FACULTY OF VETERINARY MEDICINE
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Retrospective study of testis tumors in male dogs

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1. Summary

The aim of this retrospective study was to find associations between the different types of testis tumors, interstitial cell tumors, ICT, seminoma, SEM, tumors of the germ cells a and sertoli cell tumors, SCT, and the symptoms they cause. Fifty-six intact male dogs, that were presented at the Small Animal Clinic of the UGent from 2010 till 2018 with testis tumors diagnosed through histology, were subjected to this study using data that had been acquired when they were presented. Certain parameters that in literature were found to be associated with specific testis tumors such as cryptorchidism, feminization, bone marrow suppression, hyperestrogenism and perianal tumors, were first thoroughly researched to understand the meaning of these symptoms and their impact on the patient. The data were used to assess if any associations could be found between certain symptoms and the type of testis tumor. The results reveal weak associations as most symptoms were caused by more than one type of testis tumor. Results show that dogs diagnosed with feminization and cryptorchidism had a higher chance of having SCT than ICT and SEM, but when both symptoms occurred at the same time, only SCT was diagnosed. Another association was observed between perianal tumors and ICT. Concluding from this study that the type of testis tumor cannot be diagnosed with absolute certainty based solely on the symptoms. These findings could be due to a lack of sufficient data collected from the patients or because the different tumor types cause similar symptoms, making it not possible to differentiate the tumor type from the induced symptoms.

Het doel van deze retrospectieve studie was het nagaan van associaties tussen verschillende soorten testis tumoren; interstitieel cel tumoren ICT, seminoma's, tumoren van de kiem cellen of SEM en sertoli cel tumoren, SCT en de symptomen die ze veroorzaken. 56 intacte reuen, die elk aangeboden werden aan de Kliniek Kleine Huisdieren van de Ugent tussen 2010 en 2018 met testis tumoren die gediagnosticeerd werden via histologie, werden onderworpen aan de studie en dit via de gegevens die verzameld werden tijdens hun consultaties en opnames in de kliniek. Parameters, beschreven in literatuur, die veroorzaakt zouden worden door bepaalde testis tumoren zoals cryptorchidie, feminizatie, beenmergsuppressie, hyperoestrogenisme en perianaalkliertumoren, werden eerst grondig bestudeerd om zo hun belang en impact op de patient te begrijpen en daarna verzameld. Deze data werd nagegaan of associaties zich voordeden tussen bepaalde symptomen en de type testis tumoren. De resultaten toonden wel een associatie aan SCT en het samen voorkomen van feminizatie en cryptorchidie, alsook een associatie tussen perianaalkliertumoren en interstitieel tumoren. Uit deze studie is het mogelijk te concluderen dat het type testis tumor niet met absolute zekerheid gediagnosticeerd kan worden aan de hand van de symptomen die ze veroorzaken, dit kan veroorzaakt zijn door een tekort aan verzamelde data van de patiënten door inconsiquentie of doordat de verschillende types testis tumoren gelijkaardige symptomen veroorzaken waardoor een onderscheid maken aan de hand van deze symptomen niet mogelijk is.

2. Introduction

Literature describes three main types of testis tumors can occur in the male dog; interstitial cell tumors (ICT), neoplasia of the Leydig cells, seminomas (SEM), neoplasia of the germ cells and sertoli cell tumors (SCT). The symptoms they cause depends mostly on their activity. When the neoplasms are hormone producing, bone marrow suppression, feminization or perianal tumors may occur. The severity of these different symptoms range from mild to severe, feminization for instance can be unharmful to the dog starting with bilateral symmetrical alopecia but can escalate into a secondary pyoderma that can cause wounds to the skin. Bone marrow suppression is possibly the most severe symptom and should be diagnosed and treated as quickly as possible. Bone marrow suppression can be caused by hyperestrogenism, which can be caused by an absolute increased amount of estrogens in the blood or by an imbalance of the estrogen/testosterone ratio (Mischke et al., 2002). When a testis tumor is present and these symptoms are also present, it would be suspected that hyperestrogenism is caused by sertoli cell tumors, because the sertoli cells produce estrogen, the same can be said for feminization, but the question should be asked if only sertoli cell tumors can be linked to hyperestrogenism. If hyperestrogenism can also be caused by an imbalance of the estrogen/testosterone ratio it could also be said that interstitial cell tumors could be responsible, these cells normally produce testosterone and a neoplasia could interrupt the normal production of this hormone, causing an imbalance.
Literature has remained vague about the correlations between a testis tumor type and the symptoms they cause, making it difficult to give a good prognosis and ofttimes not knowing that extra treatment is needed to cure possible secondary, chronical problems. These secondary problems could be a secondary pyoderma that needs an antibiotic treatment or bone marrow suppression with a non-regenerative character that needs extra medication to help the bone marrow produce blood cells again. But if it is not known what symptoms are caused by which testis tumor, the prognosis and further treatment cannot be predicted.

In this retrospective study we aim to address the question whether it is possible to associate the type of testis tumor with presented symptoms. If so a faster diagnosis can be made along with a prognosis and the knowledge on how to further treat the dog.
To do this, patients will be selected based on following criterium: the testis tumor must be diagnosed by histology. From these patients further data such as anamnesis, behavior, physical examination values, complete blood count, biochemistry values, hormone tests and dermatologic factors will be collected and analyzed.

3. Literature review

3.1 Testicular neoplasms

Testicular neoplasms are the second most common type of tumors in intact male dogs (Fan and de Lorimer, 2007; Gamlem et al., 2008) and the most common type of tumor of the male genitalia in dogs (North and Banks, 2009). There are three main categories of primary testicular neoplasms; sex cord/stroma tumors (interstitial cell tumors and Sertoli cell tumors), germ cell tumors (seminoma and teratoma) and epithelial tumors (rete adenoma and carcinoma) (Peters et al., 2000).

Sertoli cell tumors, seminoma's and interstitial cell tumors, also known as Leydig cell tumors are the most common types of testis tumors, and each occur at approximately the same frequency (Liao et al., 2009; Togni et al., 2015). However there have been a few studies where it was concluded that seminomas (SEM) and Interstitial cell (ICT) tumors appear more often than Sertoli cell tumors (SCT) (Ciaputa et al., 2012).

It is possible for a dog to have different types of testis tumors at the same time, and it is even possible for these different types to be present in the same testes (Grieco et al., 2008). It has also been shown that there could be a possible connection between the type of tumor and the age the dog has at the time of diagnosis. The mean age for Sertoli cell tumors, seminomas and interstitial cell tumors is respectively 8,6 years, 10,6 years and 10,9 years (Nødtvedt et al., 2010). The reason for earlier detection of SCT could be that cryptorchid testes have a higher chance of degenerating into sertoliomas and at a faster rate than descended testes would (Liao et al., 2009), therefore decreasing the mean age of detection. Twenty to thirty percent of sertoliomas are active tumors that produce estrogens and can cause feminization, skin changes and bone marrow hypoplasia making detection easier and thus resulting in an earlier diagnosis (Filho et al., 2017).

Most testis tumors are benign and asymptomatic but it is possible for seminomas and Sertoli cell tumors to metastasize. Other symptoms of testis tumors arise from hormone active producing tumors, most commonly Sertoli cell tumors and interstitial cell tumors, although there have been reports of hormone producing seminomas (Kim et al., 2004). These hormone active producing tumors can cause feminization, skin conditions, bone marrow suppression, squamous hyperplasia of the prostate and benign hyperplasia of the prostate. One of the most severe consequences is bone marrow suppression, which could lead to secondary infections, due to the suppressed immunity, and could be life-threatening.

