment [86, 87]. The name for the disease though, is attributed to Hippocrates from the Greek word "*karkínos*" (translates to "crab") that later was adapted to Latin by Celsus.

Cancerigenous processes affect also animals except humans and many of these processes are identical across different species. Today, the genetic basis of the disease is widely accepted. Also, in multicellular organisms cellular division, proliferation, differentiation and apoptosis mechanisms are regulated in the same way, therefore, there is a huge advantage in engaging firmly to comparative oncology. Factually, in the early 20th century it was studies on animal models that evidenced the relation between some substances and cancer. In 1915, Katsusaburo Yamagiwa and Koichi Ichikawa at Tokyo University, induced cancer in lab animals for the first time by applying coal tar to rabbit's skin. Most importantly in 1911 was discovered that viruses can cause cancer by Payton Rous at the Rockefeller Institute in New York. The virus that causes sarcoma to chicken, is still known as the Rous sarcoma virus. Along with the discovery of the chemical structure of the DNA by Watson and Crick in the 60's, there was enough knowledge to conclude that chemicals, radiation and new gene sequences, introduced by viruses, could lead to genetic alterations and tumorigenesis [88].

Nevertheless, it is incorrect to assume that the environment is responsible, alone for tumors, as there is experimental evidence that supports that intrinsic mutagens can interact with environmental causes to promote cancer [49]. For example, oxygen free radicals that result from chronic inflammation can act as procarcinogenic mutagens. An equally important mutagen, is the inherent error rate of enzymes that control DNA replication, which introduces from one in 10,000,000 to one in 1,000,000

mutations, for each base that is replicated, during each round of cell division[49]. Mammalian genomes include two to three billion base pairs, so after a cell division, each daughter cell, carries at least a few hundred mutations in its DNA. Most mutations though, are silent. Others can disable tumor suppressor genes or activate proto-oncogenes that are oncologic promoters. Also, the concept of "mutator phenotypes", which increases cancer predisposition to individuals that carry it, might explain why not all people or animals, exposed in similar environmental carcinogens, develop the same rates or forms of cancer [49].

Scientific advances, in the last two decades, gave fertile ground for current research that uses gene expression profiling and proteomic approaches, to focus on pathways, that help metastatic cells survive and proliferate in distant sites from the primary tumor. With the release of the canine genome sequence, the dog is now also amenable to comparative genomic analysis. Indeed, assessment of the canine genome suggests that, the dog and human lineages are more similar than the human and rodent lineage, in terms of both nucleotide divergence and rearrangement [89].

As it seems, there is big interest in using canines as platform for studies on new therapeutic goals that could benefit both humans and pets. Furthermore, in dogs and other domestic animals, the coexistence of closed populations we call "breeds" along with animals of mixed breeding, can demonstrate how homogeneous genetic background influences cancer, in out-bred populations. Finally, it is widely understood that among cancer seen in dogs and humans, many of the strongest similarities exist between canine and pediatric osteosarcoma [90]. It is important to say so for underlying that during the editing of this bibliographic review many novel studies are in progression through the blossoming field of comparative oncology. Funded clinical trials, which are currently under way, could engage a big chunk of caregivers of osteosarcoma patients [91]. As such, the reader is encouraged to be vigilant for new publications to come on canine osteosarcoma (OS).

3.2 CANINE OSTEOSARCOMA

August 8th of 2016, was the first day that the author followed the veterinarians at "A&A" as an extern. The friendly introductory conversation was interrupted, as the first appointment arrived. The patient was a neutered male, white-brindle Greyhound, named Scoops that was eight years old. Scoops was a Retired Racing Greyhound (RRG) that has been adopted by his caregivers in 2012, after four years of a competitive career. The beautiful, athletic dog came in limping on his front left, with a big, lime sized, mass on the antebrachium (Figure 14). At this point, there has already been a discussion about the topic of the case study, that the author could choose with Dr. Anastasiou. Based on the caseload of the hospital, canine OS in Greyhounds, was the first suggested. As follows, the unexpected sight of a clinical presentation so characteristic of OS and on the first patient, pushed for some medical enthusiasm. Unfortunately for Scoops, the suspicion of the clinician was confirmed with

radiographs and for the author, the idea to study OS in RRGs, had gained “flesh and bones”. Bone lysis and proliferation on the distal diaphysis of the radius, was evidently the cause of pain resulting in wide open eyes and dropped head (Figure 15). The caregivers said that after an insignificant bump while running, the dog’s leg, had swollen ever since. They were a retired couple that have been adopting RRGs, many years now, and one of their past adoptees was also diagnosed with OS. So, they were familiar with the detrimental effects of the disease. This was the last time that Scoops was seen at “A&A” since the couple moved to Virginia. They refused any treatment except of NSAIDs.

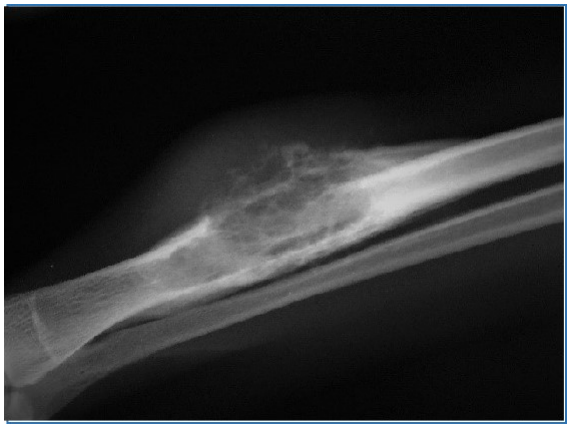

Figure 14. Lateral projection of the left radius. Cortical lysis, bone proliferation and elevation of the periosteum

Figure 15. Scoops on the Rx table. Note the mass on the left antebrachium

In a web-based health survey on RRGs, in 2007, 747 dogs were used to assess that mortality rate in a two-year period was 15% (113 of 747). The most common cause of death reported was cancer (66 dogs, 58%), and the most common type of cancer listed as the cause of death was OS (28 dogs, 25%). Finally, forty-five percent of Greyhounds was diagnosed with cancer and 6% of the overall population had OS. In the United States, 120,000 Greyhounds live in homes as pets as compared to 55,000 Greyhounds in racetracks. In the past few years, private Greyhound adoptions ranged from 15,000 to 18,000/year [92]. Considering the high number of deaths due to OS (one out of four) and how many adoptions take place yearly, it is of great importance that clinicians can diagnose and deal with OS and that are familiar with the hematologic and biochemical particularities of Greyhounds.

Among the six patients with OS there was one Pitbull and five RRGs. Scoops moved to another state and two patients that were seeking a second opinion, returned to their regular veterinarian. Additionally, the author, recovered an interesting case from “A&A” records. In conclusion, there will be three cases to discuss about. Initially though, osteosarcoma will be approached regarding the canine species as a whole and not solely related to RRGs.

3.2.1 Clinical Characteristics

Osteosarcoma is an aggressive neoplasm resulting in early mortality because of rapid progression. It is one of the most malignant tumors in veterinary medicine, accounting for 85% of all bone

tumors[93]. The outcome, is almost invariably fatal even with early detection of small masses. Differential diagnoses include: other primary bone tumors (malignant and benign), secondary bone tumors, mycotic and bacterial osteomyelitis, fracture, periostitis, arthritis, hypertrophic osteopathy, and metaphyseal osteopathy [93-95]. Remarkably, the clinical presentation of Scoops was a textbook model. Most recent textbooks, that the author consulted refer that there may be a history of a mild trauma prior to onset of lameness and a painful swelling can arise at the site. Signs, are associated with the site of the tumor but pain is a common feature in all presentations. Painful mouth opening and dysphagia is common in mandibular OS, facial deformities in nasal OS and pain on palpation on rib OS. Moreover, even in advanced metastatic disease in the lungs, respiratory signs are rare [96]. Chronic irritation, associated with osteomyelitis, or the presence of an internal fixation device, are occasionally linked to tumor development [93].

As 75% of OSs occur in the appendicular skeleton, there is frequently lameness at presentation. That, can be acute and severe in case of a pathologic fracture that yet is uncommon, accounting for just three percent of fractures seen in long bones [93, 96]. The metaphysis of long bones is usually affected and the forelimbs 1.6 to 1.8 times more often, than the hind limbs. This ratio is equal to the weight distribution between anterior and posterior body [93]. It is extremely rare for OSs to be in bones next to the elbow (Figure 16). At "A&A", the saying, "*Away from the elbow and towards the knee"* was of common use. Effectively, around 60% of the OS follows that rule. The distal radius and proximal humerus, were the only sites affected in the six cases at "A&A". Generally, in the rear limbs, tumors are evenly distributed between the distal femur, distal tibia, and proximal tibia, with the proximal femur a less common site. Primary OS, distal to the antebrachiocarpal and tarsocrural joints, is rare in dogs (Figure 16). Axial sites, commonly reported, are the mandible (27% of all axial OS), maxilla (22%), spine (15%), cranium (14%), ribs and nasal cavities (10% each) and pelvis at 6% [97]in[96]. Reports of primary OS exist in the following sites: os penis, patella, mammary tissue, subcutaneous tissue, spleen, bowel, liver, kidney, testicle, vagina, eye, gastric ligament, synovium, meninges, and adrenal gland [96].

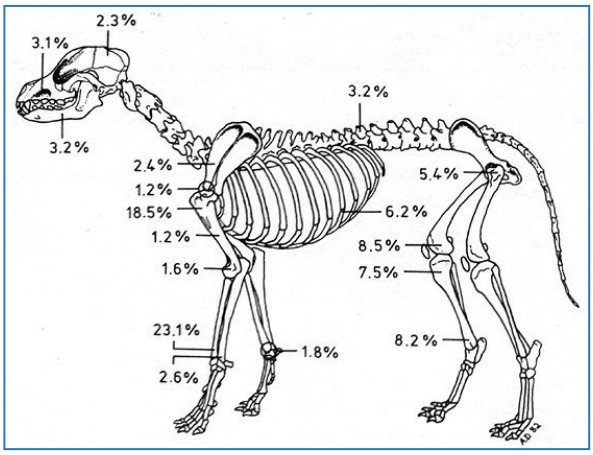

Figure 16. Distribution of 1215 primary OSs. Adapted from Kistler KR, UPenn,1981.

Reportedly, there is relation between internal metal fixators and occurrence of OS [96]. There is a study that associated tibial plateau leveling osteotomy (TPLO) and OS with a median of 5.3 years post TPLO diagnosis of OS in 29 cases [98].

As in human cancer patients, energy expenditure, protein synthesis, urinary nitrogen loss, and carbohydrate flux alterations have all been documented in dogs with OS. Also, metabolic derangements of unknown impact include, low chromium, iron and zinc levels, lower iron-binding capacity and increased ferritin levels. However, the most clinically relevant systemic alteration, with a prognostic value, are the levels of total (ALP) and bone-specific alkaline phosphatase (BALP). Median survival times (MST) for dogs, with normal or increased ALP activities before treatment, were 12.5 and 5.5 months, respectively, while for BALP activities before treatment MSTs were 16.6 and 9.5 months [99].

3.2.2 Incidence and Risk Factors Associated with Breed, Size, Age and Sex.

OS relates to 85% of skeletal malignancies while a, probably underestimated, calculation results in 10.000 canine OS cases per year in the US [100].

OS is primarily a disease of large and giant breeds. Breeds with a high risk of developing OS, except of RRGs, include: Saint Bernard, Irish Wolfhound, Great Dane, Irish setter, Rottweiler, German Shepherd, Doberman Pinscher, Boxer, and Golden Retriever. In one study, breeds weighing more than 36kg (giant) and large breeds (18– 36kg) were shown to be 60.9 and 7.9 times respectively, more at risk, of developing primary bone sarcomas than breeds weighing less than 9kg [93]. In a more recent and large study, the risk of osteosarcoma was associated, more consistently, with increased height than increased weight [101]. In a review of 1462 OS cases, only 5% of the dogs weighted less than 15kg but almost 60% of them had an axial OS.

The tumor occurs in middle-aged to older dogs with a median age of around seven years, but the range is broad and there is a small peak in incidence at two years of age. Primary osteosarcomas of the ribs are reported to occur in young adult dogs with a mean age of 4.5– 5.4 years and dogs of giant breeds tend to develop osteosarcomas at a younger age than dogs of smaller breeds [93].

There are conflicting reports on sex predilection. Some studies, state that males are slightly more affected and other studies give a ratio of one to one. Still, there is some consensus that gonadal exposure is inversely related to incidence rates of OS independently of height and weight. Some authors, give a twofold risk increase in gonadectomized pets while in Rottweilers, gonadectomy before 12 months, resulted in 25% chance of a bone sarcoma during lifetime [96, 101, 102].

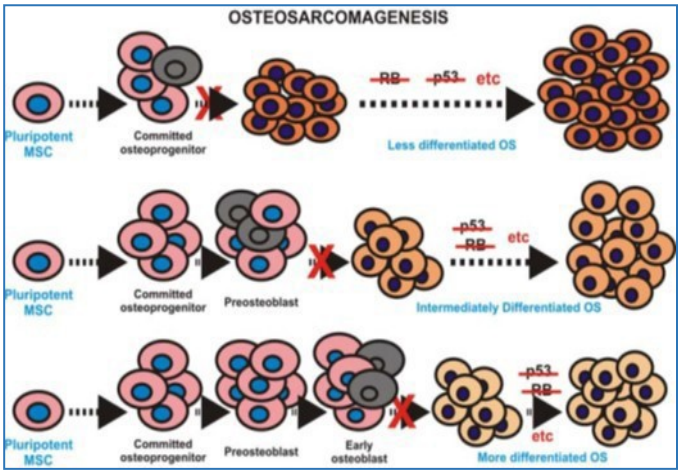

Figure 17. MSC: mesenchymal stem cells, p53 and RB: tumor suppressor genes, (©The Association of bone and Joint Surgeons)

3.2.3. Etiology

The etiology of canine OS is specifically unknown. There is speculation about viral causes but no isolated virus exists[96]. OS, can be regarded as a differentiation disease that is caused by genetic and epigenetic disruptions of osteoblast terminal differentiation. Because of the numerous genetic and epigenetic alterations, that will be discussed below, OS tumors, exhibit the characteristics of undifferentiated osteoblasts [103]. Additionally, there is evidence that osteocytes which are terminally differentiated osteoblasts, can serve as a progenitor cell for osteosarcoma [104]. Disruption of osteogenic differentiation may lead to OS development while mutational defects at the early stages may lead to the development of more undifferentiated and aggressive OS (Figure 17).

3.2.3.1 Genetic and Molecular Factors

The influence of genetic variability to disease is major topic of research in the last decade. Genome-wide association studies, seek to detect single nucleotide polymorphisms (SNPs) that contribute to cancerigenesis. The mutational landscape of osteosarcoma, is complex and varies significantly between tumors. Unlike many other sarcomas, osteosarcoma lacks a canonical translocation or genetic mutation and is typified by widespread and heterogeneous abnormalities in chromosomal number and substructure [105]. However, classically defined modes of genetic mutation are known to occur in osteosarcoma. For example, point mutations are likely the result of errors in DNA replication and subsequent proof reading, whereas aneuploidy is the result of errors in chromosomal segregation during cell division. In addition to these modes of genetic mutation, another mechanism of mutation acquisition, known as chromothripsis, has recently been identified [105]. A phenomenon, by which a lot of genomic rearrangements occur during cancer development in a cellular crisis. An important paper, published in 2011, demonstrated that chromothripsis occurs in at least 2–3% of all cancers and approximately 33% of OS[106].

In dogs, the most thoroughly described gene mutation, that contributes to OS formation and progression in dogs, is on gene *p53*. The *p53* gene's product, plays an important role in maintaining genomic stability and forms part of a stress response pathway to various exogenous and endogenous DNA damage signals, including gamma irradiation, UV irradiation, chemicals, and oxidative stress [107]. *P53* acts as a tumor suppressor, in essentially all tumor types, and its function can be affected by mutations to the gene itself or by mutations to up- or downstream mediators of its activity [105]. Studies in immortalized canine OS cell lines, demonstrated that the gene was defective and that *p53* mRNA and *p53* protein were overexpressed in 60% of cell lines. Studies, in spontaneously arising OS, that used single-strand conformational polymorphism and polymerase chain reaction (*PCR*), followed by nucleotide sequence analysis, identified that 24 to 48% samples had mutations of *p53. PCR* assessing of the entire *p53* sequence resulted in 41% of the tumors there were mutations. In their majority were point substitutions of amino acids. The normally labile *p53* nuclear phosphoprotein when enhanced by mutations, gains stability that enable detection through immunohistochemistry

methodologies. Studies have showed that overexpression of p53 protein is more significant in appendicular OS and markedly higher in Rottweilers [96]. A recent study, investigated isoforms of *p63* and *p73* which are transcription factors, belonging to the *p53* family. *TAp63* isoforms, suppress metastasis through induction of senescence. In contrast, *ΔN* isoforms, including *ΔNp63*, oppose *TAp53-*, *TAp63-*, and *p73*-mediated transcription, and therefore apoptosis and cell cycle arrest, by blocking target promoters or by forming inactive heteromeric complexes. Real-Time Quantitative Reverse Transcription *PCR* (*qRT-PCR*) assays, designed to distinguish *TA* from *ΔN* isoforms of *p63,* proved that approximately 90% of primary canine OS tumors express high levels of the ΔNp63 isoform when compared to that found in normal osteoblasts. Furthermore, *ΔNp63* was highly expressed in three out of the four cell lines examined [108].

One of the first tumor suppressor genes to be signaled was the *Retinoblastoma* (*RB*) gene. Suppressor mutations are recessive, that is why some texts refer to tumor suppressor genes as recessive oncogenes. *RB* has one normal allele that usually produces enough tumor suppressor product to continue as normal.[107] Disruption of *RB* function has been found to be a common feature of many cancers, not only retinoblastoma and it seems that can be permissive for OS. Authors state that it is probable that aberrations in the *RB* gene can contribute sporadically to OS formation and progression [96]. RB function depends, at least in part, on interactions with the *E2F* family of DNA-binding transcription factors (*E2F*)[109]. It has been lately discovered that in tumors from patients with worse prognosis the *RB-E2F* pathway is dysfunctional [110].

Another tumor suppressor gene, the phosphatase and tensin homolog (*PTEN*) is speculated to be involved in OS. In vitro studies, of canine OS cell lines, showed that 60% contained mutations on *PTEN [96].* In two projects on spontaneously arising OS, specific chromosome copy number aberrations were identified. In these studies, the use of targeted microarray-based comparative genomic hybridization studies, indicated aberrations, in 42% and 30% of the samples respectively [111, 112].Overexpression of other genes were individualized in one of those studies (genes of *Runt-related transcription factor 2 and Ras homolog gene family, member C : RUNX2* ,*RHOC*)[111]

The cell cycle is mediated by sequential activation and deactivation of a class of proteins called *cyclin dependent kinases* (*CDKs*) [107]. One of the most important studies found 33 inherited risk loci for OS, accounting for 55–85% of phenotype variance, within a breed. This study evaluated, through genome-wide association analyses, three breeds: Greyhounds, Rottweilers, and Irish wolfhounds. The *SNP* with the strongest association with OS development, was found in Greyhounds and was located 150 kilobases (*kb*) upstream of the *CDK Inhibitor 2A* and *2B (CDKN2A/B)* genes. Both *CDKN2A* and *CDKN2B* function as cell cycle regulators and tumor suppressors and play a key role in OS development and progression [113]. One risk allele, tagging the top Greyhound locus on chromosome 11, was tested by imputation and association of sequenced variants. This narrowed the peak of association in Greyhounds to a 15*kb* risk haplotype which is telomeric of the genes *CDKN2A*

and *CDKN2B*. This allele, is nearly fixed in both the Rottweilers (98% in cases and 96% in controls) and Irish Wolfhounds (95% in cases and 92% in controls) which could relate to the higher incidence of OS in these breeds [113]. The results of a recent but small study, on the *p16* gene's product expression, which is also of the *CDK Inhibitor* family, did not reach statistical significance but found just a trend between the lack of *p16* expression in canine appendicular OS lesions and a shorter Disease Free Interval (DFI) [114].

