UNIVERSIDADE DE LISBOA
FACULDADE DE MEDICINA VETERINÁRIA

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RENATA MALVEIRO GASPAR

ORIENTADOR(A):
Doutor Rodolfo Assis Oliveira Leal

COORIENTADOR(A):
Doutora Rute Marina Garcia Da Noiva

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Faculdade de Medicina Veterinária da Universidade de Lisboa, 6 de Junho de 2025

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## Resumo

### Rinite Crónica Felina versus Linfoma Nasal Felino: problemáticas no diagnóstico e limitações

As manifestações clínicas da rinite crónica (RC) e do linfoma nasal (LN) em gatos são semelhantes, e tanto os resultados obtidos pelas técnicas da rinoscopia quanto da tomografia computorizada (TC) não são patognomónicos na sua distinção. Ambos são relativamente prevalentes na prática clínica, e os únicos métodos para distingui-los baseiam-se no recurso a histopatologia e, em alguns casos, análises complementares como imunohistoquímica (IHQ). Na prática diária, é, portanto, difícil distinguir entre RC e LN; isso deve-se em parte às semelhanças nas populações celulares das amostras de biópsia. Embora o diagnóstico por imagem e a histopatologia sejam importantes e complementares para um diagnóstico rápido, existem algumas limitações comumente reconhecidas que comprometem uma abordagem médica precisa.

Este estudo pretende rever as alterações imagiológicas, de histopatologia e IHQ. Visa também rever retrospetivamente o diagnóstico final correlacionando com a histopatologia e biomarcadores de IHQ (CD3, CD20, Pax5, STAT5, MLH1, MSH6) e o desfecho, assim como caracterizar retrospetivamente a apresentação, sinais clínicos, idade e outros dados relevantes em casos de gatos com doenças nasais, entre 2017 e 2022.

Foram incluídos neste estudo 35 casos de gatos com diagnóstico de RC ou LN do Hospital Escolar Veterinário (Faculdade de Medicina Veterinária, Universidade de Lisboa). A secreção nasal (28/35; 80%) foi o sinal clínico mais comum, seguido por espirros (21/35; 60%). A histopatologia e a IHQ revelaram ser úteis na diferenciação destas doenças. Os casos de RC demonstraram predominantemente processos inflamatórios crónicos com alterações estruturais mais ligeiras, enquanto os de LN foram caracterizados por uma destruição tissular mais marcada e um perfil neoplásico linfoide distinto. Quanto à IHQ, os anticorpos CD20/Pax5 e MLH1 mostraram potencial para melhorar a precisão diagnóstica ($p$=0.024 e $p$=0.001, respetivamente), e embora não sejam estatisticamente significativos, STAT5 e MSH6 também mostraram potencial no diagnóstico de LN ($p$=0.509 e $p$=0.051, respetivamente). Estas alterações, além de serem uteis no processo de diagnóstico, aparentam ser úteis como fatores de prognóstico; no entanto são necessários mais estudos. Ainda que preliminares, estes resultados revelam que alguns destes marcadores, como STAT5 e CD20, poderão ser usados para criar novas estratégias terapêuticas em medicina felina.

**Palavras-chave:** Rinite Crónica Felina, Linfoma Nasal Felino, Rinoscopia, Histopatologia, Imunohistoquímica.

## Abstract

### Feline Chronic Rhinitis versus Feline Nasal Lymphoma: diagnostic issues and pitfalls

The clinical manifestations of chronic rhinitis (CR) and nasal lymphoma (NL) in cats are similar, and neither rhinoscopy nor computed tomography (CT) scans results are pathognomonic to one or another. Both are extremely prevalent in day-to-day practice, and the only methods to distinguish between them are nasal biopsies, histopathology and, in some cases, immunohistochemistry (IHC). In everyday practice, it can be particularly difficult to distinguish between CR and NL; this is partly due to similarities in the cell populations of biopsy specimens. Another contributor to the difficulties in diagnosis is the absence of pathognomonic findings in CT scans, many of which are linked to NL but overlap with those in CR. Although diagnostic imaging and histopathology are important for a prompt diagnosis, there are some commonly recognized pitfalls that compromise an accurate medical approach.