3.2 Histology

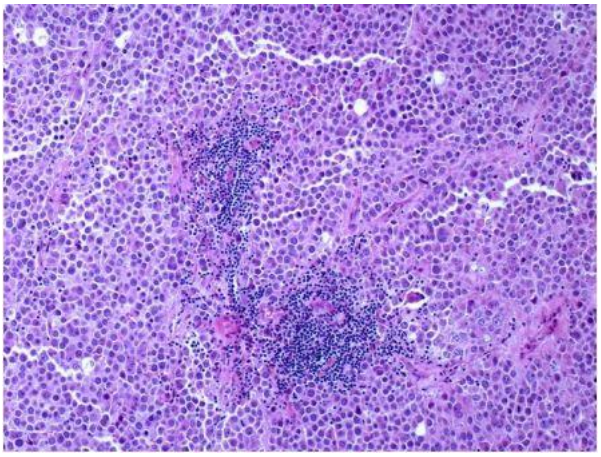

Figure 1: 'Histological pattern of seminoma' adapted from Ciaputa et al. showing aggregates of embryonal cells with oval nuclei and scanty cytoplasm and typical focal lymphocyte infiltrate

Seminomas develop along the gonadal line, originating from primary genital cells. They are common tumors in dogs and the mean age of diagnosis is 10,6 years (Nødtvedt et al., 2010). Seminomas can develop unilaterally or bilaterally with single or multiple foci in one testes. When palpated they are soft and are macroscopically homogenous, grey or cream colored (Grieco et al.,2007). Seminoma's that have grown to large sizes may contain foci of coagulative necrosis without hemorrhages (Ciaputa et al., 2012). The World Health Organization (WHO) categorizes seminomas into seminomas with a diffuse pattern, which are more likely to be malignant tumors and seminomas with an intratubular pattern which are usually benign (Kang et al., 2010). The development of seminomas is thought to start as an intratubular type which consists of aggregated embryonal seminiferous cells that replace the original lining of the seminiferous tubules (Ciaputa et al., 2012). The cells histological patterns are typical: large and demarcated, containing large and most frequently a vesicular cell nucleus with one or two nucleoli and cytoplasm that is scant and basophilic. Many of these tumors contain focal aggregates of lymphocytes as can be seen in figure 1. Diffuse types of seminoma cells will transgress the lining of the seminiferous tubuli forming bands of relative uniform texture. They are well outlined, tightly packed and contain a large nucleus with one or two nucleoli. It is possible to find vacuolated histiocytes, focal and/or perivascular aggregates of mature lymphocytes in between the neoplastic cells and mitoses of the seminoma cells are often atypical and occur frequently (Grieco et al., 2007; Ciaputa et al., 2012).

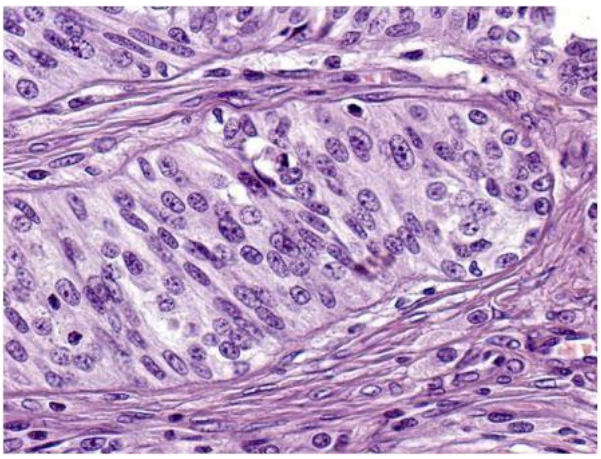

Figure 2: 'Histological pattern of sertolioma' adapted from Ciaputa et al. Hyperplasia of spindle shaped cells with hyperchromatic nuclei.

Sertoli cell tumors originate from sex cord-stromal cells, more specifically from sustentacular cells of the seminiferous tubules. These cells produce estrogens, which is why sertoliomas can be responsible for hyperestrogenism (Turek, 2003). Sertoliomas, like seminomas, also have an intratubular type, which is the benign form and a diffuse type that can become malignant (Kang et al., 2010) The intratubular neoplasms are most frequently unilateral, solid, white or grey and demarcated from surrounding tissue. If the neoplasm grows large enough it can deform the testis, making it noticeable from the outside of the scrotal sac. The cells have indistinct outlines, a round or oval hyperchromatic nucleus and their cytoplasm is vacuolated and acidophilic. They may penetrate the basement membrane of the seminiferous tubules but this is unlikely. Diffuse sertoliomas can infiltrate the tunica alba, the epididymis and spermatic cord. They form aggregates of large,

irregular cells with no evident structure and can contain erythrocyte-filled cysts (Ciaputa et al., 2012; Catoi et al., 2008), a histological example can be seen in figure 2.

Interstitial cell tumors, also known as Leydigomas, can degenerate unilaterally or bilaterally into small individual or multiple foci with a yellow or brown color. They are soft when palpated and demarcated from surrounding tissue. The cells are large and either oval or multiangular in shape, their cytoplasm is rich and contains fine vacuoles (as can be seen in figure 3) and a small to average sized hyperchromatic nucleus. In the tumor it is possible to find spaces filled with erythrocytes similar to sertoliomas (Ciaputa et al., 2012; Catoi et al., 2008). Leydig cells are the cells responsible for the production of testosterone, which can result in hyperandrogenism (Frank, 2006) and possible other symptoms that will be discussed later.

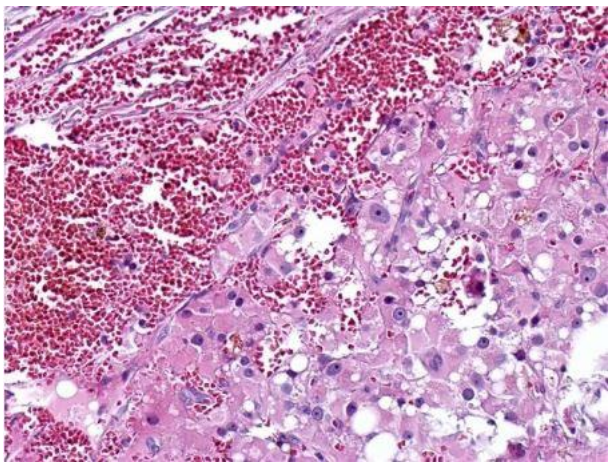

Figure 3: 'Histological pattern of leydigoma' adapted from Ciaputa et al. Large oval cells with vast cytoplasm, numerous lipid vacuoles and focal hemorrhages.

3.3 Cryptorchidism

In the normal development of the male genitalia of dogs, the testicles descend from the abdominal cavity to their physiological site, the scrotum, at an age of 10-14 days. However, when this fails to happen to one or both of the testes within the first eight weeks, the abnormality is referred to as cryptorchidism. A short gubernaculum, topographic position abnormalities or failure to regress correctly are different possible causes. Cryptorchid testes can remain in the abdominal cavity, descend into the inguinal canal or even shift to an ectopic penile position (Bufalari et al., 2015).

Undescended testes also have a higher chance of degenerating due to the difference in temperature. Abdominally retained testes are more predisposed of developing Sertoli cell tumors because the higher temperature of the abdominal cavity, in comparison to the scrotum, causes all other cells to waste except for Sertoli cell tumors (Ciaputa et al., 2012). Testes that have descended into the inguinal region, where the temperature is higher than in the scrotum but lower than in the abdominal cavity, have a higher chance of developing seminomas (Chaganti et al., 2000; Hemminki and Sweden, 2004). However this does not rule out the possibility of sertoliomas developing in inguinal retained testes. Temperature seems to have no influence on the development of interstitial cell tumors, and therefore this type of tumor is rarely seen in cryptorchidic testes (Liao et al., 2009). Different studies have also shown that there is a higher chance of the right testes to become cryptorchidic, because it lies more cranially in the abdominal cavity before descending than the left testes, and must travel a longer distance before reaching the scrotum (Kim and Kim, 2004; Ciaputa et al., 2012; Dugat et al., 2015).