Proto-oncogenes, are cellular oncogenes that do not cause tumorigenesis in their native state but when altered can provoke malignancy. The conversion of a proto-oncogene to an oncogene results from somatic events in the genetic material of the neoplastic-to-be tissue. The activated allele of the oncogene dominates the wild-type allele and results in a *dominant gain of function.* Meaning that, in contrast to tumor suppressor genes, only one allele is needed for phenotypic changes to occur [107]. The *hepatocyte growth factor receptor* (*HGR* or *MET)* proto-oncogene, has been shown to take part in OS formation and progression. Studies in both cell lines and spontaneously arising OS, through *northern blot analysis* and *RT-PCR* respectively, detected expressions of *MET/HGR,* while Rottweilers were, once more, signaling higher rates. Regional lymph node metastasis was also related to higher expression of *MET/HGR* [96].

Studies to evaluate the effects of overexpression of the Insulin-like *Growth Factor-1 (IGF-1)* have resulted to ambiguous conclusions. The cellular effects of growth hormone are regulated by *IGF-1* which in osteoblasts induces to mitogenesis and protection from apoptosis. Even if the involvement of *IGF-1* has been reported in canine OS, it appeared to be cell line specific and no clinical benefit has been proven in dogs treated with a long-lasting somatostatin analog [96, 115]. However, in a recent study of 34 naturally occurring OSs, immunohistochemical data showed that IGF-1R was expressed in 71% of the analyzed osteosarcoma samples [116]. The *erb B-2 proto-oncogene,* related to *human epidermal factor receptor-2,* was found to be overexpressed in OS cell lines and in 40% of primary tumors. Bigger studies are needed to validate these results [117]. Recently, a small study investigated the immunohistochemical expression of *Bcl-2* (*B-cell lymphoma 2),* through the use of a specific antibody, in spontaneous high grade canine non-metastatic OS [107]. The *Bcl-2* gene, encodes for a protein that is found on the outer membrane on mitochondria and plays a role in proapoptotic and apoptotic mechanisms. Its overexpression has been linked to some human tumors. There was an overexpression of *Bcl-2* in all the cases of this study in Perugia.

The *mammalian target of rapamycin (mTOR) pathway* aberrant signaling, promotes growth and chemotherapy resistance to many tumors. Studying how its downstream effector protein, *p70S6K* and *mTOR* can be effected by rapamycin, resulted in an inhibition of phosphorylation and consequently to apoptosis and reduced OS growth [118]. Another hint came from expression and inhibition studies, on *tropomyosin-related kinase* proto-oncogenes, which gave indication that this pathway, also, influences OS and can be targeted in with new drugs [119]. The enzyme telomerase,

can grant cells with infinite replicative capacity by catalyzing the addition of telomeric sequences at the 3´ ends of chromosomes. An enhanced replicative capacity is one of the *"Hallmarks of Cancer",* as stated previously, and it has been demonstrated that OS cells possess this ability endowed by telomerase [120].

Osteolysis and tissue invasion, are the foundation of OS progression while metastasis seems to be related to the ability that OS cells have in interacting with their microenvironment, both in bone and lung tissue [96]. Matrix metalloproteinases (*MMPs*), are a group of enzymes that handle the degradation of most extracellular matrix proteins during organogenesis. The expression and activity of *MMPs,* increases significantly in various pathological conditions that may lead into unwanted tissue destruction, such as inflammatory diseases, tumor growth and metastasis [121]. Canine OS cell lines were established from spontaneous pelvic and radial Oss. Zymography, showed that the cell lines produce high levels of *MMP-2* and *MMP-9*. These are enzymes directly involved in crucial aspects of the metastatic process[122]. RANK is a Receptor Activator of Nuclear factor Kappa-B *(RANK).* RANK-ligand *(RANKL),* modulates osteoclastogenesis*,* resulting in homeostatic bone resorption during health. Skeletal tumors of dogs, express *RANKL* and induce malignant osteolysis. In 15 canine OS (15/45) samples, relative *RANKL* expression was correlated with radiographic characteristics of bone pathology [123]. Lysosomal Cathepsin K is a protease with collagenolytic activity, and its secretion by osteoclasts is responsible for degrading organic bone matrix. A study found that canine OS cells contain it within their cytoplasmic vesicles [124]. Ezrin, is a protein that links the actin cytoskeleton with the cell membrane and has prognostic significance in tumor progression, due to its role in the metastatic process. Ezrin's phosphorylated form, in dog samples, was abundant, as it emerged from immunohistochemistry and western blot techniques[125]. Furthermore, there was found a significant association between high ezrin expression and poor outcome in pediatric osteosarcoma patients [126]. A study, evaluating the involvement of *cyclooxygenase-2* (COX-2) gene expression on stem cells, found that inhibition of *COX-2* in daughter cells prevented sphere formation, indicating a potential significant role for *COX-2* in tumor initiation [127]. Finally, the chemokine receptor CXCR4 participates also in metastatic OS progression[96].

3.2.3.2 Epigenetic Factors

Dr David Allis, runs a laboratory at Rockefeller University, in New York and is one of the leading scientists to explore the field of epigenetics. His work in the last 15-20 years has uncovered that besides the genetic, there is also, an epigenetic code that can be passed to the descendants. Epigenetics, can be considered the study of regulatory mechanisms affecting the expression of DNA templates without altering the sequence of the templates themselves [105]. In a simplistic explanation, epigenetics study the manner in which DNA is coiled or uncoiled, methylated or demethylated. The most well-described epigenetic mechanisms involved in cancer biology include DNA methylation, histone modification, nucleosome remodeling, and RNA-mediated events.

In human OS, promoter hypermethylation and consequent reduced gene expression have been demonstrated at the *p16INK4a* locus, which is a member of the *RB* pathway. Similar to *RB*, the primary mechanism of direct *p53* loss of function, in OS, seems to be genetic. However, expression levels of multiple members of the *p53* pathway are subject to promoter hypermethylation.[105] Normal expression, was restored through treatment with the DNA demethylating agent 5-aza-2'-deoxycytidine (decitabine), in both pathways, in OS cell lines. DNA methyltransferase, is an enzyme involved in epigenetic dysregulation when oncogenes are activated. It can be downregulated *in vitro* by ibandronate[105]. Furthermore, there are indications that histone modification derived from dysregulation of the *RB* and *p53* pathway may play a role in OS[105].

Importantly, for canine OS, there is evidence that non-coding micro RNAs *(miRNA, miR)*, which are epigenetic regulators, act to post-transcriptionally silence large numbers of genes. Dysregulated miRNA expression, results in aberrant expression of OS genes that play critical roles in tumorigenesis and progression [105]. MiR-9, was identified as being significantly overexpressed in canine OS tumors and cell lines compared to normal osteoblasts. Additionally, high miR-9 expression was demonstrated in tumor-specific tissue obtained from primary OS tumors. This study, proved that miR-9 promotes a

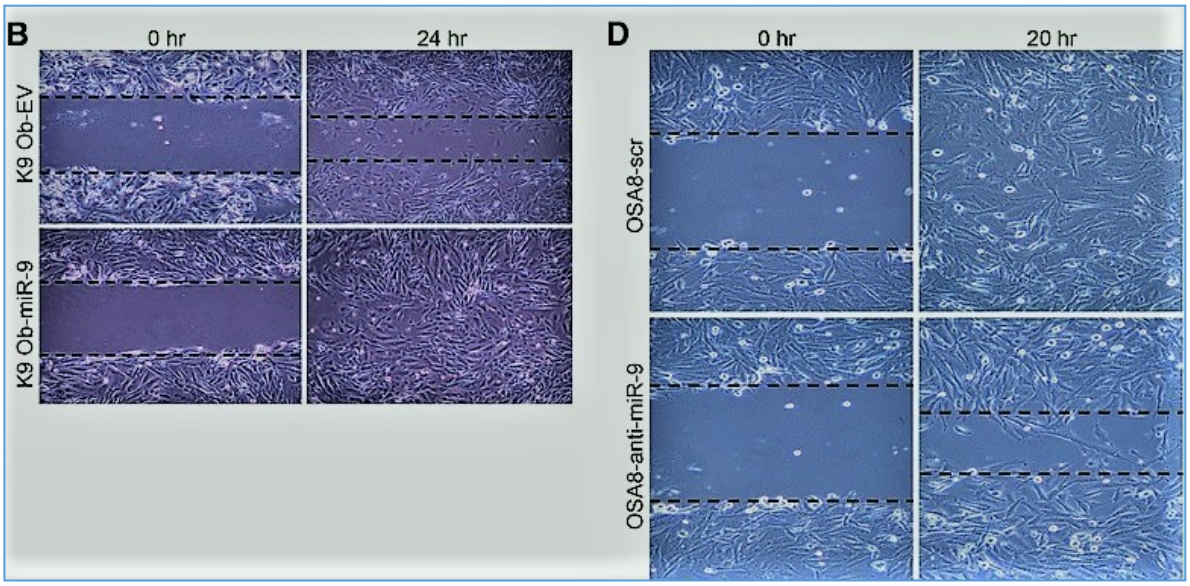

*Figure 18 MiR-9 enhances invasion and migration in normal canine osteoblasts and OS cell lines. B: Cell migration was assessed in canine osteoblasts transduced with either empty vector (K9Ob-EV) or pre-miR-9-3 lentivirus (K9Ob-miR-9) using standard wound-healing assays. After 24 h, digital photography evaluated cell migration. D: Cell migration was assessed in OSA8 cells transduced with miRZip-9 (anti-miR-9) or scramble vector (OSA8-scr) using standard wound-healing assays. After 20 h, digital photography evaluated cell migration. By Fenger, J.M., R.D. Roberts, O.H. Iwenofu, M.D. Bear, X. Zhang, J.I. Couto, J.F. Modiano, W.C. Kisseberth, and C.A. London, MiR-9 is overexpressed in spontaneous canine osteosarcoma and promotes a metastatic phenotype including invasion and migration in osteoblasts and osteosarcoma cell lines. BMC Cancer, 2016. **16**(1): p. 784*

metastatic phenotype in normal canine osteoblasts and malignant OS cell lines and as well enhances invasion and migration (Figure 18) [128].

3.2.4 Gross Morphology and Diagnostic methods

Most Osteosarcoma (OS), originates within the medullary canal of bones (intraosseous, central or medullary OS) in contrast with bone surface osteosarcoma like periosteal or parosteal OS that arise from the periosteum and is uncommon.

The gross morphology of central OS is characterized by variable bone lysis, reactive bone on the endosteum and periosteum, and neoplastic bone. Cases can show one or all of these elements. Frequently, there is cortex erosion, necrosis and extended areas of hemorrhage. An important characteristic is that OS, rarely crosses a joint to affect other bones[93]. Highly productive OS may present as dense, bony swellings involving flat or tubular bones. In this case, blood supply disruption causes neoplastic and normal bone to form around the diaphysis giving the impression of osteomyelitis[93]. OSs where osteolysis predominates, destroy bone rapidly, may be advanced already when diagnosed and, if not treated, often local progression is the cause of malaise and death, instead of metastasis (Figure 20). However, there is no evidence of higher metastatic rate of lytic

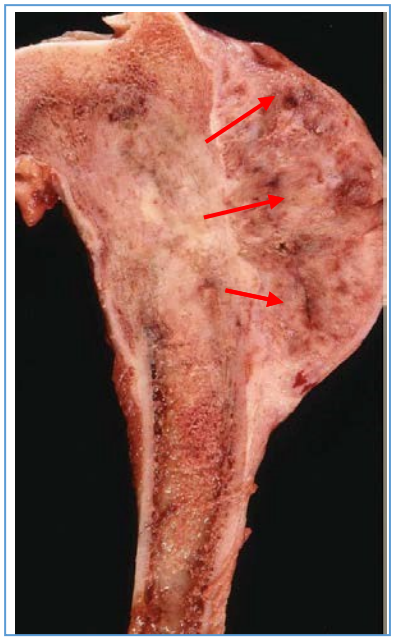

Figure 19 Proliferative tumor of a proximal humerus with abundant matrix that in cytology would yield only few cells. Adapted from Donald J. Meuten. Tumors in Domestic Animals Wiley. Kindle Edition . Red: Reactive and Tumor bone (Image was courtesy of S.S. Couto and the School of Veterinary Medicine, UC Davis.)(95)

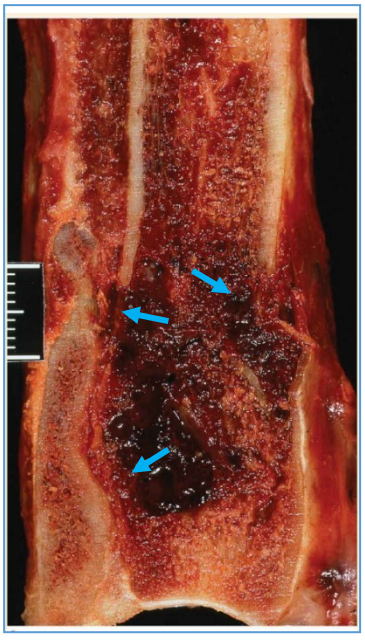

Figure 20 Telangiectatic OS on a distal radius. : Blue: Cortical lysis Note the lack of reactive bone due to the rapid progression of lysis and hemorrhagic lesions. Frequently mistaken for hemangiosarcoma. Adapted from Donald J. Meuten. Tumors in Domestic Animals Wiley. Kindle Edition (Image was courtesy of R.A. Fairley.)(95)

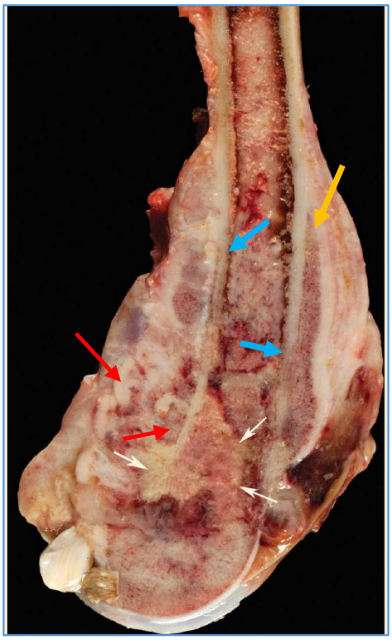

Figure 21 Arrows: *Blue: Cortical lysis. White: Necrosis. Yellow: Reactive bone and Codman's triangle. Red: Reactive and Tumor bone. Mixed lesion on a distal femur. Adapted from Donald J. Meuten. Tumors in Domestic Animals Wiley. Kindle Edition. (Image was courtesy of S.S. Couto and the School of Veterinary Medicine, UC Davis.)(95)*

tumors in comparison to mixed lesions. Factually though, giant-cell lytic OS, is slower in progression. Mixed or proliferative lesions are slower to advance also, permitting the formation of periosteal containment in response to cortical destruction[93](Figure19 and 21).

3.2.4.1 Cytological and Histopathological Means of Diagnosis

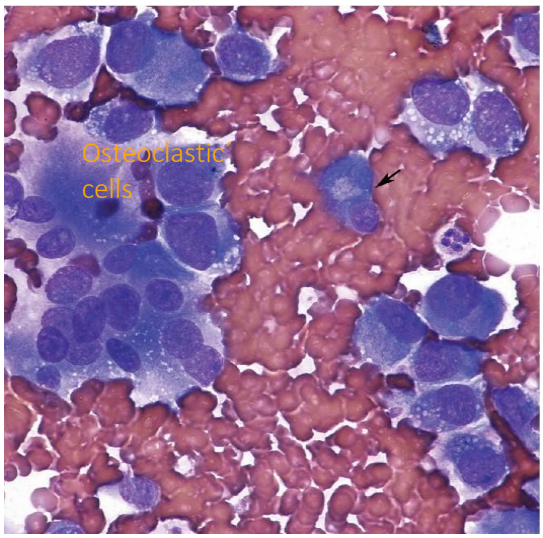

Figure 22 Leishman's stain. Arrow: One cell with amphibolous reactive or neoplastic origin. Many erythrocytes. Adapted from Donald J. Meuten. Tumors in Domestic Animals Wiley. Kindle Edition (95)

Malignant tumors of mesenchymal origin are termed as *sarcomas.* The prefix *Osteo-* indicates that bone cells are responsible for this type of cancer. Primitive bone cells that multiply in an unregulated manner can also produce an extracellular matrix, called *osteoid,* often accompanied by areas of *chondroid* and fibrous differentiation[96]. An enzyme histochemical stain, for alkaline phosphatase, can help identify cells of osteoblastic lineage in cytological diagnosis but it is the matrix's (tumor osteoid) presence, in histological sections, along with cellular morphology that determines diagnosis and differentiation of OS, from other bone neoplasms. Malignant bone neoplasms, present structural

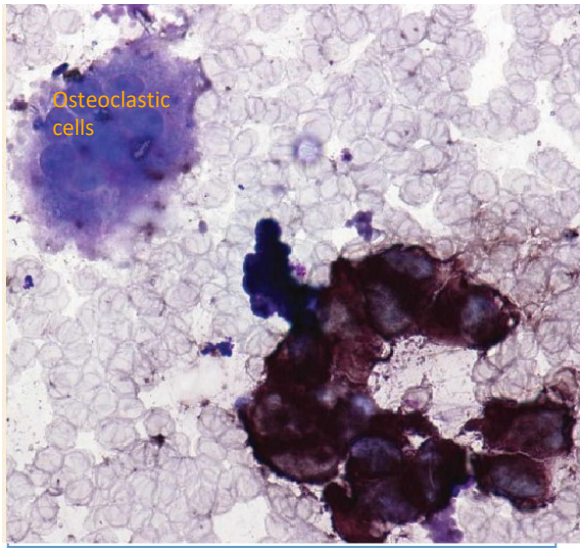

Figure 23 ALP stain of the same preparation on Figure 22. OS diagnosis. Adapted from Donald J. Meuten. Tumors in Domestic Animals Wiley. Kindle Edition (95)

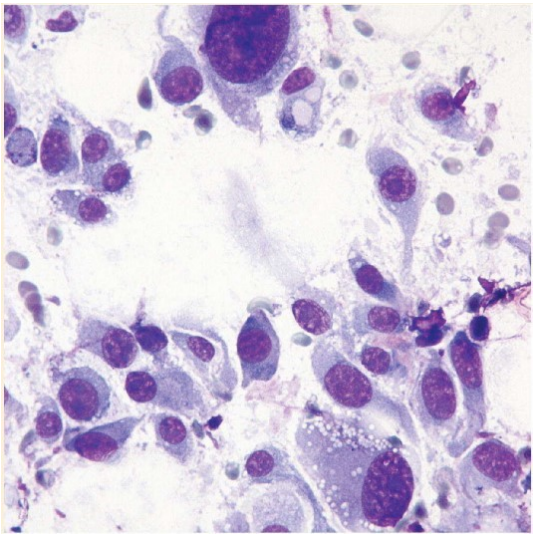

Figure 24 Anisocytosis and anisokaryosis in confirmed OS. Scarce pink islands of osteoid are clear among cells. Adapted from Donald J. Meuten. Tumors in Domestic Animals Wiley. Kindle Edition (95)

specializations that can range from sarcomas of undifferentiated mesenchyme to sarcomas closely mimicking one of the well-differentiated types of connective tissue, such as bone or cartilage. That is why, small biopsy samples can lead to erroneous diagnosis[93]. Reactive bone, chondrosarcoma, fibrosarcoma and hemangiosarcoma, can be misdiagnosed instead of OS due to great variability of histologic patterns within the same tumor and between other tumors (Figures 19, 20,21).