The current study intends to review imaging, histopathology and IHC findings. It also aims to retrospectively revise final diagnoses, correlating with histopathology, IHC biomarkers (CD3, CD20, Pax5, STAT5, MLH1, MSH6) and outcome, as well as and retrospectively characterize cases pertaining to cats with nasal disease, between 2017 and 2022, for general data, signalment, age, and other pertinent data.

35 cases of cats with a final diagnosis of CR or NL diagnosis from the Veterinary Teaching Hospital (Faculty of Veterinary Medicine, University of Lisbon) were included in this study. Nasal discharge (28/35; 80%) was the most common clinical signs, followed up by sneezing (21/35; 60%). Regarding histopathology and IHC, these revealed to be useful in differentiating these conditions. CR cases demonstrated predominantly chronic inflammatory processes with milder structural alterations, while NL was characterized by more aggressive tissue destruction and a distinct lymphoid neoplastic profile. As for IHC, CD20/Pax5 and MLH1 antibodies showed potential for enhancing diagnostic accuracy ($p$=0.024 e $p$=0.001, respectively), and although not statistically significant, STAT5 and MSH6, showed potential in diagnostic NL as well ($p$=0.509 e $p$=0.051, respectively). These findings, besides helping determine a diagnosis, appear to be useful as prognostic factors; however, further studies are needed. Although preliminary, these results reveal that some of these markers, such as STAT5 and CD20, could be used to develop new therapeutic strategies in feline medicine.

**Key words:** Feline Chronic Rhinitis; Feline Nasal Lymphoma; Rhinoscopy; Histopathology, Immunohistochemistry.

## Resumo Alargado

### Rinite Crónica Felina versus Linfoma Nasal Felino: problemáticas no diagnóstico e limitações

As manifestações clínicas da rinite crónica (RC) e do linfoma nasal (LN) em gatos são semelhantes, e tanto os resultados obtidos pelas técnicas a rinoscopia quanto as imagens imagiológicas não são patognomónicos tanto para uma como para outra. Ambos são extremamente prevalentes na prática clínica, e os únicos métodos para distingui-los são biópsias nasais, histopatologia e, em alguns casos, imunohistoquímica (IHQ). No entanto, estudos anteriores referem a dificuldade de distinguir entre RC e LN; devido à semelhança nas populações celulares das amostras de biópsia assim como as alterações estruturais dos tecidos. Outro fator que reforça as dificuldades do diagnóstico é a ausência de achados patognomónicos nas tomografias computadorizadas (TC), muitos dos quais estão ligados ao LN, mas que se sobrepõem aos da RC. Embora o diagnóstico por imagem e a histopatologia sejam importantes para um diagnóstico rápido, existem algumas limitações comumente reconhecidas que comprometem uma abordagem médica precisa. A IHQ, permite diferenciar de forma mais precisa estas duas patologias devido à presença e intensidade dos marcadores utilizados. Além de diferenciar as populações de linfócitos, esta demonstra também a sua malignidade e grau proliferação devido ao local de marcação e intensidade da mesma.

Apesar de estudos anteriores terem sido feitos neste âmbito, nenhum relacionou os achados da IHQ com o diagnóstico e caracterização das patologias. Tendo assim surgido a necessidade de caracterizar melhor a IHQ nestas doenças, assim como a utilização de novos anticorpos.

Este estudo visa também rever retrospetivamente os sinais clínicos e história pregressa, os achados da TC, rinoscopia e histopatologia de gatos com doenças nasais, entre 2017 e 2022, a fim de relacionar com o diagnóstico e prognóstico por forma a facilitar este processo.

Durante o período do estudo, os animais incluídos teriam de ter realizado biópsias nasais cegas ou guiadas por rinoscopia sujeitas a histopatologia realizada pelo laboratório de patologia, e estas amostras deveriam estar no repositório do laboratório. Apenas foram abrangidos animais com diagnóstico de RC ou LN, e casos como corpos estranhos nas vias nasais, carcinomas nasais, pólipos, fistulas oronasais e estenose nasofaríngea não foram incluídos.