3.4 Metastasis

Metastasis of testicular tumors in dogs occur at a low rate. Seminomas are reported to be the most common tumor to metastasize (Canadas et al., 2016), but this only happens in less than 10-15% of cases. When seminomas do metastasize, typical locations for secondary tumors are primarily the local lymph nodes. Further locations, that have been documented, are the eye, central nervous system, abdominal viscera of the liver, kidney, spleen and pancreas, lymph nodes, the soft palate, trachea, pericardium and skin (Dugat et al.,2015). Secondary seminomas would spread through the body by use of lymphatic vessels. Non-seminomas, ICT and SCT, metastasize via lymphatic vessels and blood vessels (Ciaputa et al., 2012). Sertoli cell tumors have been documented to metastasize with a 10-20% rate. Metastatic locations for sertoliomas are the regional lymph nodes, kidney, liver, spleen, lung, adrenal gland and the pancreas (Kang et al., 2010). Leydigomas are generally considered benign, and metastasis is considered rare, however there have been case reports of metastasized ICTs. Malignancy of leydigomas is characterized by the irregular form of the cells, higher mitotic rate and infiltrated growth, which are factors that are hard to diagnose (Togni et al., 2015). The musculature of the hind limb and cutaneous nodules are both documented cases of ICT metastasis (Togni et al., 2015; Canadas et al., 2016). Although the metastatic rate of testicular neoplasia is low (10-15%), abdominal ultrasound and thoracic radiographs should be included in diagnosis for a complete clinical staging (Turek, 2003).

3.5 Hyperestrogenism

Hyperestrogenism is a medical condition caused by an excess of estrogenic activity in the body (Lavin, 2009) and can cause an array of different symptoms in the male dog: feminization symptoms such as enlarged mammary glands, linear preputial erythema, bilateral symmetrical alopecia, pendulous prepuce, hyperpigmentation, gynecomastia and squamatic metaplasia of the prostate gland, skin changes and bone marrow suppression. The latter being the most serious problem because this can be complicated by secondary infections, most frequently to the urinary tract (Frank et al., 2006). Hyperestrogenism caused by testicular tumors is a form of paraneoplastic syndrome, the tumor causes a set of signs and symptoms (feminization, bone marrow suppression) to occur but not due to the local presence of the cancer cell (Darnell et al., 2011).

Estrogen in the male dog is produced by Sertoli cells. If these cells degenerate, 20-30% of SCT will remain active, still producing estrogens (Turek, 2003; Filho et al., 2017). Other studies and cases have proven that it is not just Sertoli cell tumors that can cause signs of hyperestrogenism, Leydig cell tumors can also cause hyperestrogenism, more specifically feminization (Huggins et al., 1945; Turek, 2003; Kim et al., 2004). Seminomas have been reported to cause feminization, but other than a couple case reports, no other studies have been able to show a connection between seminomas and hyperestrogenism (Kim et al., 2005; Plavec et al., 2007).

Another debated subject is whether hyperestrogenism is caused by an excess amount of estrogens in the body or due to an imbalance between the estrogen and testosterone levels in the body. There have been conflicting results among studies that could reject the hypothesis that solely an excess of estrogens is sufficient to cause hyperestrogenism and feminization, these studies measured estrogen levels in blood of feminized dogs (Turek, 2003). Some studies reported increased blood levels, whereas the other studies reported normal estrogen levels, these results were mostly based on case reports. Recently two comparative publications (Peters et al., 2000; Mischke et al., 2002) have shown that dogs

with Sertoli cell tumors had higher levels of oestradiol-17β in peripheral venous and testicular blood than normal dogs. They also reported that dogs with Sertoli cell tumors and feminization symptoms had higher oestradiol-17β levels in peripheral venous and testicular blood than dogs with Sertoli cell tumors but without feminization symptoms. Both reports also stated that dogs with Sertoli cell tumors had reduced testosterone concentrations compared to the dogs in the test group. It was suggested that the ratio testosterone/oestradiol would be a more reliable diagnostic tool than the individual hormone levels. These publications also reported that oestradiol-17β levels were raised in dogs with interstitial cell tumors compared to normal dogs, suggesting that ICTs can also be endocrinologically active. Seminomas, however, showed no difference nor decrease, suggesting that they are not active. Because not all dogs showing feminizing symptoms had raised oestradiol-17β levels, they also suggested the shifted balance between testosterone and oestradiol would be more important than the absolute levels of these hormones, particularly oestradiol (Mischke et al., 2002). This would mean that using oestradiol-17β levels to diagnose feminization is not sufficient, raised blood levels are a good indication for hyperestrogenism, but normal levels do not eliminate the possibility of hyperestrogenism.

3.5.1 Feminization

Feminization is one of the possible results of hyperestrogenism and 24-57% of dogs with sertoli cell tumors will show symptoms of the feminization syndrome (Turek, 2003), but as has been mentioned, it is possible for interstitial cell tumors and even seminomas to induce feminization. The feminization syndrome is characterized by both behavioral changes and skin conditions. Some behavioral changes include that the patient becomes sexually attractive to other male dogs, is attracted to other male dogs or takes a female position to urinate. Skin conditions include enlarged mammary glands, linear preputial erythema, bilateral symmetrical alopecia, originating on the neck, lumbar region, perineum and genital area, pendulous prepuce, hyperpigmentation, gynecomastia, squamatic metaplasia of the prostate gland, penile atrophy and thinning of the epidermis is possible. Histological changes to the skin are also possible and include sebaceous gland atrophy, telogenization of hair follicles, follicular keratosis, follicular dilatation and orthokeratotic hyperkeratosis. Coat color change, linear preputial dermatosis and macular melanosis around the perineal, inguinal and genital areas are also possible (Huggins et al., 1945; Turek, 2003; Paepe et al., 2016). Linear preputial dermatosis is pathognomonic for testicular neoplasia and is described as a linear narrow pigmentary change that starts at the preputial orifice and continues to the scrotum (Turek, 2003).

Bilateral symmetric alopecia is very typical for hyperestrogenism in both female and male dogs. This is a consequence of the amount of estrogen that affects the hair follicle, the hormone is known to be an inhibitor of anagen initiation (Frank, 2006). An example of a dog with bilateral alopecia can be seen in figure 4.

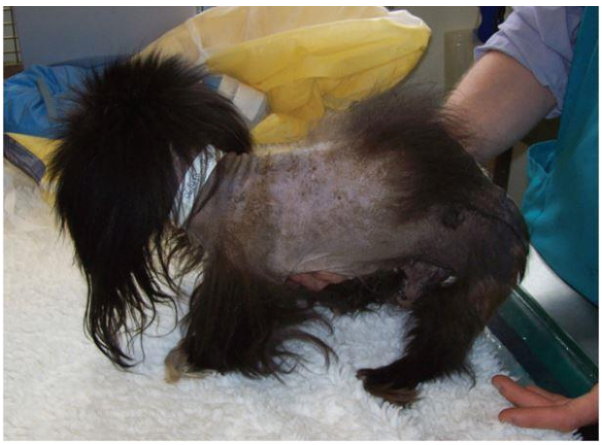

Figure 4: 'Extensive alopecia, skin thinning and pendolous prepuce of a dog with feminizing syndrome' adapted from Warland et al.

Squamous metaplasia of the prostate is also possible after exposure to estrogens or in feminizing syndromes. This arises from the production of keratins by basal cells creating a stratified squamous epithelium instead of the normal columnar glandular epithelium. The changes can range from subtle to dramatic.

3.5.2 Bone marrow suppression

Bone marrow suppression is another symptom of hyperestrogenism and is caused by a high estrogen serum level, unlike feminization, which is more likely to be caused by a shift in testosterone/estrogen balance. High estrogen serum levels are myelotoxic in dogs and can cause aplastic or hypoplastic bone marrow with pancytopenia. Pancytopenia is a medical condition where all cell lines, white cells, red cells and platelets, are decreased. The exact mechanism of how estrogens cause myelotoxicity is unknown but it is known that estrogens interfere with stem cell differentiation; they alter utilization of iron by erythrocyte precursors, stimulate the production of myelopoiesis inhibitory factor and inhibit production of erythrocyte stimulation factors (Kearns and Ewing, 2006), resulting in leukocytosis, neutropenia, thrombocytopenia and decreased erythropoiesis, which can lead to anemia, and after 3-4 weeks of exposure pancytopenia can develop. Clinical signs will first appear from leukopenia and thrombocytopenia, and this within 2 weeks, because of the short lifespan of these cells. Clinical signs will most likely be caused by secondary infections due to a lowered immune system or petechia, bruising and internal bleeding if the platelet count drops severely.