Even if cytology is less efficient than histopathology it can be of great use, especially, if clinical presentation and radiographic findings converge towards OS. In cytological preparations, OS appears to be highly cellular, when compared with soft tissue sarcomas. Malignant osteoblasts, can vary in shape but usually are hyperplastic and well-differentiated pyriform cells, with eccentric nuclei and basophilic cytoplasm, often with a clear Golgi zone near the nucleus. Standard staining technics, can evidence eosinophilic matrix (osteoid) and deep blue cytoplasm with occasional fine pink granules, in preparations with varied degree of anisokaryosis (Figure 24) [93]. However, when staining by using a particular substrate, the membrane-bound ALP, hydrolyzes the substrate to an insoluble brown product that dissolves in the cytoplasm. This technique has been shown to be 100% sensitive and 89% specific [129] (Figures 22 and 23).

Table 18. Comparison between histologic features of osteosarcoma and reactive bone. Adapted from Donald J. Meuten. Tumors in Domestic Animals. Wiley.

Reactive Bone	*Osteosarcoma*
Woven bone	Woven bone
Trabeculae interconnected	Trabeculae not interconnected
Anchored to adjacent bone	Loose from adjacent bone
Trabeculae lined with one layer of osteoblasts	Large areas of undifferentiated osteoblasts
More osteoid than cells	More cells than osteoid
Uniform osteoblasts	Variable size and form of osteoblasts
Osteoblasts producing osteoid with clear sequential maturation stages.	Undifferentiated cells forming isolated spicules of osteoid with no signs of sequential maturation

Biopsy for cytologic preparations is gaining importance, with the evolution and perfection of limp-sparing surgical techniques. Correct diagnosis of a bone tumor, is necessary before proceeding to surgery. When performing a biopsy, meticulous aseptic, hemostatic and wound closure procedures should take place. There are three methods of obtaining cytologic samples: an open incisional biopsy (more suitable for histopathology), a closed Jamshidi needle biopsy and a trephine biopsy[96]. The open incisional method, yields the best diagnostic results but involves frequent post-surgical complications as tumor seeding and hematoma. Likewise, a trephine has diagnostic accuracy rate of

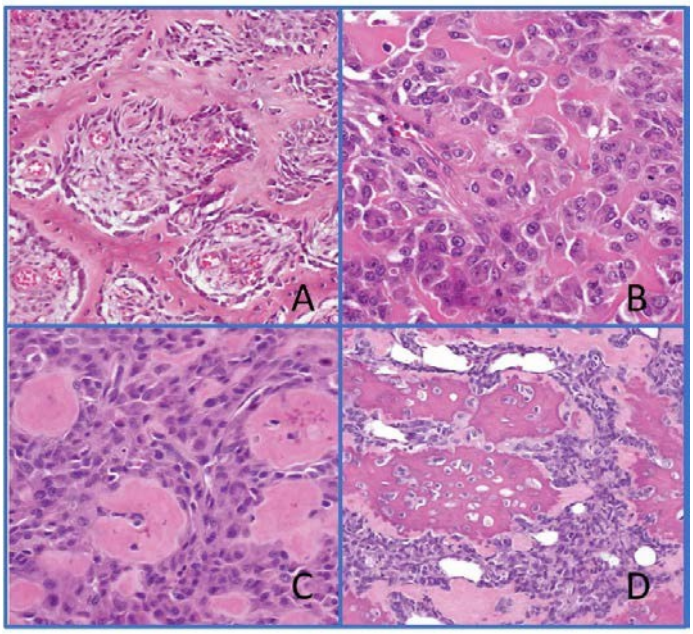

Figure 25. Histopathology of OS. Adapted from Donald J. Meuten. Tumors in Domestic Animals Wiley. Kindle Edition (95)

93.8% but can also provoke a pathologic fracture post-surgically. By using a Jamshidi needle these risks are diminished. Yet, with a decreased accuracy rate. The accuracy for tumor detection, with a Jamshidi needle, is of 91.9% and for subtyping is 82.3%[130]. A recent retrospective study though, showed no significant difference between histology and cytology in terms of diagnostics[131]. Many authors and clinicians state that an important factor, is the pathologist's experience for diagnoses from needles. Results yielding "reactive bone" in cases that Rx gives clear suggestion of OS are very common (Table 18).

The needle biopsy site, should be in the center of the lesion and the skin incision, should be carefully placed so that it can be removed with the therapeutic surgery. Using an eight or eleven-gauge needle, the surgeon should avoid vessels, nerves and joints. A couple of samples, should be collected by redirecting the needle through the same incision, penetrating only one cortex. Specimens should be, minimum, one to two centimeters long and should contain solid tissue cores. Fluoroscopy or CT, can help when operating the needle, especially in axial sites [96]. It is of good practice if the same surgeon who takes the biopsy sample, performs also the limb-sparing surgery.

Histologic evaluation, is the most reliable form of diagnosis for many authors and clinicians. Either with an open incision biopsy or by submission of the whole neoplasm, after amputation or surgery, histologic sections can determine the form of OS in question. There is poorly differentiated, osteoblastic, chondroblastic, fibroblastic, telangiectatic and giant cell-rich OS. Nevertheless, it is of common knowledge that most tumors can hold many, if not all forms, due to the highly capricious genetic and epigenetic origin of OS. New bone can form naturally due to inflammatory and other neoplastic reasons except OS. Reactive bone, formed in the tumor can mislead and examination of various sections is recommended. Differences between reactive bone and OS are related in Table 18. The distribution of osteoid and cells, can vary greatly in histologic preparations of the same tumor. In Figure 25 (A), there are interlinked trabeculae surrounded by one layer of osteoblasts, without any mitotic figures. There are vessels inside the trabeculae and all indicates this is reactive bone. In Figure

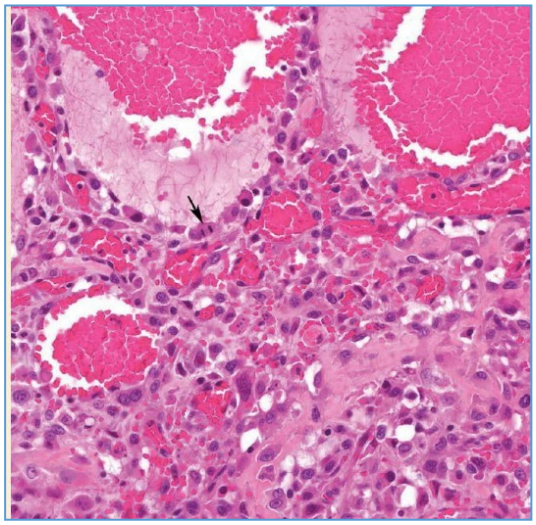

Figure 26 Telangiectatic OS. Arrow: mitotic figure. Adapted from Donald J. Meuten. Tumors inDomestic Animals Wiley. Kindle Edition (95)

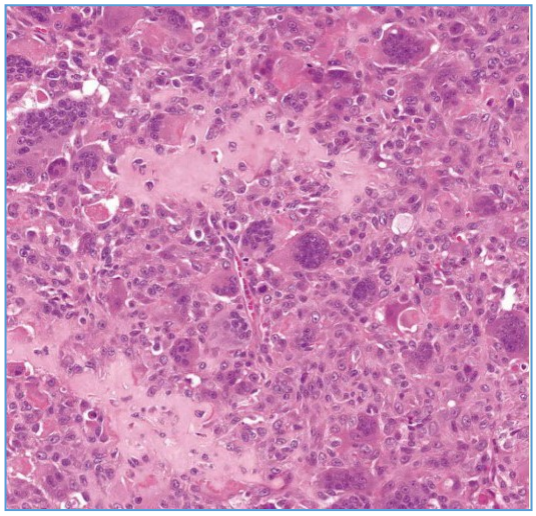

Figure 27. Giant-cell OS. Adapted from Donald J. Meuten. Tumors in Domestic Animals Wiley. Kindle Edition (95)

25 (B) osteoblasts are surrounded by elongated strands of osteoid, while in (C), the matrix, forms islands that can enclose malignant cells. The differences in matrix mineralization and formation can lead to formation of a tissue that resembles collagenous fibrous like in Figure 25 (D). Distinction, between osteoid and collagen fibers, is usually based on degree of mineralization but in rapidly advancing tumors that event may have not time to take place. There, stands the reason for the need to examine many sections of the sample. Leading authors state that, due to the higher malignancy of OS respecting chondrosarcoma, when there is, even slight, evidence of osteoid formation with signs of malignant osteoblasts, OS should be diagnosed [93, 96]. There may be, dispersed multinucleated, giant osteoclastic cells in OS preparations summoned upon osteoblastic proliferation (Figures 22 ,23). It is recommended to examine a section on lower-pawer microscopic examination for a complete picture of the extension and behavior of the tumor.

As OS is one of the most aggressive and malignant neoplasms in dogs, histopatholgists are fundamental players for the establishment of the treatment plan. Studies have shown that is very rare to call a benign tumor as malignant, which is very important due to the invasive and chemical treatments needed for OS[131].

Poorly differentiated OS, is so highly aggressive and lytic, that can cause a pathologic fracture early. Pleomorphism and osteoid in various patterns is common. Osteoblastic OS (Figure 25 B, C, D) is usually an aggressive tumor with lytic bone lesions and little periosteal response. Proliferation of osteoid varies but may largely fill the marrow spaces between pre-existing bone trabeculae [93]. Telangiectatic OS, characteristically produces an aggressive, osteolytic radiographic lesion, and the bloody, cystic lesions detected on gross examination (Figure 20). Occasional spicules of osteoid, among pleomorphic, malignant mesenchymal cells, can differentiate OS from hemangiosarcoma but that can be complicated. Frequently, due to the blood-filled spaces, only on high-power examination is possible to distinguish telangiectatic OS from aneurysmal bone cysts, by individualizing mitotic

figures and irregular production of osteoid (Figure 26). Chondroblastic OSs, produce both osteoid and chondroid matrices and while one section of a tumor could suggest chondrosarcoma, radiographic appearance and clinical presentation can give away an OS diagnosis. Fibroblastic OSs, begin as lytic bone lesions, but approximately 50% will progress to a mixed pattern when the neoplastic spindle cells increase their capacity to form mineralized matrix [93]. One study attributed a better prognosis for fibroblastic OS [132]. Giant cell tumors of bone are benign and therefore have a more favorable prognosis than giant-cell OS (Figure 27). Multinucleated giant cells, with features of osteoclasts, are common in OS that are likely to appear more aggressive and induce a periosteal reaction. Nuclear atypia, high mitotic rate or other features of malignancy and positive ALP staining in cytology should diagnose an OS. Authors state that differentiating a giant cell bone tumor from giant-cell OS is the most difficult issue in bone pathology [93].

In spite that no histological grading system has gained widespread application by veterinary pathologists, cellular atypia, mitotic count, tumor necrosis, osteoid production, tumor cell density, vascular invasion and the presence of multinucleated giant cells have been used to grade OS. Around 75% of OS in one study was given a high-grade score III (scale from I to III) while another resulted in 35% grade III, 37% grade II and 28% grade I OSs [133, 134]. Nevertheless, it is of little clinical relevance, if a patient has grade I or grade III OS, when deciding to amputate or not, even supposing higher

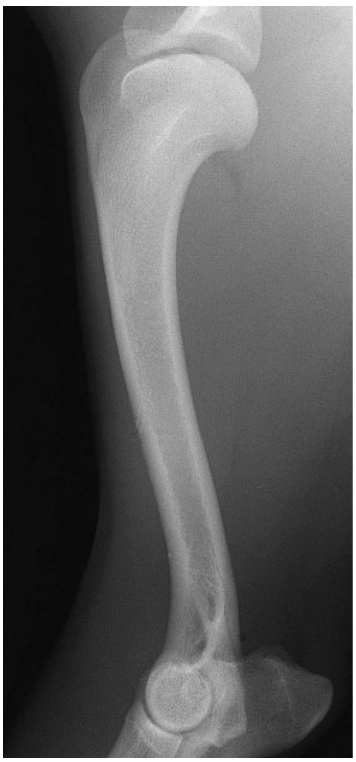

Figure 28 Normal Humerus

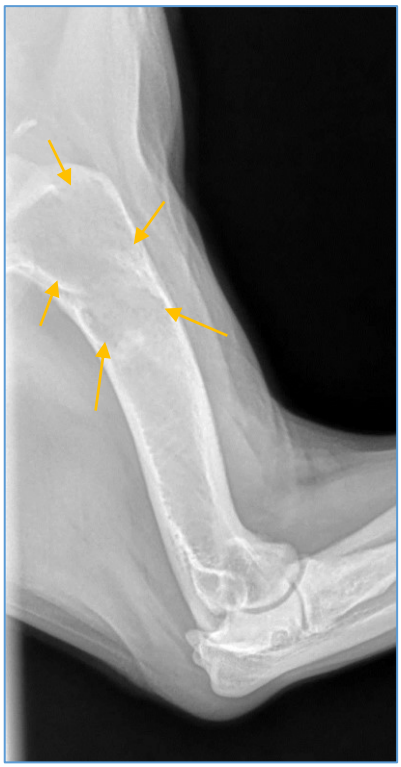

Figure 29 Signs of bone lysis on the proximal aspect. "A&A" patient, 8-year-old Pitbull.

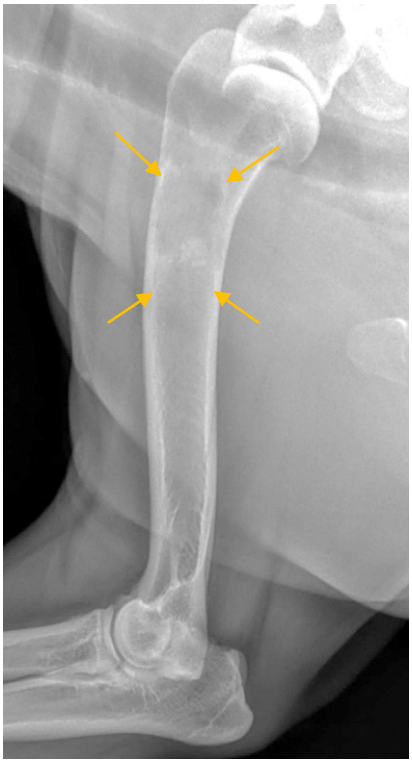

Figure 30 Signs of bone lysis on the proximal aspect. "A&A" patient, 8-year-old Pitbull.

grade OS has a worst prognosis [93]. Moreover, only few diseases that cause advanced bone destruction can be treated without any invasive method [96]

3.2.4.2 Imaging Methods for Diagnosis

Radiographic evaluation, is the first step beyond the physical exam towards a presumptive diagnosis and therapeutic recommendations. Good quality radiographs (X-ray), are important because non-experienced clinicians can easily miss subtle lytic lesions. Two views should be taken and some authors suggest three (two laterals) is ideal, particularly before a biopsy. An important characteristic is that OS, rarely crosses a joint to affect other bones[93]. As in histology and cytology, there is large variation in radiographic anomalies in appendicular OS. A practitioner, when evaluating a Rx from a member, without evident external signs, should closely examine the cortex of long bones for discontinuities. Also, the radiolucency of a normal bone should always be acquainted, for one to be able to perceive small lytic lesions, because the angles of the exposure might not permit a perfect perpendicular view (Figure 28). The Pitbull case referred in the introduction, was a good example of how a veterinarian with experience can identify abnormalities, not immediately visible in the newbie's eye. That patient, came in for a second opinion after almost three weeks of treatment with NSAIDs and apparently unremarkable Rx (Figures 29,30). Clearly in pain, with a non-weight-baring lameness on the right front, the 8-year-old dog, responded heavily on palpation. Since there were digital Rx from the member in pain, "A&A" received those radiographs with request of the caregivers. The author, accessed first to the image file, but, only after the guidance of Dr. Anastasiou could clearly visualize the presence of lysis on the proximal humerus of the unfortunate pet. Due to impossibility for treatment, the patient deteriorated quickly and was euthanized after a couple of weeks, even without definitive diagnosis.

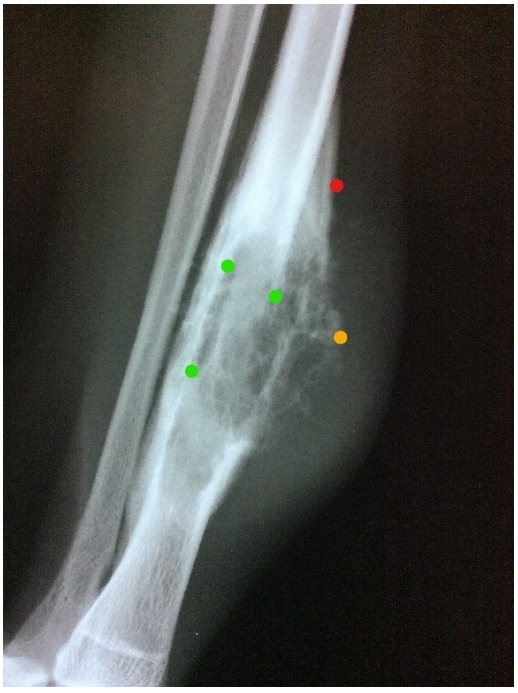

Figure 31 .Proximal radius of "Scoops". "A&A" Greyhound patient

In some cases, there is soft tissue tumefaction with reactive or tumor bone proliferation (yellow dot in Figure 31). Cortical lysis (green dots) can be devastating in a degree of an eminent pathological fracture. The "sunburst" effect of the palisading pattern on Figure 28 is frequent, according to leading authors[93, 96]. Sub-periosteal new bone, which develops beneath an elevated periosteum, forms a characteristic triangular mass of bone, referred to as a Codman's triangle, that merges with the underlying cortex at the periphery of the tumor (red dot) but is not

pathognomonic for OS[93]. Effectively, OS radiographic appearance, resembles to osteomyelitis of fungal etiology and the patients travel history should be taken into consideration. Appendicular OS can show dramatic change in radiographic appearance in as little as seven to 10 days. This can be a useful diagnostic feature in equivocal cases[96].

Table 19. Radiographic patterns compared with other causes than appendicular osteosarcomas.

Radiographic patterns	*Osteosarcomas*	*Other causes*
Osteolytic	Telangiectatic, Poorly Differentiated, some Chondroblastic, 50% Fibroblastic, Giant cell	Fibrosarcoma, Hemangiosarcoma, Giant Cell tumor of bone
Mixed	Most mixed type, Most Chondroblastic, 50% Fibroblastic, Moderately productive Osteoblastic	High grade Chondrosarcoma
Productive	Osteoblastic	Rarely Chondrosarcoma

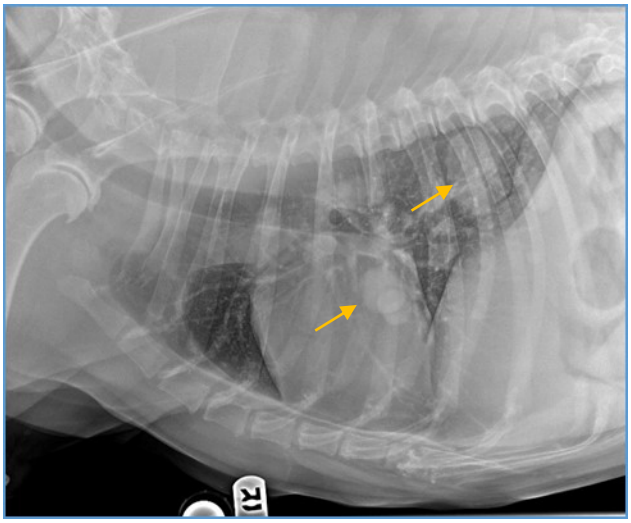

Figure 32. OS metastatic pattern. Adapted from http://animalpetdoctor.homestead.com/CancerOsteo.html

OSs can produce lytic, mixed (destructive and productive), or productive bone lesions, which are best distinguished by radiographic examination of the tumors. Unfortunately for the diagnostician, some of these patterns may also occur in other primary bone tumors, as well as in metastatic tumors of bone and certain inflammatory lesions[93, 96]. On Table 19, the various types of OS are compared with other possible causes of similar radiographic findings.