Neste estudo foram incluídos 35 casos de gatos com diagnóstico de RC ou LN seguidos no Hospital Escolar Veterinário (Faculdade de Medicina Veterinária, Universidade de Lisboa). Os dados necessários foram recolhidos a partir da plataforma *Guruvet*®, e os casos foram revistos e retrospetivamente caracterizados quanto à história pregressa, idade,

género, status reprodutivo, sinais clínicos e a sua duração, status viral (FIV, FeLV, Herpesvírus, Calicivírus), infeções bacterianas (*Chlamydophila felis*, *Mycoplasma felis*), método de colheita de biópsia, relatórios de rinoscopia, TC e histopatologia, resultados da IHQ, diagnóstico final, tratamento, *follow-up* e *outcome*. Todas as histopatologias e IHQ feitas previamente foram revistas, e as alterações histopatológicas foram classificadas quanto à sua morfologia e população celular presente. Foram realizadas novas análises de IHQ nos casos em que estas não haviam sido previamente executadas. Estas análises não tinham sido anteriormente realizadas em alguns casos de LN e em quase nenhum caso de RC. Além disso, tanto as novas IHQ quanto as realizadas anteriormente foram complementadas com novos anticorpos, como o STAT5, MLH1 e MSH6.

Tanto na RC como no LN, a caracterização da população correspondeu na sua maioria ao que está reportado em estudos anteriores. A idade média dos animais com RC foi de 6.5 anos e de 10.6 no caso do LN. Os animais mais velhos desta amostra pertenciam ao grupo do LN, à exceção de dois casos: um gato de 3 anos e outro de 7. Contudo, o primeiro era positivo para FIV e FeLV e o segundo apenas para FeLV. Estudos anteriores revelaram que a infeção por retrovírus leva ao desenvolvimento de LN em idades mais precoces, principalmente quando existe co-infeção com ambos. Quanto aos sinais clínicos e história pregressa, a presença de secreção nasal foi o sinal clínico mais comum a ambas as doenças (RC: 67.9%; LN: 85.7%), seguido por espirros (RC: 60.7%; LN: 57.1%), o que reforça a sobreposição de sinais clínicos. Este estudo revelou que a inflamação crónica pode ser indicativo de desenvolvimento de LN, especialmente do tipo B, uma vez que dois casos apresentavam sinais clínicos prolongados, durante 1 e 3 anos, e o seu diagnóstico final revelou ser LN.

Também foi possível demonstrar que apesar de não poder ser utilizada como único método de diagnóstico, a TC pode ter um papel fundamental no mesmo e na escolha do local de biopsia. Uma massa foi identificada em 83.3% dos animais que realizaram este exame, o que é bastante mais elevado que os 20% que identificaram uma massa na rinoscopia. A avaliação dos linfonodos através da TC também revelou ser importante, pois permite estadiar o LN. Foi observado que cerca de 66.7% dos animais tinha aumento dos linfonodos, dos quais cerca de 21% foram considerados como possível hiperplasia reacional ou metástase. Isto foi um achado importante na avaliação da TC, uma vez que apenas um animal com LN apresentava linfadenomegália evidente em consulta.

Através da rinoscopia, aferiu-se que os achados são semelhantes em ambas as doenças, reforçando a necessidade de ser realizada análise histopatológica das biópsias recolhidas. Quanto à histopatologia e à IHQ, estas revelaram ser úteis na diferenciação da RC e do LN. Os casos de RC demonstraram predominantemente processos inflamatórios crónicos com alterações estruturais mais ligeiras, enquanto os de LN foram caracterizados por destruição tecidular mais acentuada e um perfil neoplásico linfoide distinto. A inflamação

linfoplasmocítica predominou em ambas as doenças; a maioria dos casos de LN (71.4%) foi imunofenotipada como de células B.