After a period of 120 days, the typical lifespan of a red blood cell, it is possible for anemia caused by bone marrow suppression to develop (Kearns and Ewing, 2006). Anemia occurs when the total amount of red blood cells are decreased or when the red blood cells contain too little hemoglobin. It can be calculated by consulting the hematocrit, the fraction of red blood cells per liter. The lower the hematocrit, the more severe the anemia, as can be seen in table 1 (Gultekin et al., 2017).

	DOG
MILD	30-37%
MODERATE	20-29%
SEVERE	13-19%
EXTREMELY SEVERE	<13%

Table 1: 'Different stages of anemia' adapted from Gultekin et al.

To specify the type of anemia, different values can be examined, the MCV (mean cell volume) can be used to determine the mean volume of the red blood cells and the MCHC (mean cell hemoglobin concentration) can be used to determine the concentration of hemoglobin per mean red blood cell. When the MCV is too low, normal or too high, it is respectively referred to as micro-, normo- or macrocytic anemia, when the MCHC is too low it is referred to as hypochromic anemia. If these values arise in combination of a reduction of other cell lines, it is possible to speak of bone marrow suppression.

Another factor that can be consulted to verify bone marrow suppression (in combination with previous values) is the type of anemia; regenerative or non-regenerative. When non-regenerative anemia is present, the bone marrow isn't able to regenerate blood cells fast enough to maintain sufficient blood levels. To check the regenerative capability of the bone marrow the absolute amount of reticulocytes needs to be calculated, this can be done using following formula: $Reticulocyte\ concentration = Reticulocyte\%\ x\ amount\ of\ red\ blood\ cells\ /\ 100$. If this value is higher than 80.000/µL the anemia is regenerative, if the value is lower the anemia is non-regenerative (Gultekin et al., 2017).

3.6 Androgens

As well as hyperestrogenism, hyperandrogenism can cause an array of symptoms, but unlike hyperestrogenism, it does not occur often. Most common symptoms are hyperplasia and overproduction of the sebaceous and circumanal glands or the hepatoid (sweat) glands. As a result of these changes following clinical signs can arise: a “donut” ring around the anus, generalized seborrhea oleosa, alopecia and seborrhea of dorsal side of the tail starting one third away from the tailbase (Frank, 2006). This can also degenerate into perianal tumors, most commonly tumors of the perianal glands, perianal gland adenomas more frequently than perianal gland carcinomas, although the latter can also arise at a rate of three to seventeen percent of perianal neoplasms (Pisani et al., 2006). The study of Pisani et al. (2006) concluded that there are androgen receptors in these perianal gland tumors and that castration can help to partially regress the tumors but surgical removal is needed to completely remove the perianal gland tumors.

The presence of androgens, not necessarily in excess, can also cause prostatic hyperplasia or hypertrophy as canine prostates, in non-neutered dogs, change progressively with age. In about 63% of dogs the prostate enlarges to a size larger than normal and because this isn’t necessarily a lesion, it is referred to as ‘benign prostatic hyperplasia’. However in some dogs the prostate can reach a size that can cause clinical signs, most commonly fecal obstruction (Foster, 2012).

4. Goals

Literature has not been able to show strong correlations between the type of tumor, the clinical symptoms, the nature of the tumors, the period that the tumors have been present and how the symptoms evolve after castration. There have been studies about bone marrow suppression due to estrogens and the possibility of it being regenerative or non-regenerative but not correlated to the type of tumor. It would be interesting for the prognosis if it was possible to deduce the severity of the tumor and its symptoms from the histology and the period of time that the tumor has been present, although it is very hard to collect exact data for this last factor due to the difficulty of diagnosis. This is usually only after symptoms arise and is rarely in an early stage, bloodwork could be used as a reference because time of estrogen influence can be seen when it is myelotoxic, cell lines will begin to decrease after their life-span is over. This could give a better idea for additional medication after neutering to help recover from the bone marrow suppression.

Using the different symptoms ranging from bloodwork and urine analysis to behavior and skin conditions, this study will aim to search for associations between the symptoms of testis tumors and their histology. More specifically if feminization and bone marrow suppression occur at the same time, to which extent the occurrence of symptoms can be associated with the histology and nature of the tumor.

5. Material and method

5.1 Table of content

A first step in this study was creating a table of content with parameters that were regarded as significant to the clinical signs of the tumors.
These parameters were chosen based on other studies and literature and how important they are to categorize the different types of tumors and what symptoms they can cause.
The parameters are divided into different groups: anamnesis and behavior, physical examination, dermatology, testes examination, blood analysis with hematology, biochemistry, coagulation tests and hormonal tests, urinalysis, thoracic radiography and abdominal ultrasound.

5.1.1 Anamnesis, behavior and physical examination

These are basic parameters that are important in any disease or disorder. It is important to know the anamnesis so as to differentiate the problem from other diseases that could give the same symptoms. The types of behavior specifically important for this study are behavioral traits linked to feminization such as taking in a female position during urination or attracting other male dogs. These symptoms could be caused by hyperestrogenism, a described symptom of testis tumors.
A physical examination is important for a first screening of the dog because it is also possible to conclude that the dog might have severe anemia if the mucosae are pale or the capillary refill time is too slow. These factors have to be taken seriously so that the dog can first be stabilized before other tests are done.
The more important factors of the physical exam for this study are the perianal abnormalities and the rectal examination as studies have shown that perianal tumors and prostate hyperplasia can be caused by testes tumors.

5.1.2 Dermatology

This category is mainly for detection of feminization as most clinical symptoms of feminization can be seen as a change of coat skin or superficial underlying tissues. One of the more common signs of feminization is alopecia, more specifically bilateral symmetric alopecia, other signs are hyperpigmentation, swelling of the mammary glands, pendulous prepuce and linear preputial erythema.

5.1.3 Testicular examination

The cell type of the neoplasia is the most important factor in this category, this forms the basis of which patients will be subjected to the study. If the type of tumor isn't known, it is impossible to deduct anything that that tumor can induce. Another factor that is considered is if the neoplasia is one or both testicles, if the neoplasia is an active hormone producing tumor and if one or both of the testicles are cryptorchid. Hormone producing tumors can be deducted from other symptoms such as bone marrow suppression and feminization or by checking what the blood of testosterone and estrogens are. The last factor, the possible presence of cryptorchid testes, is important as studies have shown that cryptorchid testes have a higher chance degenerating.

5.1.4 Blood analysis

5.1.4.1 Hematology

These factors are an important screening for bone marrow suppression, especially if pancytopenia, the presence of non-regenerative anemia, leukopenia and thrombocytopenia is present. These

parameters can be used to estimate how long the tumor has been influencing the bone marrow and how much damage has been done to other organs as anemia can also cause hypoxia in other organs, causing them to degenerate.

5.1.4.2 Biochemistry, coagulation tests and hormonal tests

As stated above, biochemistry parameters are useful to see if there are changes in other organs, due to hypoxia or other factors that could cause these organs to degenerate.
Coagulation tests are needed to check if the coagulation factors work properly or not and hormonal tests can be very valuable to check if the existing testes tumor is a hormone active producing tumor or not. It will also be possible to see if there is a distinct difference between hyperestrogenism or if a deviation of the estrogen/testosterone ratio is sufficient to cause the symptoms linked to hyperestrogenism.

5.1.5 Urinalysis

As a result of leukopenia due to bone marrow suppression, there is less defense and immunity, allowing bacterial infections to arise. Analysis of the urine can give a better view to how severe the immune system has been reduced as well as if there is a problem in the urinary tract that has to be treated.
Other parameters can help exclude other systemic diseases such as diabetes mellitus and kidney failure.

5.1.6 Radiography and ultrasonography

Radiography and ultrasonography are good techniques that can be used for visualizing abdominal masses, suspicions of cryptorchid testes and potential metastasis to proximate lymph nodes in the abdominal region as well as in the thorax. Deformations and irregularities in other organs and part of the body can then also be visualized.