Full body radiographic evaluation, is recommended when skeletal OS is detected. As there is a hematogenous metastatic route, through the venous vasculature, pulmonary radiographs should be always in the diagnostic plan. Evidence of pulmonary lesions is uncommon upon diagnosis (15% or less)[96]. Radiographically, pulmonary metastasis, when big enough (8mm), is easy to detect and distinct from other abnormalities (Figure 32), for the circular radiolucent lesions in the pulmonary parenchyma.

Magnetic Resonance Imaging (MRI), Positron Emission Tomography/Computed Tomography (PET/CT) and nuclear scintigraphy have been evaluated, by various studies, for the early detection of metastatic disease, as well for the estimation of intramedullary lesion extension, prior to a limp- sparing surgery. Scintigraphy, greatly overestimated lesion size, when compared with histopathology, while radiography also but with an insignificant margin. MRI, was found to be more accurate on

detecting tumor length, with a slight overestimation, and CT gave similar results. Underestimations occurred also, that eventually, could lead to subtotal tumor resection. In contrast, overestimation of tumor length has the potential to exclude suitable patients, as candidates for limb-sparing surgery [135-137]. All these studies though, were relatively small. Another recent study, executed a retrospective evaluation of whole body CT for tumor staging in canine appendicular OS [138]. Bone metastasis was not detected in any dog on whole body CT. Pulmonary metastasis was considered definitive on CT based on board certified radiologist assessment in five percent of dogs (N=39). Two more dogs (2/39, 5%) had soft tissue masses diagnosed on CT, consistent with concurrent, non-metastatic malignancies.

3.2.5 Metastatic Behavior

Metastasis is almost certain in OS and unfortunately happens very early, which demarks OS as one of the most unpleasant diagnosis for caregivers and their pets. As stated before, only 15% of the cases, presents with metastasis on diagnosis (some authors give just a 10%)[96]. Metastatic cells though, in OS, are capable to reach the lung, evading all adversities, and establish micrometastasis that escapes detection from imaging methods. Usually, when amputation is the only treatment, around 90% of dogs will die within a year. The most striking data, is the Median Survival Time (MST) that with amputation only as a treatment, reaches to just 19 weeks for appendicular OS. Axial OS is lightly less aggressive with 22 weeks of MST[93].

Except the hematogenous route of metastasis, rarely there can be involvement of the lymphatic route. Regional lymph nodes were affected in just 4.4%, in one study (N=228), along with an associated poorer prognosis [139]. However, other authors that examined dogs with already established metastatic disease found that lymph node metastasis was around 24% although this study was smaller (n=50) [134]. In conclusion, it is possible that lymph node involvement in OS is underestimated. On the same study, vascular metastasis was found at 71%. Besides lungs and lymph nodes,other soft tissues including the brain are affected, while skip osseous metastasis also happens. When dog owners elect to treat, their cancer insulted dog, then veterinarians usually recommend amputation or limp-sparing surgery in conjunction with chemotherapy. Subsequently, MSTs, even with metastatic disease proven, get longer as well as DFIs. These important details will be discussed along with the prognostic factors and therapeutic models. However, it is known that in humans there is an increase of bone metastasis succeeding chemotherapy[140]. One plausible scenario, to explain this, is that when chemotherapy selectively targets lung metastasis, smaller osseous or non-osseous metastatic sites become clinically relevant[96]. Another view, more scientifically grounded, is based on the concept of concomitant tumor resistance. In a small study, was found that removal of the primary tumor, decreased the serum levels of Vascular Endothelial Growth Factor (VEGF) and endostatin. VEGF though, has a shorter half-life than endostatin, so this resulted in a significant elevation of systemic angiogenesis-inducing ability [141]. One study, evaluating the ways of evasion

of the immune system during metastasis, found that peripheral blood monocytes of dogs with OS, exhibit decreased chemotactic function when compared to control dogs [142]. An *in vitro* study, investigating platelet interaction with OS cell, showed that healthy canine platelets inhibited the migration of the canine osteosarcoma cells[143].

It is reported that mandibular OS is less aggressive metastatically but with contradictory scientific data. Unfortunately, exist only very rare reports of spontaneously regressing OS [96].

3.2.6 Prognostic Factors

Prognosis, is certainly related with staging of OS and except of a histological staging, referred previously, there is a surgical staging system that can be adopted from human applications. This system, includes histological grading, anatomic setting of the primary lesion and regional or distant metastasis. Three stages, are classified as follows: stage I, is attributed to dogs with low grade histologic lesions and without metastasis, stage II, to dogs with high grade lesions and without metastasis and finally stage III, is related to the presence of metastasis, regardless the histologic grade. Additionally, a subdivision per anatomic setting classifies tumors as intracompartmental (A) and extracompartmental (B). According to leading authors most patients present with stage II-B disease [96]. The designations by the letters (G) for histologic grading, (M) for metastasis and (T) for anatomical setting can also be used.

Stage III disease has very poor prognosis with a MST of merely 76 days[144]. Dogs with bone metastases had a longer survival time than dogs with soft tissue metastases. Lymph node involvement shortens furthermore the MST to 58 days compared to 318 days of dogs without[139] .

There is data comparing MST with anatomical location, age and completion of excision amongst other. Most of the studies, agree that there is improvement in survival times with adjunctive chemotherapy after surgery. On Table *20,* there is a comparison between positive and negative prognostic factors. The reader should rely on this table only as informational, because the data derives from different studies with variant methodologies and objectives [96, 145-151].

Table 20. Comparison of various parameters related to prognosis for canine osteosarcoma

Better prognosis	*MST (days)*	*Worst prognosis*	*MST (days)*
Completeness of excision	-	Younger than 7, older than 10 y.	-
Small dog size	-	Humerus	-
Flat bone location	-	Large tumor size	-
Mandible after mandibulectomy	75% at 365	Maxillae	140
Distal to carpus or tarsus	466	Ribs	90
Skull – less metastasis	204	Vertebrae	120
-	-	Extra-skeletal	30 to 90

Serum alkaline phosphatase levels as mentioned previously were associated with poor prognosis. One study found that for each 100 U/L increase in ALP or BALP activity, the risk of death in any given time interval increased by approximately 25%[99]. In another study, ALP over 110 U/L before surgical removal resulted in 117 days, of MST, while when under 110 U/L in 495 days [152]. The levels of BALP over 23 U/L resulted in 218 days of MST, and when under 23 U/L in 586 days. The same authors report that, after surgical removal, ALP usually dropped to normal. However, when after 40 days was still high, there was also a higher incidence of metastasis. Notwithstanding, one leading author, justly points out that bone specific ALP (BALP) is not available to measure in all laboratories and that ALP can be altered from other concomitant disorders [93]. A recent study found that there is no indication of genetic influence on levels of ALP in OS [153].

A very recent study found that elevated total serum cholesterol was associated with a reduced hazard ratio for overall mortality in dogs with osteosarcoma [154].

Several molecular proteins have been related to affect disease free interval (DFI) and MST in canine OS. These are: 1) *Ezrin*, an anchor site for cytoskeletal actin fibers. 2) *Recepteur d'origin nantaise (RON)* from the *MET* proto-oncogene family. 3) *VEGF* .4) *COX-2.* 5) *Survivin,* an apoptosis inhibitor *and 6) IGF-1R.* Relatively to *IGF-1R,* a recent study found through immunohistochemistry, a positive correlation between high levels of expression and poor prognosis [116]. Data relating DFI and MST were available only for five mentioned molecular prognostic factors [96, 155] (Chart5).

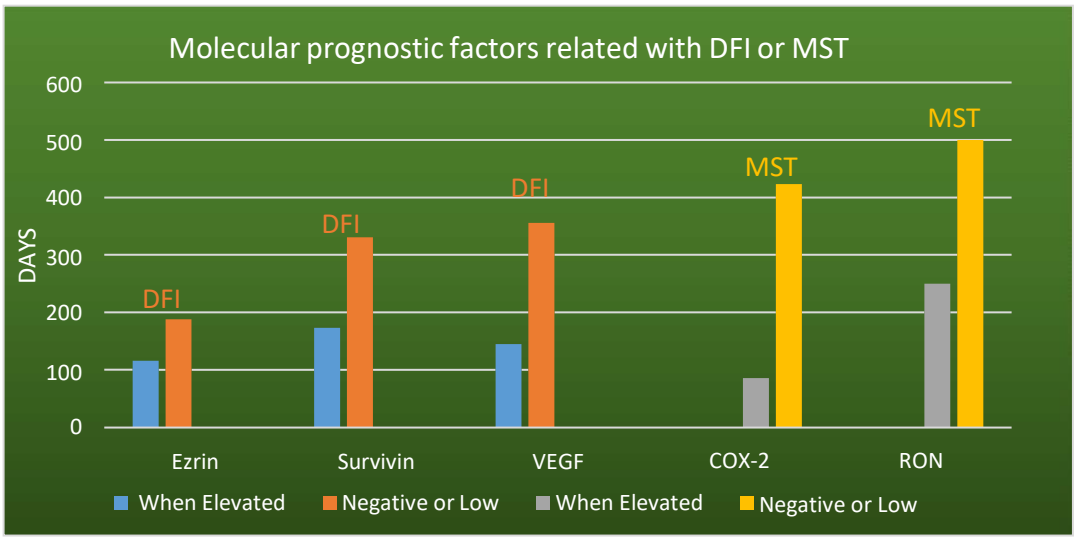

Chart 5 Molecular prognostic factors related to Disease Free Interval (DFI) or Mean Survival Time (MST)

Considering gene transcripts, through canine specific microarray analysis, a study divided a group of dogs, treated uniformly, into poor and good responders. The findings, surfaced differences in eight specific gene transcripts [156]. Another group, conducted a similar study where 51 genes were differently expressed in good and poor responders [157]. The pathways involved were related to

proliferation, immune response, drug resistance and metastatic power. Importantly, the similarities between human and canine OS, in their molecular pathways, were furthermore substantiated.

The ability to evade the immune system during all stages of metastatic progression, enables cancer cells to survive and create micrometastasis. A study found that a low ratio T-CD8 / T-Regulatory cells was associated with lower MST and DFI [158]. A prognostic tool, more accessible to clinicians, derived from a study which found a relation of relative lymphocytosis (>1000 cells/μL) and relative monocytosis (>400cells/μL) with shorter DFIs [159].

A recent validation study, suggests that mortality risk after osteosarcoma surgery can be predicted using patient characteristics [160]. By using data for age, gender, weight, ALP, tumor location and gonadal status (neutered or not), the scientific team, created a tool, that can be used by practitioners to calculate the mortality risk at five months and one year post surgery.
This tool is available, for free use, as an Excel® spreadsheet at:
http://www.sciencedirect.com/science/article/pii/S0167587716300034?via%3Dihub#upi0010

The fields of molecular tumor biology and gene expression profiling, are actually, in an effervescent state, with numerous new studies being published frequently. Through a bibliographic review, the author tried to achieve access to the most recent information available on prognostic factors of canine OS. However, the reader is encouraged to investigate for actualized information always.

3.2.7 Therapeutic approaches

A complete patient assessment should take place, before recommending and calibrating any therapeutic plans. As in any grave disorder, the more stable is the patient, less are the complications during treatment and maybe, better the outcome. In the authors opinion, a thorough clinical evaluation, enhances the practitioner with a large spectrum view. Also, it is important to provide to the caregivers with understandable and complete information. In this fashion, owners can be aided in electing a treatment, which can be very hard, with a life-threatening disorder as OS. Mostly, it would be unfortunate to start chemotherapy and during treatment, discover that the patient was already in organ failure. Cardiac disease can be detrimental during surgery or chemotherapy and an ECG should be performed always. Patients with known heart problems should be evaluated by an echocardiogram. Additionally, renal function must be evaluated. A minimum database profile with CBC, SBP , urinalysis and platelet count should be performed[96].

3.2.7.1 Options for Primary Tumor Removal

OS can affect various anatomical sites but not all can be approached surgically with ease. Vertebrectomy is not well developed and provide limited local disease control [96]. Resection of cranial OS is dependent on venous sinus involvement [96]. In both vertebral and cranial OS, radiation therapy is usually elected. Other axial sites can be treated with surgery. Pelvectomy with or without

amputation is an option. Mandibulectomies, maxillectomies and orbitectomies are also performed. Rib resection is the most common procedure, for rib OS, but requires resection of cranial and caudal ribs adjacent to OS site [96]. The purpose of this report though is to elaborate on appendicular OS which accounts for more than 85% of all bone tumors. Consequently, the author will focus on surgical and non-surgical techniques of the long bones that, statistically, develop OS more often.

3.2.7.1.1 Amputation

Patients with severe orthopedic or neurologic conditions, can yield poor postsurgical results when amputated. Otherwise, even moderate osteoarthritis rarely causes clinical issues in an amputee. Despite body size, dogs react well to a three-legged life and usually, caretakers are satisfied with their dog's life quality. It is recommended to execute a forequarter amputation (scapular removal) for the forelimb and coxofemoral disarticulation for the hind members [161]. Cosmetically and functionally, forequarter amputation yields the best outcomes. When OS offends the femur, disarticulation is obligatory. However, if distal bones are affected, the aesthetic results from an osteotomy or disarticulation of the knee are not good and the member not so functional. For proximal femoral lesions, soft tissue safety margins cannot be achieved with disarticulation alone. In such cases *en bloc* acetabulectomy and amputation should be performed [96].

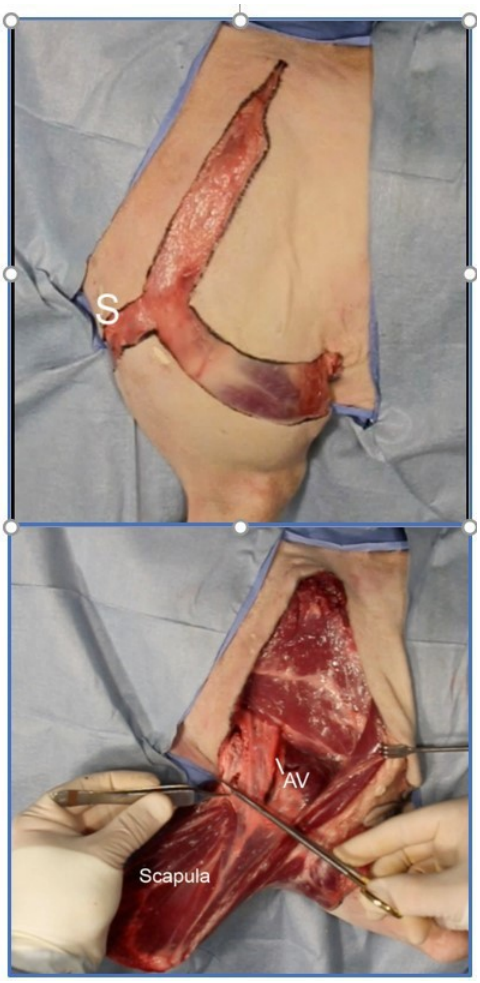

Figure 33 Two steps of a forequarter amputation. S: Shoulder, AV: Artery and Vein. Adapted from https://www.cliniciansbrief.com/article/forelimb-amputation

The owners and the veterinarian should evaluate the ability of the dog to ambulate with three limbs by using a sling for short intervals. Immediately prior to surgery, antibiotics targeting skin contaminants should be administered. Multimodal analgesia should be in the plan and some authors support preemptive analgesia also. A large catheter should be used in case there will be need for a transfusion but the patient should always be well hydrated. A surgeon does not need any special equipment except a standard surgical pack[161].

Forequarter amputation, starts with an incision on the dorsal border of the scapula, over the scapular spine and around the proximal aspect of the humerus. Transection of *trapezius*, *omotransversarius*, *rhomboideus* muscles allows lateral retraction of the scapula (Figure 33). The

brachial plexus is adjacent to the axillary vein and artery, medially of the scapular neck. Ligation of the vessels and transection of the nerves, must be performed first before transecting the *brachiocephalicus*, *pectoral*, and *latissimus dorsi* muscles. After removal of the limb and before closure, the ligated vessels should be covered by muscle approximation[161].

For a coxofemoral disarticulation, after an incision around the femur, separation of the *pectineus* and *sartorius* muscles can give access to the femoral vessels for ligation. Before ligation of the medial circumflex vessels the *pectineus*, *gracilis*, *sartorius* and *adductor* muscles must be transected. After incision of the joint capsule and the ligament of the femoral head, then laterally, all muscles must be severed, including the sciatic nerve and the rotator muscles. A flap of the *biceps femoris,* sutured to the *semitendinosus* and *gracilis* muscles, should close the wound under the skin[161].

3.2.7.1.2 Limb-Sparing Techniques

Many limb-sparing procedures are taking place lately, particularly in academic settings, that as reported, do not diminish survival times[96]. Owners that would not accept amputation as an option or patients that have orthopedic or neurologic conditions, open way to limb-sparing surgery[161].

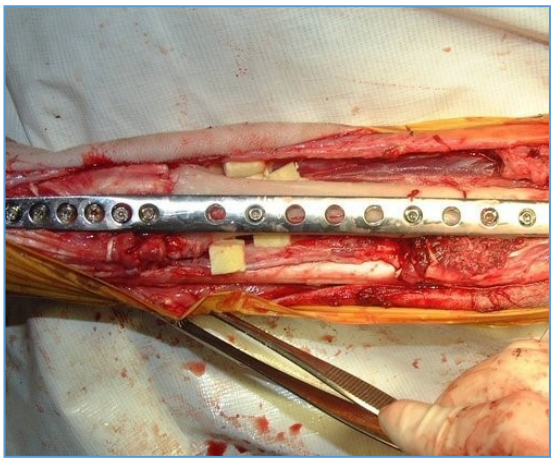

Figure 34 Allograft limb-sparing. Adapted by http://www.animalcancersurgeon.com/bone-tumors-appendicular/

When surgeons intervene drastically, they seek outcomes that offer an acceptable life quality to the patient and can satisfy the caregivers. Arthrodesis, is a fundamental part of a limb salvage procedure which depending on the joint secured, is more or less functional. Only carpal arthrodesis is considered acceptable and as follows, just distal ulnar or radius OS can be removed by a limb-sparing technique[96, 162]. This narrows down the possible candidates to almost 25% of all OS patients. Furthermore, a good general health is a prerequisite as well as the absence of a pathologic fracture. A tumor that affects more than 50% of the bone or has 360-degrees of soft tissue involvement would overturn a dog as a candidate. Also, an edematous soft tissue mass lesion is less suitable versus a firm one [96]. Finally, most experienced centers, select cases with preoperative downstaging or local chemotherapy and apply postoperative adjuvant chemotherapy [96]. The use of preoperative radiotherapy and cisplatin has facilitated limb-sparing surgeries [96, 163]. In all cases, a meticulous aseptic operation and cephalosporin IV, pre-, intra- and 24 hours post-operatively have to be used [96].

An allograft is a tissue for implantation, exchanged between individuals of the same species while an autograft is a tissue from the same individual used to heal another part of the body[85]. Metal

endoprosthesis is also used in limb-sparing surgery. All these techniques, require removal of the bone segment affected by the tumor through, relatively, the same procedure.