Os anticorpos CD20/Pax5 e MLH1 utilizados na análise de IHQ, revelaram ser estatisticamente significativos para os casos de LN. Isto é devido à sua elevada frequência e intensidade de marcação nestes casos. O STAT5 e o MSH6 também demonstraram algum potencial no diagnóstico desta doença pelas mesmas razões apesar de estatisticamente não o demonstrarem. Estes achados, além de ajudarem a atingir um diagnóstico correto, também fornecem um fator prognóstico. Pensa-se também que, no futuro, alguns podem ser usados para criar novas estratégias terapêuticas ou medicamentos, como STAT5 e CD20, respetivamente.

Quanto à evolução da doença, esta foi mais favorável nos casos de RC, uma vez que a grande maioria dos animais teve uma recuperação completa ou manteve-se controlado com medicação, ao contrário do que foi observado nos casos de LN, em que apenas 1 animal se manteve controlado com a medicação até ao final deste estudo.

Este estudo conclui que não existe um exame de diagnóstico ideal para estas doenças, e que estes devem ser utilizados em conjunto para chegar a um diagnóstico definitivo. Revelou também que a técnica de IHQ é uma ferramenta importante no diagnóstico, uma vez que os marcadores STAT5, MLH1 e MSH6 têm potencial enquanto marcadores de diagnóstico.

Por fim, tendo em conta as conclusões retiradas, devem ser realizados mais estudos neste tema com uma população maior, mais consistente e homogénea, a fim de ultrapassar as limitações deste estudo.