5.2 Patients

The main goal of this study is to search for links between certain tumor types and the clinical symptoms they cause. To properly underline that goal, the patients subjected to this study need to be chosen specifically so that links can be made. For this reason the patients had to fulfill one requirement: histology had to be done on the tumor to specify the cell type; sertoli cell, interstitial cell or germ cell. All patients were chosen out of the database of the small animal clinic in Merelbeke from the university of Ghent (UGent). Search terms as “sertoli cell”, “Leydig cell”, “seminoma”, “interstitial cell”, “testis tumor”, “testis neoplasia”, “feminization” and “hyperestrogenism” were used to find different patients, then the files of these patients were checked to see if histology was done and if so, the patient was added to the list. These patients were both primary and referral cases. .
A total of 56 dogs have been subjected to this study, all these patients were diagnosed and treated at the clinic between 2010 and 2018.

After having searched for all patients in the database that fulfill the histology terms, they were inserted into the table of content and all the factors and parameters that were available were also inserted into the table. Because of the retrospective aspect of this study, missing data were present.

5.3 Results

After having collected the data, these were placed into four big groups: sertoli cell tumor, seminoma, interstitial cell tumor and mixed tumor, the last group being any dog with more than one tumor type. The most valuable data values were placed on the other axis and were then filled in with: yes, no, does not apply or not available, these values are presented in following tables along with the used reference values.

Table 2: Values used to generate results

Testes	**Hematology**		**Biochemistry**	
Cryptorchid	RBC deficit	<5.60 x10^{12}/l	Urea deficit	<2.5 mmol/l
Atrophy of the contralateral testes	Hemoglobin deficit	<7.5 mmol/l	Urea surplus	>12.07 mmol/l
	Decreased hematocrit	<3.3%	Hypoalbuminemia	<22 g/l
	Regenerative anemia		Albumin surplus	>45 g/l
	Leucopenia	<5.00 x10^{9}/l	Creatinine surplus	>1.10 mg/l
	Leucocytosis	>15.0 x10^{9}/l	Sodium deficit	<140 mmol/l
	Neutropenia	<2.9 x10^{9}/l	Sodium surplus	>155 mmol/l
	Neutrophilia	>11.64 x10^{9}/l	Potassium deficit	<3.6 mmol/l
	Lymphopenia	<1.00 x10^{9}/l	Potassium surplus	>5.8 mmol/l
	Lymphocytosis	>5.10 x10^{9}/l	Calcium deficit	<2.3 mmol/l
	Monocytopenia	<0.15 x10^{9}/l	Calcium surplus	>2.75 mmol/l
	Eosinopenia	<0.06 x10^{9}/l	Chlorine deficit	<102 mmol/l
	Eosinophilia	>1.25 x10^{9}/l	Chlorine surplus	>122 mmol/l
	Basophilia	>0.10 x10^{9}/l	Decreased liver values	ALT <9 U/l AF <13 U/l
	Thrombocytopenia	<50.000/µl	Increased liver values	ɣ-GT >16 U/l AST >62 U/l ALT >125 U/l AF >212 U/l
	Thrombocytosis	>484.000/µl		

Table 3: Values used to generate results (part 2)

Hormone tests		**Coagulation tests**		**Urinalysis**	
Increased estradiol	>50 pg/ml	Cit-PT decreased	<11 sec	Increased UPC	>0.50
Increased testosterone	>400 pg/ml	Cit-PT increased	>17 sec	Increased pH	>7.5
		Cit-aPPT decreased	<8 sec	Decreased pH	<4
		Cit-aPPT increased	>102 sec	Protein strip positive	
				Infection	
				Sediment test	
				Pyuria	>27/ml
				Hematuria	>27/ml
				Increased plate epithelial cells	>15/ml

Table 4: Values used to generate results (part 3)

Dermatology	**Other**
Feminization	Hernia perinealis
Pyoderma	Perianal tumor
Other dermatologic deviations	Mass
	Benign prostate hyperplasia
	Prostate cyst
	Prostatitis
	Tumor elsewhere in body
	Other

6. Results

6.1 Seminoma

Of the 56 dogs used in the study, 9 dogs had seminomas. Only one of these nine dogs was cryptorchid, whilst two of the nine dogs had atrophy of the contralateral testes. The mean age of diagnosis of the tumor was 11 years and 11 months.

6.1.1 Hematology

In total, five dogs had a blood analysis done, of these five dogs, only one dog had anemia and a hemoglobin deficit (10 g/dl). The anemia was mild and regenerative.
Three dogs had altered white blood cell values; one dog had leukocytosis ($26x10^9$/l) but this was the only value that was tested, the second dog had lymphopenia ($0,91x10^9$/l) and the third dog had neutropenia (83,5/µl with reference values at least 3000/µL), lymphopenia (988/µl (<1000/µl) and eosinopenia (99/µl (<100/µl), all other white blood cell values were normal for the three dogs.

6.1.2 Biochemistry

Of the five cases where blood analysis was done, three dogs had an altered result. One dog had increased sodium (155 mmol/l) and increased liver values: ɣ-GT was 16 U/l (this is also the dog with lymphopenia, eosinopenia and neutropenia), another dog had increased potassium (6,29 mmol/l), this dog also had lymphopenia and the last dog had increased liver values: ALT was 147 U/l (as well as lymphopenia).

6.1.3 Coagulation tests and hormone tests

None of the nine dogs had any coagulation nor hormone tests done.

6.1.4 Dermatology

One of the nine dogs had feminization symptoms, this being urinating in the position that a bitch would. Five other dogs had other dermatologic deviations; nodules with a high probability of being skin tumors, dermatitis, furunculosis, rectal rupture/atrophy this could be a result of a hernia perinealis and lipoma.

6.1.5 Urinalysis

Three of the nine dogs were subjected to a urinalysis, and two of these dogs had an additional sediment test done. Of these two, one dog had an increased UPC (0,89), an increased pH (7,5) and a positive protein strip test. The sediment test resulted in increased white blood cells (46/µl) but otherwise there were no deviations. The second dog showed no deviations on the urinalysis, but the sediment test resulted in increased white blood cells (54/µl) as well as red blood cells (5284/µl). The third dog that had no sediment test done, had an increased UPC of 0,61 but otherwise no other deviations.

6.1.6 Other

All dogs with seminoma are listed in following table along with any deviations.

Table 5: Deviations of other symptoms

Patient number	Hernia perinealis	Perianal tumor	Mass	BPH	Prostate cyst	Prostatitis	Tumor elsewhere in body
1	No	No	No	No	No	No	Yes
2	No	No	No	No	No	No	No
3	Yes	No	No	Yes	No	No	No
4	No	No	No	Yes	No	Yes	Yes
5	No	No	No	Yes	No	No	No
6	No	No	No	No	No	No	No
7	No	No	No	No	No	No	No
8	No	No	No	No	No	No	No
9	No	No	No	No	No	No	Yes

6.2 Sertoli cell tumor

Eleven of the 56 dogs had a testes tumor consisting of solely sertoli cells. Of these eleven dogs five dogs were cryptorchid and one dog had an atrophic contralateral testes. The mean age of diagnosis was 10 years and 9 months.

6.2.1 Hematology

As regards to red blood cell values, only three dogs had results. Of these two only one dog had decreased red blood cells ($1{,}2x10^{12}$/l) , a hemoglobin deficit (3 g/dl) and decreased hematocrit of 9,10% resulting in a very severe non- regenerative anemia.
4 of the 11 dogs had white blood cell values measured and of these four only two dogs had deviations, the one dog having lymphopenia ($0{,}84x10^{9}$/l) and the other dog having a white blood cell deficit ($1{,}2x10^{9}$/l), neutropenia ($0{,}57x10^{9}$/l), lymphopenia ($0{,}04x10^{9}$/l), eosinopenia ($0{,}03x10^{9}$/l) and thrombocytopenia ($0\ x10^{9}$/l).

6.2.2 Biochemistry

The same four dogs that had blood work done, also had biochemistry done, and three had deviations. One of the three dogs had increased liver values (ALT:374 U/l), another had increased sodium levels (156 mmol/l) and decreased potassium levels (3,2 mmol/l), the other had solely increased sodium levels (159 mmol/l).

6.2.3 Coagulation tests and hormone tests

Two of the eleven dogs had coagulation tests done, both the cit-PT test and cit-aPTT test were performed. One dog had no deviations, the other had a decreased cit-PTT time of 26 seconds.
Two dogs also had hormone tests done but all results were within the normal reference values.