Initially, the tumor pseudocapsule has to be reached, dorso-laterally, without penetrating into the mass. Using an oscillating saw, the radius is cut three to five cm proximally to the tumor margins. Soft tissue margins, of two to three cm, have to be kept when transecting extensor muscles but additionally to the flexor muscles, these muscle groups have to be spared when the mass is in the mid-diaphysis. The joint capsule is incised close to the proximal carpal row. The medial ulnar cortex should be sectioned with the radius and removed *en bloc* together. While preserving vessels is important, all veins and arteries associated with the mass have to be ligated divided. A radiographic and histologic evaluation of the specimen can detect incorrectly placed margins. In veterinary medicine, intraoperative frozen section histology is unreliable for bone specimens [96].

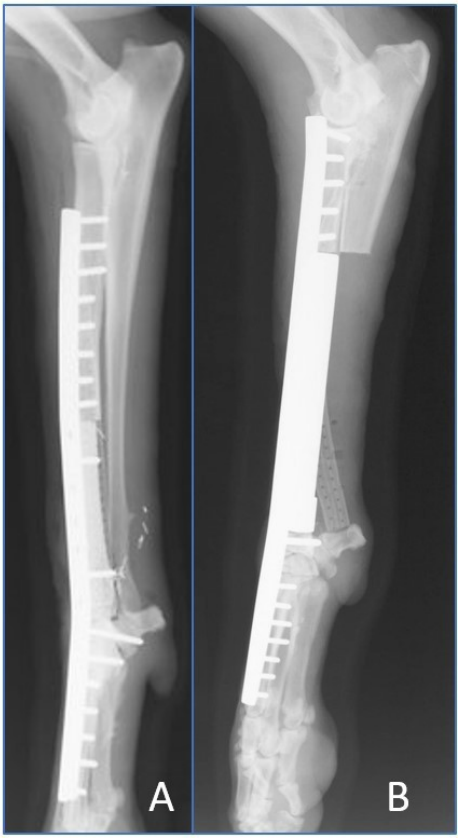

Figure 35 Rx of (A) an allograft and (B) of metal endoprosthesis. Adapted by www.animalcancersurgeon.com/bone-tumors-appendicular/

An allograft technique advances by thawing a fresh-frozen allograft, in a saline solution with neomycin, polymyxin B and potassium penicillin. Before cutting the graft to fit, the articular cartilage is removed. Importantly, before stabilizing the allograft in compression, the cavity of the specimen is reamed from fat and debris. After considering the best positioning, a dynamic compression plate is fastened in the patient and onto the allograft, with two to three screws. Consequently, the allograft is removed and filled with a bone cement, embedded with amikacin, that provides support, acts as an antibiotic reservoir and is reported that decreases failure. Replacement and fixation with four screws distally and proximally is the last orthopedic step (Figures 34, 35A). Good lavage with saline should precede any use of local chemotherapy with polymers. Preventing self-mutilation should be a primary goal post-surgically while light limb use, is encouraged for the first six weeks but without restrictions afterwards. This will help prevent flexure contracture of the digits. Two weeks from surgery, a dog is expected to walk and have no swelling. The advantage with the allograft, is that the caregivers are not particularly involved with the healing process. However, the infection rate reaches 50% with very difficult resolution. As little is the caregiver's involvement if a procedure goes well, this advantage

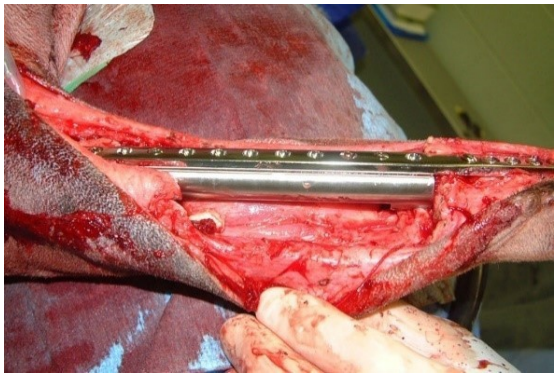

Figure 36 Metal endoprosthesis. Adapted by www.animalcancersurgeon.com/bone-tumors-appendicular

fades away, if the implant fails and there is need for revision surgeries or even worst, an amputation. [96] One compelling alternative to an allograft, is metalendoprosthesis. Surgery and fixation is nearly identical as well as the complications. A metal implant though, is always available and can be used for distal radius lesions very successfully (Figures 35B, 36). [96] Another technique reported, have used pasteurized tumoral autografts. By removing the bone segment affected and pasteurizing it at 65 C° for 40 min, then, it can be re-implanted.

The advantages are that the apposition is excellent and there is no need for a metal implant or an allograft. Furthermore, the small study, showed lower infection rates (30%) than with an allograft. [164]

By using the ipsilateral vascularized ulna, as an autograft, a distal radial defect can be corrected by rotation and fixation with a plate. This autograft, can heal earlier and cause less infection because is vascularized. Nevertheless, there can be biomechanical complications, due to the smaller relative size of the ulna to the radius. [165]

An external fixation technique, uses the Ilizarov circular fixators for longitudinal bone transport osteogenesis. After the tumoral bone removal, as described previously, a normal bone segment, termed as transport segment, is attached to the central transport ring. Four more, fixed, rings have to be placed, two distally and two proximally. During the first post-operative week, all fixators stay immobile. After that, by conveying the transport segment a millimeter per day, distally in the defect, trailing distraction osteogenesis takes place. New bone forms behind the transport segment and when the latter reaches the radiocarpal bone, it is compressed to form an arthrodesis. Prolonged recovery time, presence of external fixators and extended owner involvement are the main disadvantages of this procedure. [96]

3.2.7.1.3 Intraoperative Radiation Therapy and Stereotactic Radiosurgery for Limb-Sparing

The use of intraoperative radiation therapy (IORT) for OS in human medicine is frequently considered. In veterinary medicine, high complication rates associated with orthopedic implants and infection in radiated bone have labeled this therapeutic choice as non-recommendable from leading authors. Despite the adversities, small studies have been conducted. In IORT, the osteotomized tumoral bone is pivoted over the adjacent joint and the healthy neurovascular net, as well as the muscle and skin, are separated. The patient is transported to a radiation suite to receive just one dose of radiation. Anatomical replacement and surgical fixation of the tumoral fragment are executed as in other limb-sparing techniques. An alternative, is to excise completely the tumor and transport

just that fragment for radiation, for a procedure known as extracorporeal IORT. Survival times (MST=298 days) do not justify the risks associated, that include, an almost 70% rate of surgical revisions, amputations and common pathological fractures.[166] [96]

Another atypical technique, for common veterinary centers, is Stereotactic Radiosurgery (SRS). The employment of image guidance can assure sparing of healthy surrounding tissue through a sharp regulation on dose intensity. This is not an interventional treatment and in a small study, yielded an MST of 363 days. Other authors, that recurred to preemptive orthopedic fixation, report an 83% limb survival rate and an MST of 275 days for patients treated with SRS. [167] [96]

3.2.7.1.4 Synoptic Comparison of Tumor Removal Options

Choosing the correct recommendation for the caregiver of a dog with OS can be tumultuous for a practitioner who is unfamiliar with every possible therapeutic option. The use of RT is considered suboptimal when using high dose external-beam but acceptable to good when the dosage is moderate along with adjunctive chemotherapy. Another novel approach, is the use of Nano-pulse stimulation for tumor ablation that the authors of a very small study, claim that can remove primary OS with success [168].

Factually, there is no significant difference between an amputation or limb-sparing attempts in terms of MST or DFI, when patients receive the same adjuvant chemotherapy. An 80% good to excellent limb function is attributed to limb-sparing techniques. Complications can be various with the most representative being the occurrence of deep infection that can reach almost 50%. Despite the high rate of infections, post surgically, in most cases, limb function was not affected significantly. As frequently happens in scientific research, a positive outcome derived from an unwanted collateral effect. [96] Patients with deep postsurgical allograft infections had a considerably prolonged survival time[169]. A recent retrospective study on 125 cases of OS retrieved just six cases with a chronic local infection[170]. A result of 100% of a five-year MST and DFI rate, in dogs with local infections is pleasantly surprising, particularly when compared to a 54% and 43% (MST and DFI) in dogs without infections. Similar results have been reported in humans and murine models[171].

Following an extensive, but not exhaustive review, the author would resume that an amputation is the most cost-effective and accessible measure of tumor ablation in an average veterinary setting. As an alternative, and when there are the structural and financial means, limb-sparing techniques could yield excellent results but could also have very unpleasant outcomes. Metal endoprosthesis or a pasteurized autograft would be the most readily accessible techniques for limb salvage. Considering the increase of MST when allografts develop infection it can be tempting to purposely let that happen. However, that would not be ethically nor medically correct because there is not enough data to support the relation, neither any leading author recommends something similar. All the afore mentioned, non-amputational techniques, are considered functionally acceptable only for radial OS

[96]. A case by case evaluation is always necessary, to decide which treatment would better fulfill the caregiver's expectations and ameliorate the pet's life quality.

3.2.7.2 Management of Micrometastatic Disease

The hardest battle, a patient with OS must face, is that against metastasis. As mentioned, many factors influence the metastatic rate and extend. Genetic, molecular, epigenetic and immunologic key determinants, influence the overall MST and DFI in OS patients. In spite that new emerging therapies and numerous innovating clinical trials under way, chemotherapy (CHTH) is still the foundation for metastatic management. Micrometastasis is frequently present at diagnosis but rarely there is gross metastatic evidence. There are different methods of applying CHTH for micrometastatic disease and for gross metastatic disease. Unfortunately, in most cases, the battle against metastasis is lost and patients eventually succumb to OS.

3.7.2.2.1 Adjuvant Chemotherapeutic Management of Micrometastasis

Platinum coordination complexes, are the most used and studied agents. They form chemical bonds with nucleophiles such as nitrogen in nucleic acids and sulfur in proteins. Antitumor action of these drugs is due to formation of interstrand or intrastrand DNA cross-links. Cisplatin and carboplatin are the two agents that are widely used. Cisplatin is contraindicated in cats for causing fatal pulmonary toxicity. Side effects include nephrotoxicity, that is dose related and can be irreversible, ototoxicity, anaphylaxis, neurotoxicity and severe nausea. GI effects can include diarrhea, vomit and anorexia. Good hydration in the post-infusion period is crucial. Administration, by a rapid IV infusion is necessary. Aluminum needles should not be used because they inactivate the drug. Carboplatin is

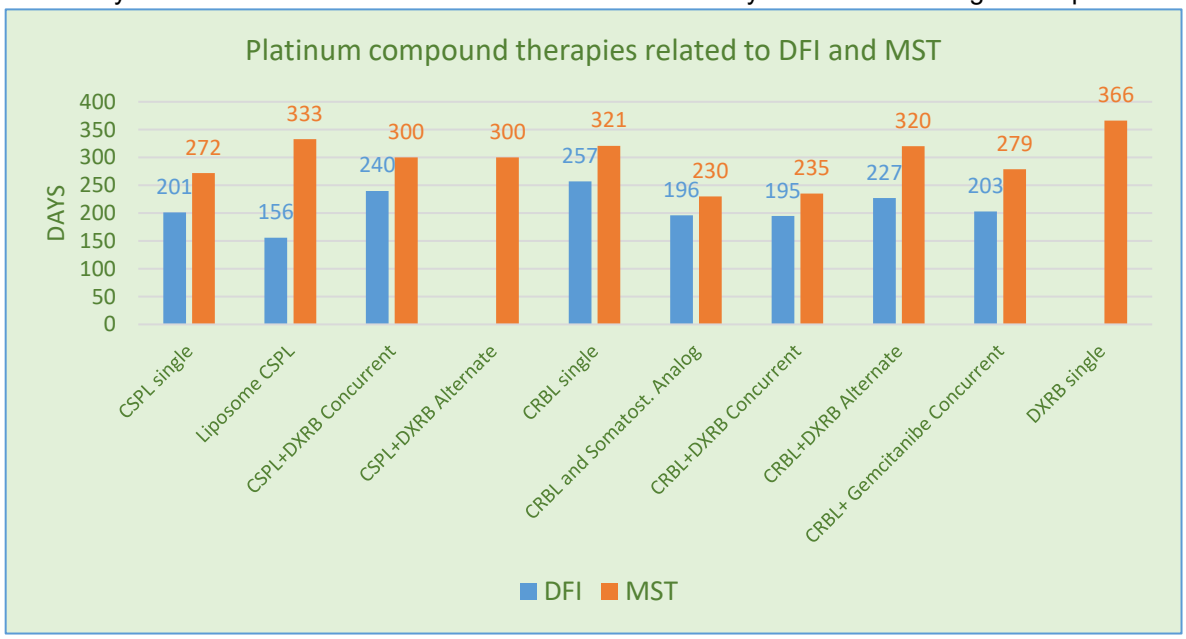

Chart 6. Platinum compound therapies related to DFI and MST. CSPL: cisplatin, CRBL: carboplatin, DXRB: doxorubicin. Data from studies with at least 20 dogs

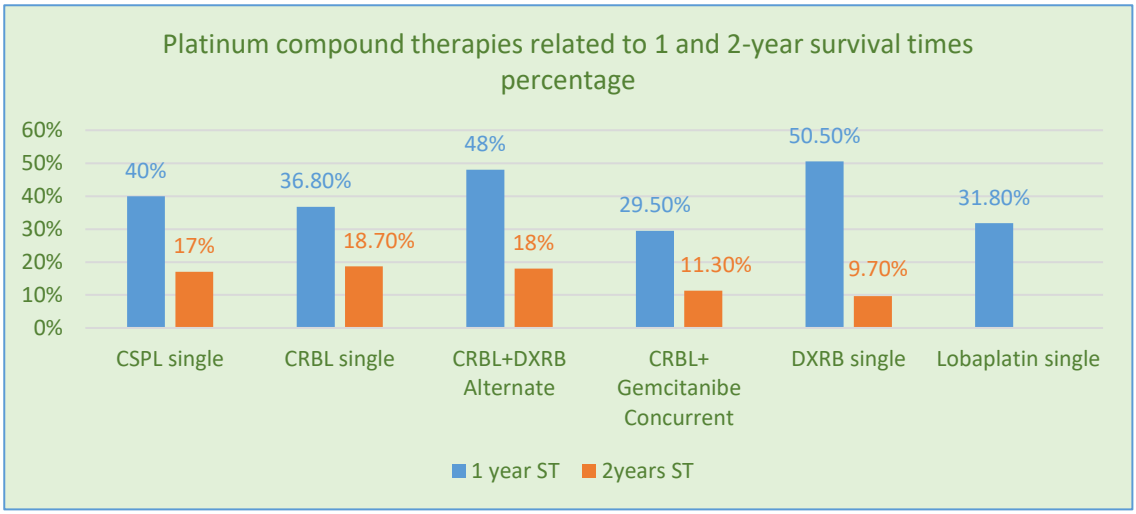

Chart 7 Platinum compound therapies related to 1 and 2-year survival times. CSPL: cisplatin, CRBL: carboplatin, DXRB: doxorubicin. Data from studies with at least 20 dogs

an analogue of cisplatin with the advantage that is less nephrotoxic and can be used on an outpatient basis. Also, has a milder nauseous effect but is a stronger myelosupressor than cisplatin[172]. Lobaplatin, a third-generation platinum compound, was studied for OS treatment. Lobaplatin provokes less vomiting and transient hematologic toxicity. [96]

Doxorubicin is an anthracycline derivative, isolated from *Streptomyces spp.* Its antitumoral activity, relies on damaging the DNA strands through free-radical generation. Detection of DNA damage by the cell and effective activation of apoptotic pathways is crucial for the cytotoxic effect of doxorubicin. This agent, presents high pharmacokinetic variability. Reaches low concentrations in the CNS but can present acute toxicosis. Head shaking, urticaria and redness along the vein of administration (on the axillae frequently), can be acute symptoms, as well as collapse if rapidly administered. Histamine related signs may decrease with previous use of diphenhydramine hydrochloride (Benadryl ®). Mid-term reactions, can be weight loss, alopecia, anorexia, diarrhea, vomiting, myelosuppression, bone marrow hypoplasia and lymphoid atrophy. Chronic toxicosis, can present through testicular atrophy and cardiotoxicity that can lead to cardiomyopathy. Neutropenia, is common and dogs that develop toxicosis at the first treatment, are 17 times more likely to develop again toxicosis after the second one. [172] Gemcitabine, is a cytosine analog that can be incorporated into RNA and DNA producing nucleic acids that cannot be replicated. Gemcitabine, is known to have activity against solid tumors in people. [172]

Protocols for CHTH, can include one or more agents in an alternating or concurrent combination. In almost all studies, the outcomes are compared by looking at the DFI, the MST or the survival rate at one or two years (Charts 6 and 7). Dosages, were proven to be relevant with toxicity in various studies. However, clinical results, of different dosing and cycle scheduling, were not significantly

diverging, when dosing did not cause unacceptable toxicity. In retrospective cohort study, evaluating different carboplatin and doxorubicin protocols, dosage intensity was not associated with development of metastases or death[173]. A protocol of carboplatin, at 300mg/m^2 for four or six cycles, resulted in less side effects.

Cisplatin, as a single agent and when alternated with doxorubicin is usually administered at 60mg/m^2. In a concurrent administration with doxorubicin, cisplatin dosage can be as low as 15mg/m^2. When used alone, cisplatin is administered on one to six occasions, 21 days apart. In concurrent combination with doxorubicin, there is an interval of one to 24 hours between administration of the two agents. When alternating, a therapeutic cycle starts with doxorubicin and 21 days later ends with cisplatin. There is an interval of three weeks between cycles. Carboplatin alone, is administered at 300mg/m^2 every two weeks for five cycles. [96] Combinations with doxorubicin follow a similar therapeutic calendar as cisplatin (dosage is still 300mg/m^2). Doxorubicin, as single agent or in an alternative pattern, is administered at 30mg/m^2 , while when concurrently used with a platinum compound then the dosage is halved[96]. Comparison of CHTH to MST and DFI, in Charts six and seven, is not direct, due the heterogeneity of the studies in terms of dosage, sample size and overall methodologies. However, outcomes of all protocols are similar with a MST, merely reaching close to a year (Chart 6). After that, there is an abrupt drop in survival rates and just a few patients reach the two-year threshold (Chart 7). Still, in a recent study, dogs receiving carboplatin alone had a significantly longer DFI (425 versus 135 days) than dogs receiving alternating carboplatin and doxorubicin [174].The references for the data on Charts six and seven are: cisplatin as single agent [175, 176], carboplatin as single agent [146], doxorubicin as single agent [177], doxorubicin and cisplatin in concurrent usage [178], lobaplatin as single agent [179], doxorubicin and carboplatin in concurrent administration [180], doxorubicin and cisplatin alternating [181] and carboplatin with concurrent gemcitabine [182].

A study, published in 2011, evaluated satraplatin, a platinum compound with high oral bioavailability, as a chemotherapeutic for various tumors in dogs. Twelve dogs in this study had appendicular OS, out of which, six micrometastatic and six gross macroscopic. In the six dogs receiving satraplatin in the adjuvant setting MST reached 577 days and DFI was also high. Oral satraplatin was administered daily, for five days and every three to four weeks, for a total of four cycles. [96, 183] The author did not find any more recent publications on this potentially promising type of chemotherapy.

3.7.2.2.2 Possible Molecular-Targeted Therapies for Micrometastasis

As the molecular pathways, that contribute to OS development, are under investigation thoroughly in the last decade, there are studies that test novel therapies against metastatic factors. The roles of growth hormone (GH) and IGF-1, have been related to human OS as there is a peak of incidence in the adolescence. In dogs, because of the high incidence of OS in giant breeds, has been

also hypothesized that GH and IGF-1 might be important. A randomized clinical trial in which dogs were treated with amputation and carboplatin, investigated the influence of a long-acting analog of somatostatin to the circulating IGF-1 and survival times. Unfortunately, and even if IGF-1 levels decreased, the DFI and MST was not different comparing to controls[115].