6.2.4 Dermatology

In following table all dogs with SCT are listed along with the results of the dermatologic values.

Table 6: Dermatologic values of dogs with SCT

Patient number	Feminization	Pydoderma	Other
10	No	No	No
11	No	No	No
12	Yes	Yes	Pruritis, colarettes, papulas
13	Yes	No	Itch: traumatic dermatitis in the region of the muzzle periocular, axilla and interdigital, secondary infection with Malassezia
14	Yes	No	Change of coat color
15	No	No	Erythematous preputium
16	No	No	Nodules
17	No	No	No
18	Yes	No	No
19	No	No	Nodules
20	No	yes	No

6.2.5 Urinalysis

Only two of the eleven dogs had a urinalysis done. The first dog had a positive protein strip and on the sediment test it was concluded that the dog had increased white blood cells (515/µl), red blood cells (40/µl) and plate epithelial cells (96/µl). The second dog had no deviations on the urinalysis and a sediment test was not performed.

6.2.6 Other

Two dogs had deviations, the first had prostatitis whilst the second had a medial iliac abdominal mass and prostatitis combined with benign prostate hyperplasia.

6.3 Interstitial cell tumor

Out of the 56 dogs subjected to the study, 25 dogs had an interstitial cell tumor, and of these 25 dogs only one dog was cryptorchid and five dogs had an atrophic contralateral testes. The mean age of diagnosis was 11 years and 5 months. Three of these dogs also had a severe systemic disorder, all having problems with the intestines: pancreatitis and duodenum perforation, mechanical obstruction of the intestines and erosive colitis with intestine obstruction. Because of this, it is possible that the deviations in blood analysis could be caused by these systemic deviations and not primarily due to the tumor, for this reason the dogs will be mentioned if there are deviations but separately from the dogs without severe systemic disorders.

6.3.1 Hematology

Of the 25 dogs, 20 dogs had bloodwork done, 5 did not. Of the 20 dogs that had blood work done, six dogs had a red blood cell deficit, decreased hematocrit resulting in five non-regenerative anemic dogs and one regenerative anemic dog and all but one dog also had a hemoglobin deficit. Two of the non-regenerative dogs have a severe systemic disorder.

As regards to white blood cells, six dogs had deviations, two of them had a systemic disorder. One dog with the systemic disorder had increased white blood cells, neutrophilia, lymphocytosis, monocytosis and eosinophilia, the other dog had an increased amount of white blood cells, neutrophilia, monocytosis and eosinophilia. Of the other four dogs, the first also had an increased amount of white blood cells, neutrophilia and eosinopenia, the second had an increased amount of white blood cells, neutrophilia, basophilia and thrombocytosis, the third only had an increased amount of white blood cells and the last dog had a leucopenia, neutropenia, lymphopenia, eosinopenia and thrombocytopenia resulting in pancytopenia (along with a decrease in red blood cells, hemoglobin and mild non-regenerative anemia). Results are listed in following table.

Table 7: Deviating blood values of dogs with ICT

	27	**28**	**31**	**34**	**37**	**41**	**45**	**Mean**
Systemic disorder		X		X	X			
RBC deficit	X		X	X	X	X	X	$4.47x10^{12}$/l
Hematocrit deficit	X		X	X	X	X	X	31.20%
Hemoglobin deficit	X							9.8 g/l
Anemia level	Moderate		Mild	Mild	Mild	Mild	Moderate	
Anemia regenerative or non-regenerative	Regenerative		Non-regenerative	Non-regenerative	Non-regenerative	Non-regenerative	Non-regenerative	
WBC deficit							X	1.18 $x10^9$/l
WBC surplus	X	X	X		X	X		31.9 $x10^9$/l
Neutropenia							X	0.01 $x10^9$/l
Neutrophilia	X	X	X		X			27.5 $x10^9$/l
Lymphopenia							X	0.97 $x10^9$/l
Lymphocytosis		X						7.95 $x10^9$/l
Monocytosis		X	X		X			3.04 $x10^9$/l
Eosinopenia		X					X	0.005 $x10^9$/l
Eosinophilia					X			2.02 $x10^9$/l
Basophilia			X					0.19 $x10^9$/l
Thrombocytopenia							X	0 $x10^9$/l
Thrombocytosis			X					571 $x10^9$/l

6.3.2 Biochemistry

19 of the 25 dogs also had biochemistry done, of these 19 nine dogs had aberrant values. All these dogs are listed in following table with the mean values listed below.

Table 8: Deviating biochemistry values of dogs with ICT

Patient number	Albumin deficit	Sodium surplus	Potassium deficit	Calcium deficit	Chlorine surplus	Liver values raised (which)
22				X		ALT
24						ALT, AF
28		X	X	X	X	
29		X		X		
31						ALT
37	X		X			
38		X				
41						ALT
45				X	X	
Mean value	18 g/l	158 mmol/l	2.9 mmol/l	2.12 mmol/l	185 mmol/l	

6.3.3 Coagulation tests and hormone tests

3 of the 25 dogs had their coagulations times tested, all results were within the normal reference values.

Hormone levels weren't tested in any of the dogs with interstitial cell tumors.

6.3.4 Dermatology

Of all 25 dogs, one dog had feminization symptoms: bilateral symmetric alopecia, gynecomastia, and a pendulous prepuce, this dog also had other symptoms such as pruritus, higher epilability of the hairs, a coarse coat and skin flakes.

Seven other dogs also had other skin conditions such as fistulas of the anal sac, alopecia, hyperpigmentation and alopecia due to skin tumors, skin tumors, bilateral enlarged anal sacs and itchiness.

6.3.5 Urinalysis

Three of the 25 dogs had a urinalysis done and of these three one dog also had a sediment test done. One dog had an increased UPC (10,92), increased pH (8) and a positive protein strip test. The second dog had no deviations of the urinalysis but the sediment test showed an increased amount of white (500/µL) and red blood cells (250/µl), plate epithelial cells remained below the threshold. The last dog had only one deviation from normal values and this being an infection of the urine tract with *Escherichia coli* (>1000/µl).

6.3.6 Other

In following table all patients with ICT are listed along with the results found of other deviations.

Table 9: Other deviations of dogs with ICT

Patient number	Hernia perinealis	Perianal tumor	Mass	BPH	Prostate cyst	Tumor elsewhere in body	Other
21	No	No	No	No	No	No	No
22	No	No	No	No	No	No	No
23	No	Yes	No	No	No	Perianal tumor	No
24	No	No	No	No	No	No	No
25	Yes	No	No	No	No	No	No
26	No	Yes	No	No	No	Perianal tumor	No
27	No	No	No	No	No	Hemangiosarcoma of the spleen	No
28	No	No	No	No	No	No	Pancreatitis, duodenum perforation
29	No	No	Yes	No	Yes	No	No
30	No	No	No	No	No	Skin tumor	No
31	No	No	No	Yes	No	No	No
32	No	No	No	No	No	No	No
33	No	No	No	No	No	Skin tumor	No
34	No	No	No	No	No	No	Mechanical obstruction of the intestines
35	No	No	No	Yes	No	Perianal tumor	No
36	No	No	No	No	No	No	No
37	No	No	No	No	No	No	Erosive colitis, intestine obstruction
38	No	Yes	No	No	No	Perianal tumor	Cardiomegaly and early stage of long congestion
39	No	No	No	No	No	Scrotal mastcell tumor	No
40	No	No	No	No	No	No	No
41	No	No	No	No	No	No	No
42	No	Yes	No	No	No	Perianal tumor	No
43	No	No	No	No	No	No	No
44	No	No	No	Yes	No	No	No
45	No	No	No	No	No	No	Prostatitis

6.4 Mixed tumor

Of the 56 dogs used in this study, eleven dogs had two or more types of tumors present in the testes with a mean age of 12 at time of diagnosis. One of the eleven dogs had one cryptorchid testes and none of the dogs had atrophic testes.

6.4.1 Hematology

Eight of the eleven dogs had blood work done, and of these eight, three dogs had values that were not within the reference values. One dog had a red blood cell deficit (4,05x10^{12}/l), hemoglobin deficit (9,7 g/dl) and a hematocrit of 25,80% resulting in a non-regenerative mediocre anemia, along with this, the dog also had a white blood cell deficit (2,09x10^{9}/l), neutropenia (0,04x10^{9}/l), eosinopenia (0 x10^{9}/l) and thrombocytopenia (12K/µl). The other dog had lymphopenia (0,95x10^{9}/l) and the third dog had thrombocytosis (499K/µl).

6.4.2 Biochemistry

Seven of the eleven dogs had their biochemistry checked, of these seven dogs, five dogs had values outside of the reference values. In the following table is an overview along with the mean values.

Table 10: Deviating biochemistry values of dogs with mixed cell testis tumors

	48	**50**	**52**	**54**	**55**	**Mean value**
Urea surplus			X	X		20 mmol/l
Albumin deficit			X			3.37 g/l
Sodium surplus					X	159 mmol/l
Potassium deficit		X				3.1 mmol/l
Potassium surplus	X					6.7 mmol/l
Calcium deficit	X					2.31 mmol/l
Increased liver values (which)			AST		AF	

6.4.3 Coagulation tests and hormone tests

One dog had its coagulation times checked, the cit-PT time was normal but the cit-aPTT time was prolonged, 131 seconds instead of less than 102 seconds.
No dogs had hormone levels tested.

6.4.4 Dermatology

In the following table all patients with mixed cell tumors can be found along with the dermatologic results

Table 11: Dermatologic results of dogs with mixed cell testis tumors

Patient number	Feminization	Pyodermia	Other
46	Yes	No	Higher epilability of hairs, papula
47	No	No	Maculae, pruritus
48	No	No	No
49	Yes	No	Linear dermatosis ventral of the penis
50	Yes	No	Seborrhoea
51	Yes	No	No
52	No	No	Alopecia on the back
53	No	No	No
54	No	No	No
55	No	No	No
56	No	No	Alopecia and nodules

6.4.5 Urinalysis

Five of the eleven dogs had their urine analyzed, of these five, four also had a sediment test done as can be seen in following table.

Table 12: Urinalysis results of dogs with mixed cell testis tumors

	48	49	50	52	54	Mean value
UPC increased	X	X	X	X	X	1.14
pH increased		X		X		7.5
Protein strip positive	X	X	X	X	X	
Infection of the urine tract		X (Gram positive germs)				
Sediment test		X	X	X	X	
WBC increased		X	X	X		1673/ml
RBC increased		X	X		Not available	124.5/ml
Plate epithelial cells increased		X	X		Not available	266.5/ml

6.4.6 Other

Of the eleven dogs, there was one case of a hernia perinealis, one perianal tumor, five dogs had benign prostate hyperplasia, of which one dog also had cysts and a mass of the prostate which was probably a neoplastic process of the prostate. One of the dogs also had a neoplastic process in the liver.

7 Discussion

The aim of this study was to describe possible associations of symptoms with the tumor cell type that is inducing these symptoms. Whether the age, the existence of the tumor, the grade of bone marrow suppression and thus the type of anemia, the level of reduction of the white blood cell lines and other secondary responses to the eventual hormones produced by the tumor, can be associated to the type of cell that has caused the neoplasia.

Of all the dogs in this study, 16% had seminomas, 20% had sertoli cell tumors, 44% had interstitial cell tumors and 20% had mixed cell tumors. From the consulted literature (Grieco et al., 2008) the three different types of tumors should be represented equally more or less. Based on our study findings seminoma, SCT and mixed cell tumors would appear at the same rate and ICT would appear more often.

The results showed that SCT were diagnosed at the youngest age of 10 years and 9 months, followed by ICT at 11 years and 5 months, SEM at 11 years and 11 months and mixed cell tumors at 12 months. In literature the age of diagnosis lower than what this study showed, SCT at 8,6 years, SEM at 10,6 years and ICT at 10, 9 years, no age was given to mixed cell tumors (Nødtvedt et al., 2010).
As regards to cryptorchid dogs, this study shows the same results as other studies, SCT have the highest percentage with 45% cryptorchid dogs, followed by seminoma with 11%, mixed cell type tumor with 9% and ICT with 4% (Liao et al., 2009; Ciaputa et al., 2012; Chaganti et al., 2000; Hemminki and Sweden, 2004). Atrophied contralateral testes were found most commonly with seminoma positive dogs (22%), followed by ICT (20%), SCT (9%) and mixed cell tumor with no positive dogs.

Concerning bloodwork, bone marrow suppression can be determined by pancytopenia and predicted by a decline in multiple blood cell lines, although this last factor could be influenced by other factors as well (Weiss et al., 2009). Other studies have shown that primarily SCT would be responsible for bone marrow suppression due to an increase of estrogens produced by the sertoli cells but ICT's could also induce bone marrow suppression by creating an imbalance between estrogens and testosterone, because of this mixed cell tumors with either ICT or SCT could also induce bone marrow suppression, this study has shown the same results.
Of the dogs with SEM, 55% had bloodwork done and 11% had anemia, but it was regenerative anemia, there was no leukopenia although 11% had a decrease in lymphocytes, neutrophils and eosinophils. Twenty-seven percent of dogs with SCT had bloodwork done and of these 27%, 24% had pancytopenia consisting of non-regenerative very severe anemia, leukopenia, neutropenia, eosinopenia and thrombocytopenia, another 9% had only lymphocytopenia. Eighty percent of dogs with ICT had bloodwork done and 30% had anemia of which 83% had non-regenerative anemia although 33% of these dogs also had systemic diseases that could interfere with these values. Five percent of dogs of with ICT (of the 80% with bloodwork done) had pancytopenia consisting of non-regenerative moderate anemia, leukopenia, neutropenia, lymphopenia, eosinopenia and thrombocytopenia, another 5% also had eosinopenia. Concerning dogs with mixed cell tumors 72% had bloodwork done and of this 72%, 13% had pancytopenia consisting of non-regenerative mediocre anemia, leukopenia, neutropenia, eosinopenia and thrombocytopenia.
In the following table is an overview of the percentages of deviations based on the animals with bloodwork done, in the second table is an overview of absolute percentages.

Table 13: Overview of the percentages of deviations of patients with bloodanalysis done

	Bloodwork	Anemia	Non-regenerative anemia	Panctyopenia (Leukopenia, non-regenerative anemia, thrombocytopenia	Decrease in blood cells without pancytopenia
SEM	55%	20%	None	None	60%
SCT	27%	33%	33%	25%	25%
ICT	80%	30%	25%	5%	5%
Mixed	72%	13%	13%	13%	25%

Table 14: Overview of the percentages of deviations of all dogs

	Total percentage in study	Anemia	Non-regenerative	Panctyopenia (Leukopenia, non-regenerative anemia, thrombocytopenia	Decrease in bloodcells without pancytopenia
SEM	16%	11%	None	None	33%
SCT	20%	9%	9%	9%	9%
ICT	44%	24%	20%	4%	4%
Mixed	20%	9%	9%	9%	18%

It is expected that the amount of dogs with pancytopenia would be highest in dogs with SCT and this study confirms this statement both with the absolute percentage as the percentage of the dogs with bloodwork.

Coagulation tests can be done to check if the coagulation factors function normally and this was checked in a couple of dogs: dogs with SEM weren't checked, two dogs with SCT were checked but both values were normal, three dogs with ICT were checked and all values were normal and one dog with mixed cell tumors was checked and this dog had an increased time of the cit-PTT time. It is difficult to know if the increased time is due to influences of the tumor or due to other deviations the dog might have, as well as that no other dogs had deviations of their coagulation times so that associations cannot be made.

Biochemistry values are more difficult values to use for predicting the type of tumor and mostly give extra information about the status of the organs like kidney and liver, but this isn't necessarily due to the tumor. Of all dogs with seminomas, 20% had increased liver values and of the dogs with SCT, 9% had increased liver values, of the dogs with ICT, 16% had increased liver values and of the dogs with mixed cell tumors, 18% had increased liver values.

If bone marrow suppression has already occurred, this would mean that there could be a decreased amount of white blood cells, leaving the body more accessible to infections, more specifically via the urine tract. In the following table is an overview of the results per tumor type, the percentages are based on the percentage of dogs that had a urinalysis done.