Another aspect of metastasis, that oncologists are focusing on, are the obligate molecular steps for tumoral successful cell invasion. As mentioned in paragraph 3.2.3.1, MMPs, have a proteolytic effect and have been linked to local invasion and metastasis. A prospective randomized placebo control study (n=223), evaluated the *MMP-2* and -9 inhibitor *BAY 12-9566*, in dogs with OS. All patients were treated with amputation and doxorubicin but there was not any improvement between trials *BAY 12-9566* and placebo control[184].

A team studied the effects of Tyrosine Kinase Inhibitors (*TRK*s), dasatinib and crizotinib on *MMP*s and *HGF*[185]. Initially, the study investigated the ability of *HGF* and *MMP*s to induce invasion and migration in OS cell lines, resulting that only HGF could influence positively these metastatic attributes. Importantly, dasatinib was found capable of suppressing OS cell viability and *HGF*-induced invasion and migration. In four case studies associated within the publication, the authors state MSTs of 15, 28, 29 and 33 months (last dog was still alive at publication). These results could justify the failure of *BAY 12-9566*, as *MMP*s were found not related with migration and invasion. In addition, a recent small study evaluated the effect of *TRK* toceranib, on *VEGF* and *MMPs* but failed in delivering a higher than usual DFI and MST [186].

Transcription factor Zhangfei, inhibits the growth and the unfolded protein response, in canine osteosarcoma (OS) cell lines, by stabilizing the tumor suppressor protein *p53* [187]. A study showed that by infecting four OS cell lines with an adenovirus expressing either Zhangfei or the control protein β-galactosidase, cells failed to divide as early as day one after infection with Zhangfei virus [188]. In this study, though, cell lines presented a functional *p53* which is not the case, at least, in many human OSs. The proportion of functional *p53* in dogs with OS is not known.

Fluoroquinolones are a class of antibiotics that have showed independent anti-tumor activity. The p53 pathway has been suggested to play a role in mediating response to fluoroquinolones, with several studies reporting that the cytotoxic effects of fluoroquinolones may be cancer specific [189]. An *in vitro* study claims that enrofloxacin enhances the effects of chemotherapy with carboplatin or doxorubicin in canine OS cells with mutant and wild-type p53 [189].

Etoposide is a synthetic derivative of podophyllotoxin, an extract of the mandrake plant and has been important in human neoplastic treatment [172]. Piroxicam in an NSAID that has been reported to have antitumor activity [172]. A study in OS cell lines found that, etoposide alone significantly suppresses cell growth and viability, whereas etoposide in combination with piroxicam exhibited concentration dependent cytotoxicity [190]. Also, the NSAID meloxicam, caused apoptosis in canine OS cell lines [191]. Baicalein, a specific flavone primarily isolated from plant roots (*Scutellaria*

baicalensis), is used in Eastern medicine for its anti-inflammatory and antineoplastic properties. One study, reports that baicalein was effective in inducing apoptosis in canine OS cell lines and did not prevent doxorubicin cell proliferation inhibition [192].

There are very promising results, for the micrometastatic stage of the disease, coming from the field of immunotherapy. Related studies and results, will be discussed, in a separate section to come.

3.2.7.3 Management of Macroscopic Metastatic Osteosarcoma

With the occurrence of macroscopic metastatic disease, cancer, reaches a level of progression that medicinal methods have difficulty in containing. In canine OS, the main core traditional strategies, are based on surgical removal and on the cytotoxic effects of CHTH. As discussed previously, the metastatic cells of OS, target mainly the pulmonary parenchyma, as well as other bones.

Resection of pulmonary metastasis, has been reported frequently in humans but there are very few reports on dogs. Authors describe the removal of small sub pleural nodules, using pursestring sutures, at the level of normal tissue. Deep, larger lesions though, must be removed by partial or total lobectomy. An overall MST of 487 days was achieved in a group of dogs, that underwent a metastasectomy, but with heterogeneous form of treatment before the evolution of macrometastatic disease. There have been established important selection criteria for pulmonary metastasectomies. A case for metastasectomy includes, a) remission of primary tumor, preferably with a DFI of more than 300 days, b) presence of one to two nodules on Rx, c) a negative metastatic bone scan, d) a long lesional doubling-time, over 30 days, without new metastasis evident. [96]

Even if bone metastatic OS gives larger MST when there is not any pulmonary involvement, it is not recommended to treat these patients only with limb-sparing surgery or SRT. Furthermore, a whole-body PET/CT is advised always, prior to any surgeries. A clinician, has to consider that metastatic bone lesions can be stabilized palliatively. [96]

Conventional cytotoxic agents are considered not efficacious for managing macroscopic metastasis of OS. As *in vitro* studies, have shown that *TRK* inhibitors have antitumor activities, the *TRK* toceranib, was administered orally, to evaluate its effects in a clinical setting. The partial response was at 4.3% and the rate of stable disease at 43%. A small study evaluating the effects of valproic acid and doxorubicin reports that achieved durable disease stabilization. [96]

Since the pulmonary parenchyma has a large absorptive surface with high blood flow, there has been an interest in testing aerosol drug delivery, for the management of macroscopic OS metastasis. Aerosolized doxorubicin alone resulted in measurable anticancer activity without any clinically evident dose-related toxicity. However, on necropsy toxin induced pneumonitis and interstitial fibrosis was discovered. A taxane plant alkaloid, named paclitaxel, is used frequently in humans against neoplasia [172]. Paclitaxel, was also used in an aerosolized form, on canine OS patients. While giving no indication of dose-related toxicity, resulted in fair duration of complete remission. [96]

3.2.7.4 Immunotherapeutic Options for Canine Osteosarcoma

The capacity of the immune system to recognize and eliminate cancer cells is well known for over a century. The use of a vaccine known as “Coley´s toxins” was found to be efficacious for a variety of sarcomas in 1891[193]. Even more, the pleasantly unexpected, increased survival times of dogs that experienced a post-surgical infection, has driven funds to the research of immunologic means for canine OS treatment. Nowadays, scientists are using *in vitro* and *in vivo* studies, to discover how the immune system targets neoplastic cells and cease that power in ways that are clinically applicable.

Bacillus Calmette-Guérin (BCG) immunotherapy, was investigated initially in the 1930’s. Today, plays a role in bladder cancer treatment for humans. Studies, in where dogs with OS, were receiving BCG, indicated a delay in metastatic development and achieved survival times of 54 weeks versus 14 in controls. There are thoughts, that BCG functions by activation of circulating monocytes or tissue macrophages in reticuloendothelial organs. [194]

The liposomal form of muramyl tripeptide phosphatidylethanolamine (*L-MTP-PE*), is a lipophilic derivative of muramyl dipeptide, which is a synthetic analog of a *Mycobacterium* cell wall. *L-MTP-PE* stimulates in vivo macrophages and monocytes but it does not exert a direct cytotoxic effect. Its use in humans with OS has resulted in prolonged survival times in both initial and relapsed settings. The most relevant study, used *L-MTP-PE* in combination with cisplatin. Patients, without macroscopic metastatic disease, when treated with an alternating combination, reached a MST of 14.4 months versus 11.2 months of controls. Still, when concurrently administered, cisplatin and *L-MTP-PE*, resulted in no survival benefit. As such, *L-MTP-PE* was not approved, by the US Food Administration, for pediatric OS treatment. However, *L-MTP-PE* is approved for use against non-metastatic pediatric OS in the European Union.[96, 194]

Interleukin-2 (IL-2), is a pleiotropic cytokine in charge of establishing cell-mediated immune responses. When evaluated in vivo for canine OS treatment, resulted in only modest evidence of regression in just half of the patients and eventually all dogs developed antibodies anti-IL-2. Is spite that, autologous stimulated lymphocytes derived from dogs with lung tumors can be preferentially reactivated by IL-2, to exert cytotoxicity against naturally occurring metastatic OS. Further analysis on the potential of IL-2 on lung-specific immunotherapy could be important in the future. [96, 194]

Vaccination against various tumors have been under scientific research. For canine OS, an attenuated and genetically modified, *Salmonella typhimurium* (VNP20009) IV administration, was found to be prohibitively toxic. Other vaccination strategies were investigated without significant clinical relevance when compared to historic controls. [96, 194]

The most optimistic clinical results overall, derived from a study that investigated ways to develop specific immunity against a tyrosine kinase receptor that belongs to the family of Epidermal growth factor receptors (HER2/neu)[195]. The team, conducted a clinical trial to evaluate the safety

and efficacy of ADXS31-164, which is a highly attenuated, recombinant *Listeria monocytogenes*, expressing a chimeric human HER2/neu construct. The goal was to prevent metastatic disease, in dogs with appendicular osteosarcoma, following amputation and chemotherapy with carboplatin. Eligibility criteria were: a) a histopathological and immunohistocompatibility diagnosis of HER2/neu osteosarcoma, b) primary tumor removal either by amputation (n=17) or limb-sparing surgery (n=1) and c) administration of four doses of carboplatin. Screening of the patients, took place three weeks after their last carboplatin treatment. Before ADXS31-164 administration, dogs received a single dose of the 5HT3 antagonist, ondansetron IV, to prevent nausea and vomiting and Benadryl® intramuscularly to prevent anaphylaxis. ADXS31-164, was diluted in 0.9% NaCl and administered intravenously over 30 minutes. Dogs, were re-staged three weeks after their third ADXS31-164 administration and every two months thereafter, until disease progression. All dogs tolerated ADXS31-164 well with only transient, low-grade toxicities observed on the day of administration along with mild pyrexia in all subjects. There was a significant difference in the magnitude of WBC, neutrophil, and monocyte responses and a trend toward significance in platelet responses, in dogs that survived greater than 18 months, compared with dogs that died within 18 months of diagnosis. [195]

For the 18 dogs with had no evidence of metastatic disease at enrollment and were treated with ADXS31-164, the median DFI was 615 days and MST was 956 days. Overall survival rates at one, two, and three years, for dogs treated with ADXS31-164, were 77.8%, 67%, and 56%, respectively. The MST of all 23 dogs (including five pets with pre-existing metastatic disease) was 738 days with overall survival rates at 1 and 2 years of 60.9% and 52%, respectively. [195]

Although, this study did not allow to determine whether the survival benefit afforded by ADXS31-164 was HER2/neu dependent, controlled experiments using this approach in mice, reveal that the antitumor effects and prolonged survival are HER2/neu dependent and not associated with a Coley's toxin effect [196] A larger, randomized *Listeria*-only controlled study, should be performed to confirm a HER2/neu-dependent mechanism of action in the dog.

3.2.8 Palliative Care for Bone Cancer Pain

The most important clinical symptom of any bone lesion is intense pain. Veterinary textbooks, describe the pain of neoplastic bone as excruciating or severe [197]. When an internal or external stimulus is harmful for the body, there must be a defense mechanism that helps sense, integrate and perceive this stimulus in order to act for avoidance and future prevention. In the first line of the sensation of pain, lye specialized efferent nerve endings, termed nociceptors. The greatest the density of nociceptors in a tissue, the greatest the impulse generated. The periosteal surface and medullary cavity of bones, possess the highest density of nociceptors. [96] In canine OS, the painful stimuli origin from the invasive growth of malignant osteoblasts and from the dysregulated and

pathologic osteoclastic bone resorption. [96, 198] As follows, an effective analgesic protocol should include eradication of malignant osteoblasts and inhibition of the osteoclastic bone resorption.

In human OS, palliative Radiation Therapy (RT) is most effective treatment for osteolytic lesions, through induction of osteoblastic and osteoclastic apoptosis. In canine trials, symptomatic improvement reached a maximum of 130 days. However, leading authors suggest that when RT is combined to CHTH the analgesic response is enhanced as well as the its duration. [96]

The use of Sm-EDTMP, which is the combination of the radioisotope Samarium and a bisphosphonate, is available for palliative treatment of breast or prostate cancer pain in humans. In dogs, there are reports of alleviation of OS bone pain with IV administration of Sm-EDTMP. In less than five percent of dogs, Sm-EDTMP, resulted in complete involution of skeletal lesions. It is described as well tolerated but with a resulting decrease in platelet and WBC count. [96]

Aminobiphosphonates (NBPs), were initially used for diagnosis through bone scanning because they preferentially get absorbed in sites of bone mineral remodeling. NBPs are considered as first-line treatment for malignant osteolysis and metastatic bone cancer in humans. They function by inducing osteoclastic apoptosis that in dogs resulted in subjective pain alleviation. Pamidronate is the most commonly agent used in an IV administration. In clinical studies, it reduced numeric lameness scores, compared to controls, while it decreased malignant bone resorption of the primary tumor. Another NBP, zoledronate, is reported to be 100 times more antiresorptive than pamidronate[96].

3.3 RETIRED RACING GREYHOUNDS WITH OSTEOSARCOMA

Greyhounds, have been used in racetracks since the beginning of the 20th century and the invention of the mechanical hare. Today, there are around 20 racetracks left in the U.S., most of them in Florida State. Since the 90's, there have been over 180.000 RRGs adoptions in the U.S. and "A&A" has actively been a part of adoption programs for more than two decades [199]. Every year, "A&A" staff, runs a two-week marathon of gonadectomies on RRGs. Most RRGs, are received in "A&A" with five or six years of age. Neuters and spays are performed and the retired racers are placed for adoption through cynophile associations. Thankfully, the majority of the RRGs get adopted and enjoy comfortable lives with their caregivers. Many of these dogs return as patients and stay in touch with "A&A" veterinarians for all their life. Frequently, individuals have a history of many RRGs adoptions and as follows, know well the breed and the disorders that affect it. When a RRG presents lame, OS, is an important concern for well-informed caregivers. Such a preoccupation, is justified, when considering that one out of four deaths of RRGs , reportedly is due to OS [92]. Racing Greyhounds (RGs) are the least inbred, when compared with American Kennel Club (AKC) Greyhounds,

Rottweilers and Irish Wolfhounds [113]. AKC Greyhounds, are a close but distinct genetic population from RGs[113]. Yet, there are not references of any difference, in OS incidence, among AKC Greyhounds and RGs [113]. The author, could not find any evidence that suggests some connection between a racing career and OS. "A&A" clinicians, as well, did not give account of any proved relation.

Coccidioidomycosis, is amongst the most important diagnostic differentials for OS. *Coccidioides* spp. is a fungus that, from the soil, produces airborne arthroconidia, which can be inhaled and reach terminal bronchioles. When arthroconidia contact with the alveolar epithelium, a sequence of events starts with septation-vacuolization and concludes with endosporulation. Endospores, entering the lymphatic and blood stream, can reach also to bones causing an infection that could lead to osteomyelitis. Consequent hilar lymphadenopathy or diffuse pulmonary interstitial disease can cause dry or moist cough respectively and signs such as fever, anorexia and weight loss. The disease, is called "valley fever" which, when worsening, can develop to pneumonia and lytic and/or proliferative bone lesions. However, incidence of Coccidioidomycosis, is restricted to areas of the southwest U.S. and Mexico. As mentioned, most of the racing tracks are in Florida State but there are also in Houston and Harlingen in Texas, in a high, Coccidioidomycosis incidence, area. The main radiographic difference from OS, is that Coccidioidomycosis, can spread over joints or to other distant bones. [200] Therefore, a respiratory tract examination, the radiographic presentation and travel history of a RRG, with suspicion of OS, can help rule out an infection from the insidious *Coccidioides spp.*

Greyhounds, have been subjected to careful selection for achieving great speeds. This has resulted to a larger muscle mass than most breeds, higher hematocrit (Hct), lengthened carpal, tarsal, metacarpal and metatarsal bones as well as a keen sense of sight[199]. There are many hematologic and biochemical factors that have been studied and found to have reference intervals, different from other breeds (Table 21).

Table 21. Analytes and features characteristic of Greyhounds compared with other breeds. (199)

Higher values	Lower values
PCV-Hct	White Blood Cells count, Neutrophil count
Red Blood Cells count	Platelet count
MCV, MCHC	Fibrinogen
Hemoglobin affinity for O_2	Clot kinetics and clot strength
Creatinine	Potassium, Phosphate, Calcium, Magnesium
Glomerular Filtration Rate	Serum total protein
Total CO_2 Bicarbonate	Total globulins
ALT, ASP	α- and β- globulins, IgM and IgA
Sodium, Chloride	Total T4 and free T4

3.3.1 Case Study "*Juliette*"

Juliette, was adopted by a family with long history of involvement in RRG rescues (Table 22).

Table 22. Information related to the Case Study "Juliette"

Breed	Racing Greyhound
Date of Birth	August 26th 2003
Gender	Spayed Female
Date of Adoption	March 2008
Date of Clinical Suspicion of Osteosarcoma	September 24th 2016
Date of Osteosarcoma Radiographic Diagnosis – Years of Age	October 4th 2016 – 13 years of age
Lesion Location	Proximal metaphysis of left humerus
Weight on Diagnosis	27 kg
Date of Euthanasia	October 15th 2016
Survival Time from Radiographic Diagnosis	11 days

She always had an updated immunization and heartworm prophylaxis record, while she was submitted to annual dental cleanings until 2013. In 2011 after a few episodes of erratic aggression, she was placed on amitriptyline therapy, that was given through her life thereafter, when episodes of anxiety would reappear. In December 2012, there was evidence of cervical and lumbosacral pain on palpation that reemerged in 2014 but, both times, resolved with deracoxib. She was on chondroprotective supplements and had an interdigital cyst removal in August of 2015. Throughout her life, paw pad corns were common. In February 2016, the owner complained about painful signs when the dog was trying to stand up or move suddenly. Once more, lumbosacral pain was elicited on palpation and the possibility of a progressive lumbosacral stenosis was discussed with the caregiver. Otherwise, a senior profile performed on the same month was unremarkable.

3.3.1.1 Anamnesis and Clinical Progression Related to Osteosarcoma

During the last days of August 2016, Juliette, developed mild diarrhea. On September 24th, when the caregiver returned for a reevaluation and after treatment with metronidazole, the GI tract was back to normal. However, the owner related that she had noticed some weakness on the left front limb during the past two days. On the physical exam, some swelling and erythema of the distal radius and carpus was evident. There was no pain on any joint movement, neither on palpation of any bone. Also, there was no clinical sign of lameness. Juliette was medicated with deracoxib (54mg PO, SID) but the caregiver, three days later reported no improvement on inflammation or intermittent lameness. On September 29th, there was a decrease in inflammation and no erythema present. Still, five days

later, the pet returned due to an increased, firm, edema that now was extending to proximal antebrachium with a positive painful response on palpation and flexion of the carpus. Both the owner and the attending clinician knew that they had to proceed to an Rx exam from previous discussion.

Juliette, was sedated and Rx of the left foot, antebrachium and shoulder were taken. The areas of the metacarpus, carpus and antebrachium were unremarkable. However, there was indication of bone lysis, on the proximal metaphysis of the humerus but without proliferation (Figure 37). This finding, conducted to chest Rx screening for possible metastatic lesions. Chest Rx, were unremarkable. The patient was in pain and a brief discussion with the caregiver highlighted the fact that their family had already experienced twice the same disease in other RRGs. Nevertheless, Juliette was placed in Aminocaproic Acid (ACA) pre-surgical treatment, in case the caregiver would elect to proceed to amputation. There is evidence that, amongst Greyhounds, exist “bleeders” (26%) which have lower platelet counts and antiplasmin activities but high fibrinogen and Hct. This suggests that excessive postoperative bleeding in RRGs is not attributable to a defect in primary or secondary hemostasis, but it may be related to enhanced fibrinolysis[[199]]. As a fibrinolysis inhibitor, ACA, is administered to Greyhounds at “A&A” a few days prior to an amputation, which is a highly invasive procedure [[201]]. Additionally, Juliette received prednisone, in an antinflammatory dose, for five days with conditional tapering thereafter.

On October 6th, the caregiver was contacted and there was not any deterioration but neither recuperation. Finally, on October 15th of 2016, the patient returned for a Rx reevaluation. The painful response was more evident, now on palpation of the humerus, the edema was unchanged and the patient was lame. Radiographs revealed a slight augmentation of bone lysis on the humerus (Figure 38). On the same day and following a discussion in depth, on the various possible actions, the owner elected to proceed to humane witnessed euthanasia.

3.3.1.2 Discussion

In this case, the strikingly short survival time, of just 11 days, was influenced from some key factors. Firstly, the age of the patient was advanced (13 years), which as mentioned in section 3.2.6, is a negative prognostic factor but also, plays an important role in the mindset of the caregiver. Another negative prognostic factor, the anatomical location of the humerus was also considered. Additionally, the effects of a possible lumbosacral stenosis to a three-legged life were certainly not favorable. Palliative care treatment was excluded because of the severe pain the patient was already experiencing. Finally, previous unpleasant experiences, of copious treatment of another pet with OS, as well the economical weight of it were inhibiting factors for owners.

Even if the patient did not survive enough time, to compare laboratorial results to a DFI, it is worthy to mention some clinical data from analysis of August 2016. Juliette, presented a ALP of 110 U/L which is the exact value from which good or bad responders were categorized in one study. Dogs having a higher value than that, survived much longer. Furthermore, a study that focused on

determining breed specific reference intervals for Greyhounds, relates that in 100 healthy dogs, TALP was between 19 and 90 U/L (15-120 U/L non-breed specific)[199]. If these results reflect breed values in general, then TALP in this case was higher than normal. Also, a low platelet count (163 *10^3/μL) on the same analysis could predict a worse prognosis as it has been related that number of platelets are inversely related to metastatic progression. However, the same study mentioned above, relates lower platelet counts for Greyhounds, so Juliette's value would be normal (Greyhounds platelet count 145-309 *10^3/μL). Moreover, the radiographic presentation of an exclusively lytic lesion it is all but not a favorable sign. Predominantly lytic lesions, represent poorly differentiated or telangiectatic OS, which both are highly aggressive forms. Lastly, when applying Juliette's data on the mortality risk (MR) prediction tool, referred also in section 3.2.6, she is given a 53.3% five-month MR and a 66.3% .3one-year MR if amputated and submitted to CHTH. [152] [93] [160] [199]

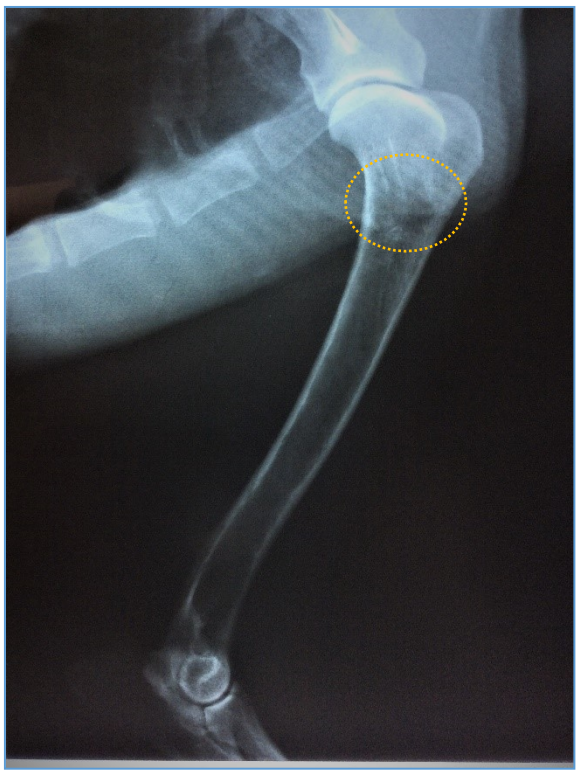

Figure 37. Radiograph of Juliette's left humerus on October4th 2016

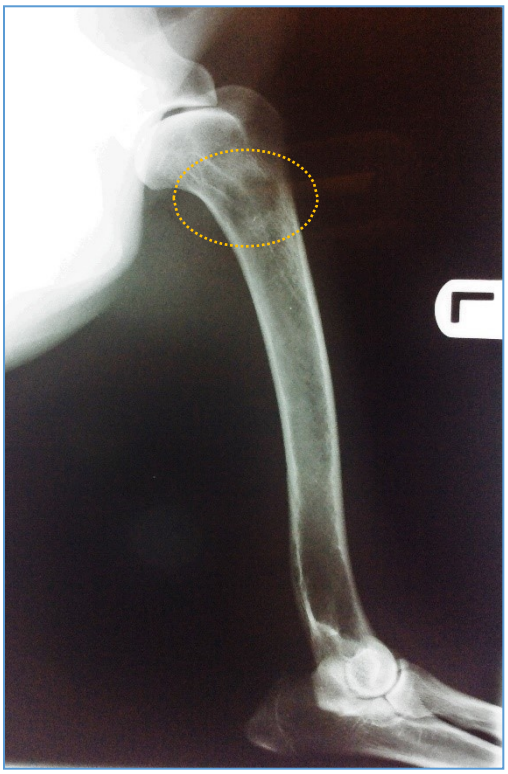

Figure 38. Radiograph of Juliette's left humerus on October 15th 2016

3.3.2 Case Study "Avishay"

Avishay, a tall brindled Greyhound, raced until 2010 and then was adopted to live in a home, where other RRGs were previously adopted. Records, from "A&A" files, depict a healthy overall life, from 2011 to 2016 (Table 23). Detartrations, were less frequent, than in the previous case study, with one dental checkup leading to several teeth being extracted. Vaccination, as well as parasite and heartworm control were mostly on schedule, throughout the pet's life.

Table 23. Information related to the Case Study "Avishay"

Breed	Racing Greyhound
Date of Birth	December 9th 2005
Gender	Neutered Male
Date of Adoption	2011
Date of Clinical Suspicion of Osteosarcoma	April 25th 2016
Date of Osteosarcoma Radiographic Diagnosis – Years of Age	April 29th 2016 – 10 years of age
Lesion Location	Proximal metaphysis of right humerus
Weight on Diagnosis	35 kg
Date of Euthanasia	September 24th 2016
Survival Time from Radiographic Diagnosis	152 days

3.3.2.1 Anamnesis and Clinical Progression Related to Osteosarcoma

On April 25th of 2016, the caregiver of Avishay, brought the dog with a complain of intermittent front limping but without, him, being able to specify on which limb. During the examination, the patient was not lame but a positive painful response on cervical manipulation raised suspicion for an intervertebral disk injury that could cause a root signature. There were not any painful bones or joints. There was a high accumulation of dental plaque as well. Thus, the owner elected to proceed to a sedated detartration and a radiographic screening at the same time. Avishay, was sent home with recommendation for movement restriction and was medicated with deracoxib.

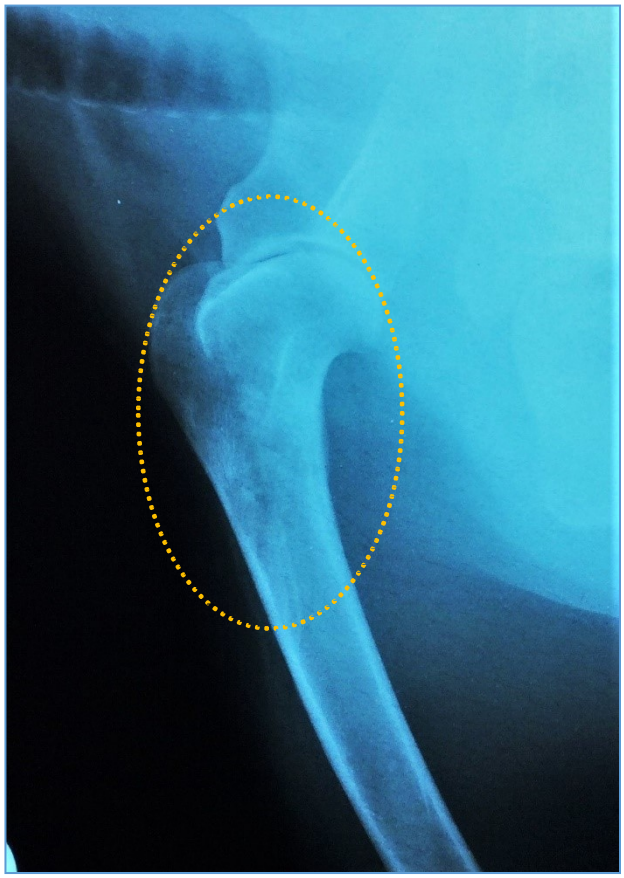

Figure 39. Early signs of bone lysis on proximal humerus of Avishay. Radiograph of April 29th 2016.

Four days later, the patient came in for a scheduled dental cleaning. This time, there was a right front lameness and a positive pain response on palpation of the humerus. There was some resentment on neck manipulation on lateral movements as well during ventroflexion. The

cervical region was screened with Rx, indicating a slight variation of the intervertebral disk spaces particularly between C3-C4 and C4-C5. Additionally, there was evidence of mild alteration of the proximal right humerus along with an increased medullary density on the distal radius (Figure 39). Subsequently, the owner was consulted and permission to proceed to chest radiographs was granted. The chest Rx, was unremarkable and the patient was submitted to a detartration.

Following a discussion on a possible tumor lesion of the proximal humerus, the caregiver solicited the opinion of a radiologist to clarify the nature of the lesions. The radiographs were sent to a board-certified radiologist and a report was released on May 4th of 2016. The findings were similar and the expert commented that the medullary density on the radius was non-pathologic and probably not related to a primary bone tumor. The report, concluded, by stating that even if the alteration on the proximal humerus might not be dramatic, an early bone tumor could not be excluded.

The need to act on the primary tumor was appointed to the owner, as well the possibility to use other diagnostic tools available. In the meantime, a long term deracoxib therapy was instituted. Lameness was still emerging occasionally but Avishay, seemed to be comfortable when under the NSAID. As a result, the owner opted to reevaluate radiographically in a month's time.

On June 10th 2016, the Rx was repeated and unfortunately the findings were not good (Figure 40). Mottled osteolysis appeared more pronounced and extended, than a month before. Once more, the

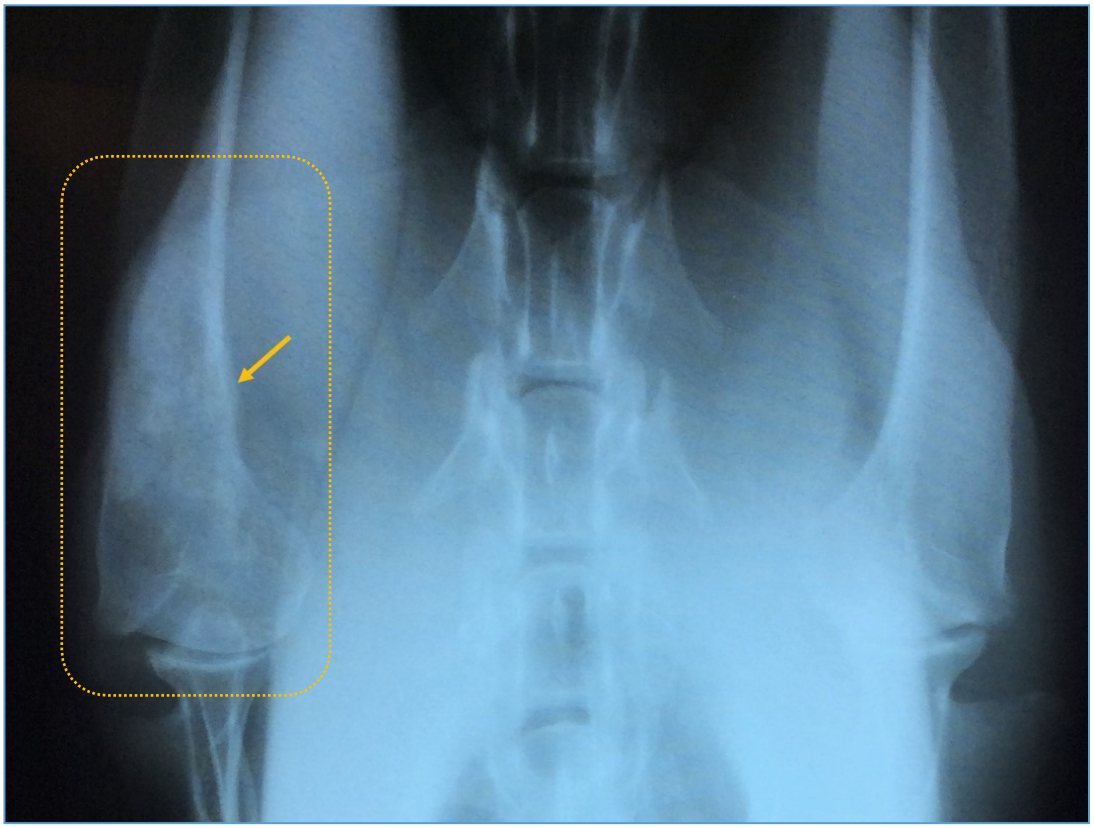

Figure 40. Ventro-dorsal view of Avishay's both humeri. The left humerus is normal. The right humerus shows signs of trabecular lysis and proliferation that could evolve to a Codman's triangle (yellow arrow). Rx of June 10th 2016.

possibility of an ongoing bone tumor like OS, as the cause of the lesions, was underlined to the owner. However, he expressed the need to contact a board-certified oncologist. Therefore, all data on the case and radiographs were released from “A&A”, that was in continuous contact with the reference center. Finally, the patient, after the initial oncologic visit that confirmed OS suspicion, was scheduled for full body CT scan.

The author, did not have access to CT images but the report, issued on June 17th of 2016, confirmed the presence of osteolytic and proliferative lesions, compatible with OS, without any signs of metastasis elsewhere. Also, the oncologist prescribed tramadol and gabapentin for pain control. Eventually, the caregiver of Avishay, decided to proceed for an amputation and adjunctive chemotherapy with carboplatin, as both “A&A” clinician and the oncologist had recommended. Despite that the decision was certain, during a discussion with the surgeon, emerged some possible practical downsides of a three-legged life in an apartment on the fourth floor. An elevator existed but Avishay was not always eager to use it, while the staircase was marble made and slippery. Nevertheless, the caregiver continued with surgery.

3.3.2.2 Amputation, Chemotherapy and Relapse

A forequarter amputation took place on June 23rd at “A&A”, almost two months from the initial radiographic diagnosis. The fibrinolysis inhibitor ACA, was started preoperatively and continued after the amputation for five days. Pre- and postoperative antibiotherapy, was implemented, with cephalexin IV and postoperative analgesia with buprenorphine IM. A protective sleeve covered the surgical wound and an Elizabethan collar was placed.

Table 24. Five cycles of Carboplatin. One cycle every three weeks.

Date	Weight	m^2	Carboplatin dose
June 29th	29 kg	0.94	282mg
July 16th	28 kg	0.93	279mg
August 6th	30 kg	0.96	288mg
August 27th	30 kg	0.96	288mg
September 17th	31.3 kg	0.99	297mg

The patient seemed uncomfortable in the immediate post-surgical setting and additional buprenorphine was administered. Yet, on June 24th, Avishay, was able to stand and could walk with assistance on the 25th. On the 26th was eating and finally on the 28th could walk unassisted. The first dose of carboplatin was administered on June 29th 2016 (Table 24). Usually, the first dose of carboplatin is administered after surgery. The owner though asked for a seven-day hospitalization due to personal commitments, so the CHTH delayed until the patient was stable.

During CHTH with carboplatin, there were no signs of acute toxicity. The patient, tolerated well the protocol without any significant adverse effects, except of occasional loose stool and mild nausea, controlled with maropitant. However, on the last day of CHTH, the pet was reluctant to walk and the owner reported some tremors and loss of equilibrium when trying to stand.

Therefore, on September 17th, before CHTH, a physical examination revealed low lumbar pain and a radiographic metastatic checkup took place. There were no signs of nodular metastasis in the lungs but there was a slight narrowing of the intervertebral space between L6-L7, gas in the small bowel and feces accumulated in the colon. Carboplatin was administered and except deracoxib, tramadol also, was given to the owner in case any pain would occur.

The following week was not good for Avishay. The RRG, was lethargic for two days and would walk only to eat. After that, the patient's appetite begun to diminish and would not stand for hours. On September 23rd, the caregiver related that Avishay was anxious, had tremors and was slipping on his front leg when trying to stand.

On September 24th, the patient was brought to "A&A". A neurologic exam on the front limp revealed negative reaction on superficial and deep pain tests, as well as a delay in proprioceptive positioning reaction in the hind limbs. The animal, most of the time was in lateral recumbency and when it was trying to lift the head, slight shaking was evident. Muscle twitching and tremors were also common. On the same day, the caregiver chose to proceed to humane euthanasia.

3.3.2.3 Discussion

Time and space, are measures related to life and exceptionally important when life is threatened. In OS, as well as in most types of neoplasia, the size of a tumor and the time it is given to act uncontested, are inversely related to life expectancy. Avishay, was one of the cases in which there was early detection, and presumptive diagnosis of a cancerigenous process. However, the size of the initial lesion was small enough to cause a dilemma for the caregiver. Many people are reluctant to perceive an amputation as a therapeutic procedure, which is natural when referring to humans. Dogs though, are very easy to please and are capable to live happy, with just three legs. Thus, on the authors opinion, Avishay was lucky to get diagnosed early, but, in a way, unfortunate for the lesion was too small to alarm his caregiver enough. Two months, in canine oncologic terms, are a substantive period of time. So, it is probable that the delayed removal of the primary tumor was detrimental in this case.

The neurologic symptoms, the patient presented at the completion of CHTH with carboplatin, could possibly be due to a platinum compound neurotoxicity. Clinical symptoms presented in humans, detectable on dogs, include sensory loss in the feet and legs that can cause sensory ataxia and gait disorders, as well as vestibular dysfunction and hearing loss. However, carboplatin is used exactly because it less toxic than cisplatin and the author could not recover any reports of case studies on carboplatin neurotoxicity in dogs [202]. Furthermore, most authors, attribute to carboplatin, only GI toxicity and myelosuppression with the most characteristic clinical finding being thrombocytopenia. Still, the animal in case, showed normal CBCs prior to all CHTH cycles.

Another explanation for the neurologic signs could be lesions present on the cervical and lumbar spine. As mentioned, there were signs of probable intervertebral disk disease in the cervical section

in April 2016, as well a suspicion of narrowing space between L6 and L7 in the last Rx screening. Nevertheless, OS can metastasize to the brain via the hematogenous route and that could also explain many neurologic signs [93].

Whatever was the cause of the neurologic symptoms of the pet, it was irrelevant, for his life quality was deteriorating too quickly to justify further diagnostic investigation. An expensive MRI, could resolve the case in medical terms but it would just prolong a suffered life for Avishay, and create more angst to the caregiver.

When reviewing bibliography on OS, it becomes obvious that a cytologic or histologic diagnosis is the only certain one and of course, this is true. Yet in practice, a biopsy is not an easy procedure nor it is straightforward to convince an owner to go for it. Particularly, with OS that is so highly variable cytologically that results frequently are non-diagnostic[93]. In academic or scientific settings, results can be very good for both cytology and histopathology. A study, reports an 83% and 82.1% of accuracy for cytology and histology respectively [131]. However, practitioners, may find these results overestimated. As follows, an invasive and expensive procedure, that has fame of little reliability, is very hard to "sell" to a caregiver, particularly when the treatment is a dead end for an amputation or limb sparing surgery. Also, when reflecting on the practical value of a histopathological diagnosis after an amputation, one could see that, more wisely, the expenses could be saved and directed towards a treatment. The only argument though, that validates the previous statements, is that OS displays such a degree of characteristic clinical and radiographic presentations, that these factors alone can yield a diagnosis.