Table 15: Overview of the urinalysis of all dogs with urinalysis performed

	SEM	SCT	ICT	Mixed
Percentage of dogs with urinalysis	33%	18%	12%	45%
UPC increased	66%	/	33%	100%
pH increased	33%	/	33%	40%
Positive protein strip	33%	50%	/	/
Infectious germ detected	/	/	33% (E.coli)	20% (Gram positive germ)
WBC in sediment	66%	50%	33%	60%
RBC in sediment	33%	50%	33%	40%
Plate epithelial cell in sediment	/	50%	/	40%

The most obvious results are from the ICT and mixed cell tumor, both have active infections that have been able to discern the infection. This being said, raised white blood cells and red blood cells in urine can be signs of an active infection or inflammation that the body is trying to combat so it could be possible that there is a beginning infection that is not yet detectible with a urinalysis but that the body is already dealing with. Raised UPC values and positive protein strip could be because of the increased amount of blood cells, presence of bacteria or because there is a problem with the kidneys which could be releasing too much protein.

The most valuable information that could have helped this study, could have been hormone tests, more specifically estrogen blood values and testosterone values, and thus also the estrogen/testosterone ratio but only 2 dogs in the whole study, both with SCT, had these tests done. Both had normal results, so it is difficult to say if these tests really are good values to use to deduct what kind of tumor the dog has. These tests could be an interesting way to possibly diagnose SCT or ICT but more studies concerning these values should be conducted with consistent blood hormone values and histology of the tumor.
Because feminization is a symptom of hyperestrogenism or an estrogen/testosterone imbalance, it is most likely to find feminization symptoms in dogs with SCT , ICT or a mixed tumor with one of these cell types present. This study supports the theory partially: SCT had the most cases of feminization with 36% feminized dogs, mixed cell type tumor second with 27%, seminoma had 11% and ICT had 4%. These two last values are surprising since there should be no reason that seminomas should induce hyperestrogenism or an imbalance in estrogen/testosterone, yet there is and ICT should have a higher amount as these cells influence the testosterone levels.
Because SCT would have higher chances of having feminization symptoms and cryptorchidism, the chances of having them together is even larger than for all the other tumor types. The results confirm this as only two dogs of the 56 had feminization and cryptorchidism, one dog had solely SCT whilst the other had a mixed tumor of SEM and SCT.

The last part of the results shows the different types of deviations that are not linked to the other symptoms that have been discussed above, and can be placed into three categories: perianal deviations, prostatic disease and tumors elsewhere in the body.
It has been described in literature that androgens receptors are present in the perianal hepatoid glands of the dog (Pisani et al., 2006) and so a neoplasm of active androgen-producing cells could induce

hyperplasia or neoplasia of these hepatoid glands. The results of this study confirm this statement: 20% of dogs with ICT had perianal tumors and 9% of dogs with mixed cell tumor, the neoplastic cells being interstitial cells and sertoli cells, there were no dogs with SEM or SCT that had perianal tumors. Benign prostate hyperplasia has also been linked to androgen production and is common in male intact dogs (Foster, 2012); the results showed that 33% of dogs with SEM had BPH, 9% of dogs with SCT had BPH (although this dog also had prostatitis so it is possible that it could have been hyperplasia due to prostatitis), 12% of dogs with ICT had BPH and 45% of dogs with mixed cell tumors had BPH (40% of these dogs had SCT and SEM, 40% had SCT and ICT and 20% had SEM, ICT and SCT). It is difficult to conclude anything from this seeing as intact male dogs without neoplasms can also have BPH.
17% of all dogs in the study had tumors elsewhere in the body, aside from perianal tumors, but without histology done of these tumors, it is difficult to say if these are metastases or other primary tumors.

Having analyzed all the results, an obvious problem is the lack of consistency in the gathered data and because of this, it is difficult to be sure if percentages expressed in this analysis are correct. An option to solve this problem would be to only use patients with adequate and consistent data and to extract results from this but then the test group would be too small to offer decent results.
Another option would be to use this study as a basis to form an improved prospective study where all patients that come into the clinic with testis tumors have histology, bloodwork (hematology, biochemistry and coagulation tests), hormone tests and urinalysis done along with the physical examination, prostate examination, dermatologic examination and possibly medical imaging to detect potential metastases or other masses intra-abdominally and pulmonary. An important factor would also be to do follow up examinations to observe if exclusively castration and supportive treatment are sufficient to heal the dog or if additional treatment is needed to cure eventual non-regenerative bone marrow suppression that continues to be present after removal of the hormone producing cells.

Another problem that was experienced while collecting data was that frequently testis tumors were diagnosed without histology being done. Most of these cases were patients with feminization symptoms with the testes tumor being presumed to be a sertoli cell tumor, but because no histology was done these cases were not able to be used for this study.

8 Conclusion

The aim of this study was to determine if it is possible to associate certain symptoms to a specific testis tumor type confirmed with histology. This study used various symptoms and compared the results from one cell type to another to find following conclusions.

Based on age of diagnosis it is possible to say that SCT were more often seen in younger dogs where SEM and ICT were presented with the same mean age at diagnosis and are typically older dogs. In this study there have been dogs with SCT, SEM and ICT diagnosed respectively at the youngest ages of 8 years and 7 months, 8 years and 5 months and 8 years and 1 month and also respectively at the oldest ages of 14 years 3 months, 15 years 9 months and 15 years and 2 months. For this reason the association between age and testis tumor types is very weak.

If cryptorchidism is used as a parameter, the results have shown that dogs with cryptorchid testes have a higher chance of having SCT but ICT and SEM or mixed tumor cells can also be seen in cryptorchid testes. This can also be said for feminization although the chances of a SCT being present in a dog with feminization is much higher than the other types of tumors, it is possible to see feminization in dogs with SEM or ICT. But when a dog with cryptorchidism and feminization is presented, this can be presumed to be a SCT.

No conclusions can be made about the hormone tests or coagulation tests. If there is a link to a certain type of tumor it should be studied more thoroughly with adequate data, which in this study is not the case. Too little tests were done on the patients taken up in this study. The same can be said about metastases but this is due to no histology having been done to the other tumors so that there is no certain idea about the neoplastic cell type causing an impossibility to say if it is a metastases or coincidence. As for benign prostate hyperplasia, all intact male dogs have the possibility to acquire BPH but the results show no higher chances of developing BPH with a certain neoplasia.

As regards to perianal tumors, the results of this study describe that only dogs with ICT, or mixed tumors with ICT, have perianal tumors, so if a dog was presented with testis tumors and perianal tumors, it is most probably an ICT. The reverse is not true though, a dog without perianal tumors can have SCT and SEM but also ICT.

Dogs presented with pancytopenia (non-regenerative anemia, leucopenia and thrombocytopenia) are most likely to have SCT, ICT or mixed tumor type with one of these two tumor types. This study describes that SEM do not cause pancytopenia but can cause anemia, although always regenerative, and can also cause white blood cell lines to decrease without an absolute reduction of white blood cells. Non-regenerative anemia can be caused by SCT and ICT or both but regenerative anemia cannot be caused by SCT.
The urinalysis can show an active infection, and does so in two dogs, one with ICT and another with mixed cell (SEM and SCT). The dog with ICT also had pancytopenia, the dog with mixed cell tumor had no deviations of the blood analysis.

The goal of this study was to research if associations can be made between a certain type of testis tumor in a dog and the clinical symptoms of the dog. A few associations were observed, but less than were expected after consulting other literature. This can be related to the fact that different types of tumors cause similar symptoms.
More importantly this can be due to lack of having adequate patients with consistent data to identify these differences between the different kinds of testis tumors; some of the patients had full blood analysis done whilst other only had partial or no bloodwork done, hormone tests were only done on two dogs, urinalysis were only done on thirteen dogs. Because of these inconsistencies there could be

a potential bias of the results that only apply to dogs that had clinical signs leading to blood analysis or different tests being done. Because of these two reasons further studies with consistent data should be done to confirm if it is or isn't possible to diagnose a testis tumor type by the presented symptoms.

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