Finally, the use of the MR prediction tool on Avishay's case, resulted in a 48.2% and 68.5% for five-month and one-year MR respectively [160].

3.3.3 Case Study "Zander"

Zander was adopted in 2012, from a very committed and attentive caregiver, with history of other RRGs adoptions (Table 25).

Table 25. Information on Case "Zander"

Breed	**Racing Greyhound**
Date of Birth	December 4th 2007
Gender	Neutered male
Date of Adoption	2012
Date of Clinical Suspicion of Osteosarcoma	January 31st 2015
Date of Osteosarcoma Radiographic Diagnosis – Years of Age	February 25th 2015 – 7 years of age
Lesion Location	Proximal metaphysis of left humerus
Weight on Diagnosis	36 kg
Date of Euthanasia	March 12th 2016
Survival Time from Radiographic Diagnosis	381 days

Since the early days of his life in this new family, Zander showed that his racing career affected negatively the function of his limbs. Many minor lameness episodes, drove the pet to the doctor's office, almost on a monthly basis. Corns on the right hind, minor carpal, tarsal and metatarsal sprains, caused frequent distress to the pet. Retired Racing Greyhounds, suffer from many tarsal, carpal, and metatarsal fractures [203]. The counterclockwise direction of the races influences the anatomic signalment of these fractures. The left metacarpal bones and the right metatarsal are the most affected [203]. Additionally, Zander had recurrent allergic pododermatitis and acute episodes of pruritus. Otherwise, until January of 2015, the patient was in a good condition. This case study has been recovered from "A&A" file records and the author relied also, on the attending clinician's information.

3.3.3.1 Anamnesis and Clinical Progression Related to Osteosarcoma

On January 21st 2015, the patient visited the hospital for an intermittent lameness of unspecified origin. In fact, a gait exam revealed both right front and left hind lameness. On one left hind paw pad, there was discovered a corn, which is was a usual finding for Zander and for RRGs in general. The corn was removed and administration of antinflammatory dexamethasone IM, as well as prescription of carprofen PO was the therapy instituted. On the 31st of the same month, the patient returned with a complain of an ongoing intermittent lameness of the right front. However, there was no lameness present on the physical exam and no pain on palpation of long bones. Nevertheless, Zander was crying when palpated on both scapulae and also on the caudal cervical area. Dexamethasone IM, was administered once more.

On the February 25th, the caregiver came in with Zander for an Rx screening, due to the unsolved lameness of the right front limb. There was a positive pain reaction on the humerus and radiographs were taken. A zone, of bone proliferation and mild lysis was evident on the proximal humerus (Figure 41). Additionally, all long bones and the chest were screened. The chest was clear of visible metastasis.

3.3.3.2 Amputation, Chemotherapy and Relapse

The caregiver, was advised to proceed to amputation and CHTH as soon as possible and the dog was medicated with ACA preventively as well with deracoxib. Rapidly, the decision to proceed to an amputation was reached and Zander was hospitalized and amputated on March 2nd 2015.

As in Avishay's case, Zander also was medicated perioperatively with cephazolin IV (instead of cephalexin), buprenorphine IM and ACA PO.

On March 3rd, the patient received his first treatment with carboplatin, was medicated with tramadol PO and continued cephazolin IV as well as ACA. There were signs of irritation on the left front, above

the catheter and for that, it was pulled out. However, the overall condition was excellent and the pet could stand alone, from the first night after the surgery. On the 4th of the same month, postoperative antibiotherapy was initiated with cefpodoxime PO, antinflammatory therapy continued with deracoxib PO and pain control was attempted with tramadol PO.

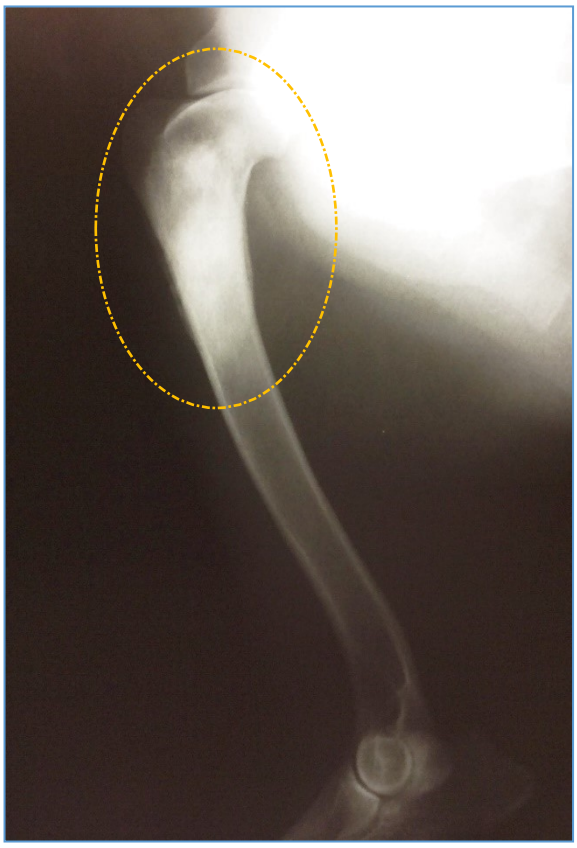

Figure 41. Right humerus of Zander on February 25th 2015

The patient, was discharged on March 7th but returned the 16th with issues on the surgical incision. Effectively, both vertical and horizontal incisions were infected with the horizontal (around the humerus) presenting some necrotic tissue and openings. Consequently, the patient was readmitted and the wound was cleaned and resutured under sedation. A sample was submitted for a culture and results yielded growth of two organisms. *Pseudomonas spp.* presented heavy growth and Meth-Resist Coagulase Negative *Staphylococcus spp*., was also recovered. As both microorganisms were susceptible to marbofloxacin, antibiotherapy was initiated on March 22nd. Apparently, the failure of successful wound healing was due to some hours during the day that the dog was left without an Elizabethan collar. The caregiver, when was home with the pet, would take the collar off, with the premise that she was watching over. The necessity for the collar to stay on, at all time, was stressed to the owner, that eventually complied, so the treatment was successful.

successful.

Table 26. Carboplatin protocol for Zander

Date	Weight	m^2	Carboplatin dose
March 2nd	34 kg	1.06	300mg
March 24th	32 kg	1.01	300mg
April 15th	32 kg	1.01	300mg
May 5th	31 kg	0.99	300mg
May 26th	31 kg	0.99	300mg

All five cycles of carboplatin treatment, were finalized without any side effects. Biochemistry profiles and CBC was, in all five cycles, normal and the patient was in good condition. Zander, was having a good functional life with three legs.

The usual metastatic checkup, on the last day of chemotherapy was not performed but the owner requested to look into options for metronomic chemotherapy. Even if the attending clinician explained that the DFI and MST referred on relevant studies were not increased, compared to treatment with carboplatin alone, Zander was eventually placed on cyclophosphamide and piroxicam [146, 204]. Cyclophosphamide was given at 12,5mg and Piroxicam at 10mg, both SID and PO.

Additionally, the caregiver has consulted an expert in traditional herbal Chinese veterinary medicine, that recommended an herbal solution for treatment of OS. The brand specifies, on their website, that the powdered solution contains : " *oldenlandia, white peony, atractylodes/white, fritillary bulb, tortoise shell, citrus, red peony, arisaema, angelica radix, hornet nest, poria, licorice, uncaria, trichosanthes, sargassum, astragalus root, scutellaria, dioscorea bulbifera bulb, fungi silkworm, sage tangle, buttercup herb, raw oyster shell, scorpion, ginseng, glehnia root, rehmannia/raw, peach kernel, asparagus, eupolyphaga, centipede, prunella, panicled swallow wort root, scrophularia*" [205]. Zander, was administered seven grams of the "herbal" powder, BID with his food. The author, searched for references on antineoplastic properties of some of the ingredients. Some studies presented with abstracts that indicated activity against bone resorption, however there was no indication of specific results neither details on methodologies [206].

Eleven months, following the radiographic diagnosis, the patient was doing well but a prominent, firm nodular mass appeared on the caudal aspect of his right thigh. After a physical exam, another firm, intramuscular mass, was palpated on the region of the amputation incision. A fine needle aspiration sample, from the mass on the thigh, was submitted for cytologic evaluation.

The microscopic description, reported a highly cellular sample on a blood background with blood leukocytes. Many variable sized aggregates and individual neoplastic spindle cells were present. The neoplastic spindle cells, presented: marked anisocytosis, anisokaryosis, binucleation/multinucleation, round to slightly oval nuclei, stippled chromatin, one to five prominent blue round angular nucleoli, micronuclei and moderate to abundant dark blue cytoplasm with a few punctate vacuoles. Frequent mitotic figures were also observed and no infectious agents were identified. The microscopic findings were consistent with sarcoma, while the pathologist commented that the morphologic features were more consistent with osteosarcoma. Whether the lesion represented metastasis, from the resected tumor or a de novo mass, was related as unclear.

On the 22nd of January 2016, a radiologic screening revealed a perihilar nodular lesion on the chest radiograph of Zander. The major suspicion was naturally that of a macrometastatic mass (Figure 42).

On the same date, records of "A&A" register that cyclophosphamide was continued weekly but there are no records related to piroxicam therapy. From this point on, Zander's condition deteriorated but he never visited the hospital again. He was euthanized, at home, by the attending clinician on March 12th of 2016.

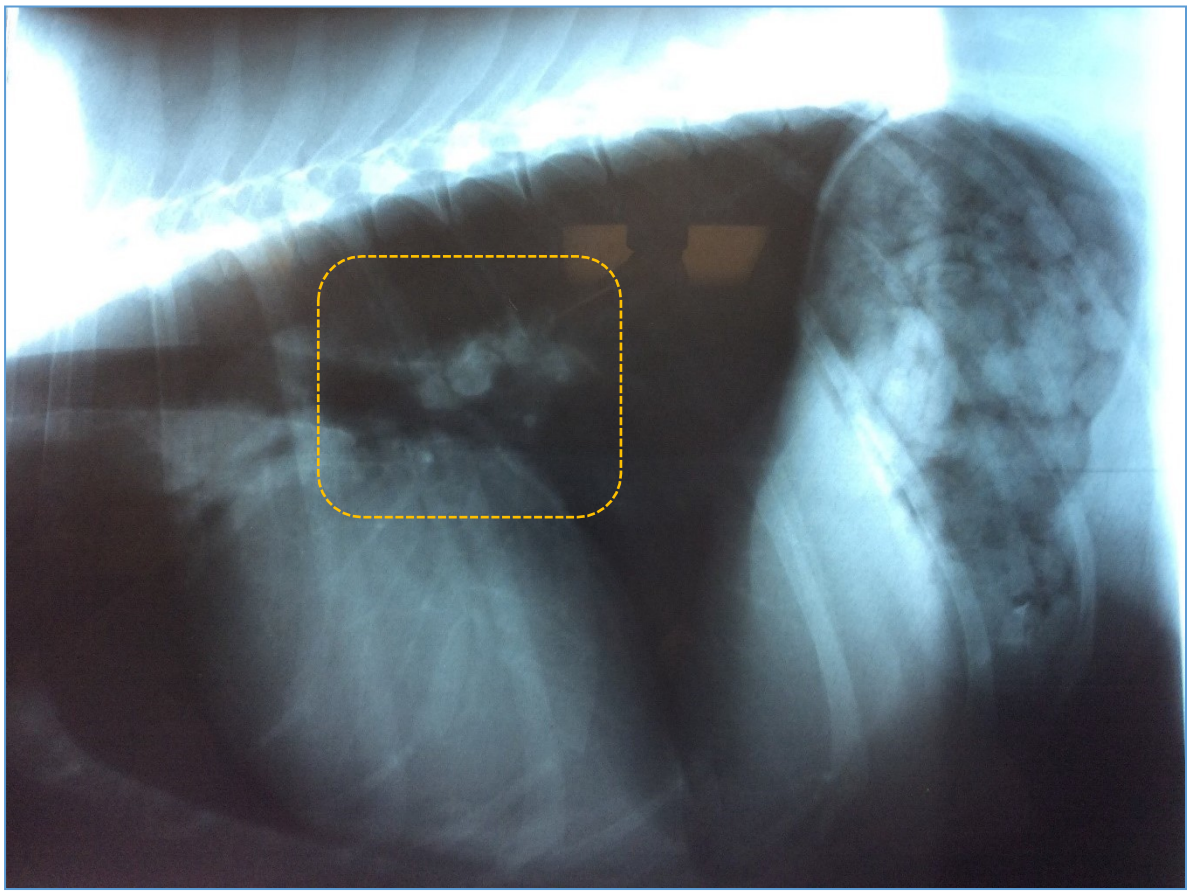

Figure 42. Chest radiograph of Zander. Possible metastatic, circular, radio-dense lesion, near the pulmonary hilum.

3.3.3.3 Discussion

Zander, overcame the MST stipulated for treatment with carboplatin, as a single agent or even the MST of treatment with carboplatin in combination with metronomic chemotherapy of piroxicam and cyclophosphamide. The MST, reported for carboplatin was 321 days and when in combination with piroxicam and cyclophosphamide was 242 days in one study (Zander's MST was 381 days) [146, 204].

This pet, had a few good prognostic factors on his side. He was only seven years old and the lesion was not very advanced when diagnosed. The amputation and chemotherapy succeeded the diagnosis almost immediately, while the levels of ALP were normal before the surgery. There were no signs of toxicity from chemotherapy neither there was any other physical impediment, such as an orthopedic or neurological disorder. Importantly, the caregiver provided the best possible care for her pet. Even if, the effort to contain the metastatic process with additional metronomic CHTH or herbal solutions, was not scientifically established or anecdotal respectively, demonstrated the eagerness to offer the best healthcare possible to the sick animal.

This was the only case that there was a cytological evaluation of a mass related to OS. There are reports of primary extraskeletal OS in the subcutaneous tissue [96]. Notwithstanding, as the pathologist commented, the fact of the metastatic spreading being hematogenous, suggests that the mass on the thigh or in the muscle could be metastatic. Additionally, the perihilar lesion in the lungs further supports the suspicion that the metastatic process was overcoming the defenses of the organism. Finally, the malaise and lethargy of Zander, that pushed the caregiver to solicit a euthanasia, were signs of a general “shut down”, which is common in patients with widespread metastasis.

The prognostic tool of MR, gave 47.4% and 68.2% for five and one-year MR for Zander.

3.3.4 Why Diagnosis of Osteosarcoma and not Another Bone Tumor?[93,96]

In all three case studies, there was a presumptive radiologic diagnosis. One, could argue that the basis of the diagnosis was not effectively established. Still, there are a number of factors that justified the therapeutic actions and diagnosis of OS.

Chondrosarcoma could be another possible bone tumor. However, 61% of chondrosarcomas are located in flat bones and considered to have a slow metastatic rate. Cytologic fine needle aspirates, yield more matrix than cells, while in OS occurs the opposite. In general, patients live longer than patients with OS. Chondrosarcoma, may start as lytic lesion but eventually proliferate to a great extent.

Fibrosarcomas, are rare in dogs accounting for just 5% of all bone tumors. Although, anatomical location can be similar to OS, destruction of subchondral bone leads to extension of the neoplasm into the joint cavity, through fractures in the unsupported articular cartilage. Tumor tissue, may also invade the fibrous layer of the joint capsule and involve an adjacent bone without involving the joint cavity. A complete surgical resection, can be curative for a fibrosarcoma, while it is reported that even tumefied lesions can be non-painful.

Primary bone Hemangiosarcoma, develops highly, aggressive lytic lesions, very similar to telangiectatic OS, but represents less than 5% of all bone tumors. The metastatic pattern of hemangiosarcoma does not differ significantly from OS.

Nevertheless, an amputation or another surgical procedure would always be needed, to eliminate the primary lesion in any of these potential malignancies.

Finally, there are two important determinants that bend the scale towards an OS diagnosis. Firstly, when there is a painful, aggressive and life threatening possible diagnosis, there is an ethical and medical obligation to treat for the worst disorder, if there is a dilemma. The second factor, is clinical experience. A practitioner with many years of clinical experience, on a specific disorder, can be better than a textbook. At “A&A”, veterinarians are in constant contact with Greyhounds, while OS cases, every year are so frequent that the author encountered six patients in just four months.

4. Epilogue

Veterinary Medicine, is a practical profession that requires extended theoretical preparation. Veterinarians, are well trained to deal with multiple species in a variety of habitats and conditions. Still, companion, production, exotic and aquatic animals are so different, that it is almost impossible for an individual to master the arts of science regarding all of them. As such, by the end of their academic career, many students have already distilled their preferences and on which species they like to work the most. Before commencing a professional career, there stands one last obstacle to overcome. The integration of the theoretical preparation, in a practical setting, where a student can focus on the species of his immediate interest.

The “A&A” externship, was an extremely positive experience that served its exact scope. It helped integrate the theory to practice, revealed weaknesses and strengths of the authors academic preparation and jumpstarted the urgency to search for new knowledge on a daily basis. Beyond the medical aspects of interest, the personal contact with the caregivers and staff, revealed the need to shape excellent interpersonal communication skills and was the most valuable professional tool acquired. Finally, this report, ministered for many hours of study that will certainly be invaluable for the authors professional future.

Canine Osteosarcoma (OS), encompasses a complex etiology that its comprehension prerequisites understanding of how neoplastic and metastatic processes operate in general. Evidence of a highly similar, biologic behavior of canine OS to that of pediatric OS, emerges from many current studies and this is a major reason for which there are new promising trails. An extrapolation of positive canine results could benefit children as well. However, the lack of change in MST and DFI over the last 30 years, after many treatment approaches resulting in minimal improvement, shows a need for stepping back and, maybe, redesigning studies for a different end-point.

In the 2016 May issue, of *Veterinary and Comparative Oncology* journal, Dr. Chand Khanna, was the author of a very interesting editorial. There it is stated, that after the failure of delivering a prolonged MST it is reasonable to ask, if the endpoint best suited for the evaluation of novel adjuvant therapies should be focused at the more consistent endpoint of two-year DFI, rather than the MST. The two-year DFI occurs at the ‘plateau’ of the survival curve. So, in a disease that is mainly afflicting large-breed, old dogs, when a patient is still alive after that interval, a veterinarian could consider that the patient is cured. This, could be a radical change in the mindset of veterinarians, that are historically trained to deal with cancer as an uncurbable disease, and could alter the convey of a treatment discussion with the owners. Also, focusing on novel biological endpoints of disease burden, i.e., ‘cell free’ circulating tumor DNA, or novel imaging platforms could help design studies that escape the human “phase II” failed model.[90]

In simple words, OS metastatic spread depends on the success of just a few cancer cells that are able to evade the immune system and “colonize” new anatomic locations. Treatments that inhibit the proliferation of these cellular bastions and prolong DFI to two years may be less frequent (20-30%), but focusing on how this is achieved could eventually help all dogs and perhaps humans.

Maintenance of life relies on the success of many protective layers. So, when a disorder is capable of penetrating all of these defenses, it can worthily be labeled as incurable. We humans though, have a remarkable capacity to uncover the scientific truth by trial and error. By understanding how neoplastic pathways truly work, we can follow the track back to the dysregulated molecular origin. Thus, by enhancing the organism’s defenses with “intelligence-like” information through immunotherapy or by using more “ballistic-like” therapies such as cytotoxic agents and molecular targeted treatments, we can then probably call a veterinary cancer, curable.

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