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## List of Abbreviations

AST – Antimicrobial Susceptibility Testing

BRMs – Biological Response Modifiers

CBC – Complete Blood Count

CHOP – Cyclophosphamide, Hydroxydaunorubicin, Oncovin and Prednisone

COP – Cyclophosphamide, Oncovin and Prednisone

CR – Chronic Rhinitis

CT – Computed Tomography

ELISA – Enzyme-linked Immunosorbent Assay

FCR – Feline Chronic Rhinosinusitis

FCV – Feline Calicivirus

FeLV – Feline Leukemia Virus

FHV-1 – Feline Herpesvirus 1

FIV – Feline Immunodeficiency Virus

FLR – Feline Lymphoplasmacytic Rhinitis

FNA – Fine-needle Aspiration

FOCMA – Feline Oncornavirus-associated Cell Membrane Antigen

IHC – Immunohistochemistry

IHQ – Imunohistoquímica

ISCAID – International Society for Companion Animal Infectious Diseases

LN – Linfoma Nasal

MALT – Mucosa-Associated Lymphoid Tissue

MMR – Mismatch Repair

MRI – Magnetic Resonance Imaging

NSAIDs – Nonsteroidal Anti-Inflammatory Drugs

NL – Nasal Lymphoma

PARR – PCR for Antigen Receptor Rearrangement

PBS – Phosphate Buffered Saline

PCR – Polymerase Chain Reaction

PO – *per os*

RC – Rinite Crónica

rt-PCR – Reverse Transcriptase PCR

SC – Subcutaneous

STAT – Signal Transducer and Activator of Transcription

SUB – Subcutaneous Ureteral Bypass

TC – Tomografias Computadorizadas

TCR – T cell antigen receptor

Th1 – T-helper type 1

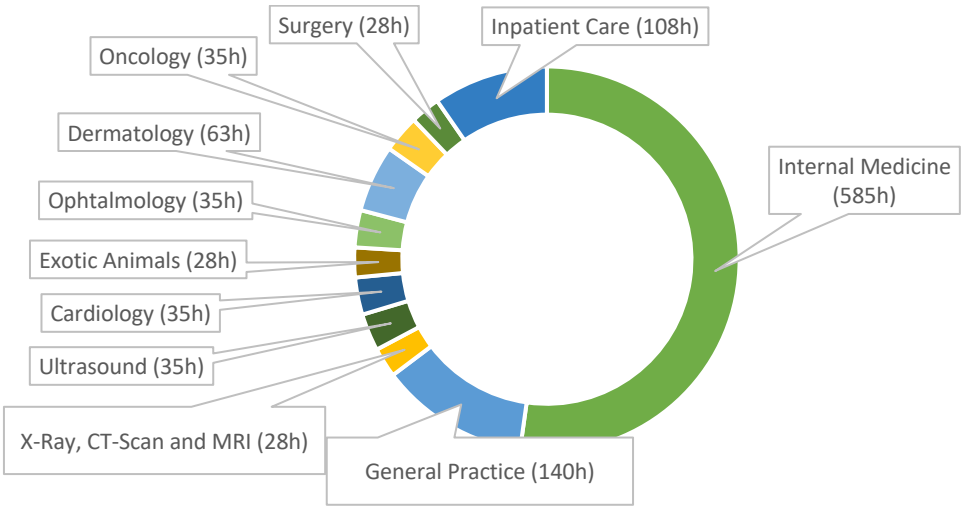
## List of Symbols

® – Registered Trademark

## 1. Traineeship Report

My final curricular traineeship took place between March 6th and September 1st, 2023, at the Veterinary Teaching Hospital of the Faculty of Veterinary Medicine of the University of Lisbon, making a total of 25 weeks and approximately 1120 hours. It consisted of two parts: three months were spent in the internal medicine service, and the other half I was listed on a rotation through almost all the other specialties of the hospital, represented in Graph 1.

**Graph 1 – Time spent in each department**




**Legend: h – Hours**

The hospital's operations involved a multidisciplinary team composed of veterinarians, veterinary nurses, and veterinary assistants. The facility included several consultation rooms, dedicated hospitalization areas for cats, dogs, and an Isolation and Biological Containment Unit for the infectious and contagious patients. Additionally, it was equipped with an ultrasound room, a radiology room, a Computed Tomography (CT) room, a magnetic resonance imaging (MRI) room, and two surgical rooms. The main departments for weekly rotations included General Medicine, Diagnostic Imaging (X-Ray, CT scan and MRI), Ultrasound, Cardiology, Exotic Animals, Ophthalmology, Dermatology, Oncology, Surgery, and Inpatient Care. I was able to participate in first and second opinion consultations, specialist consultations, follow-up and reassessment appointments, as well as palliative care and euthanasia consultations for dogs and cats. Emphasis was placed on the overall evaluation of the patient, obtaining a thorough medical history, proposing possible differential diagnoses and appropriate complementary tests, performing these tests, and subsequently selecting and implementing the most suitable therapeutic plan. In addition to the previously mentioned specialties, the consultations department also included neurology, behavior, reproduction, and dentistry appointments.

Part of the practical component of this masters' project was presented as a poster with the title "Current epidemiology and therapeutic trends of feline chronic rhinitis – a retrospective

study” on the 33rd Annual Congress of the European College of Veterinary Internal Medicine – Companion Animals (ECVIM-CA), which took place in Barcelona on 21-23 September 2023 and also, an oral presentation with the title “Inflammation or Proliferation? Exploring New Diagnostic Markers in Feline Nasal Lymphoma” for the II Congress BioMedLab, which took place in Coimbra on 1-3 March 2024 (Annexes 1, 2, 3 and 4). Under the MSC Project typology – Master's Projects – this study was completed as an internal project of CIISA with funding from a Scientific Initiation Scholarship (BIC) for graduates (scholarship code: MSC22Nov-03).

## 1.1. Internal Medicine

Over the course of 3 months, I joined the Referral Internal Medicine Service (SMIR) team, making a total of 585 hours.

The days started with medical rounds, where the hospitalized cases were discussed with the veterinarians of the different departments. Then I accompanied Professor Dr. Rodolfo Oliveira Leal, Dr. Joana Dias or Dr. Beatriz Mendonza in their morning consultations, where I was allowed to collect the anamnesis of the patient and perform the physical examination. The differential diagnoses and treatment were then discussed with the responsible clinician. After consultations, I participated in procedures such as the collection of blood and urine samples and blood pressure measurements of the consulted animals. In the afternoon, we reviewed the day's clinical cases, went over the list of differential diagnoses, decided on the appropriate complementary exams to conduct, adjusted the treatment plan, if necessary, and contacted the owners with the decided plan and exam results.

Twice a week, I assisted complementary exams such as rhinoscopies, tracheobronchoscopies with bronchoalveolar lavages, gastroduodenoscopies, colonoscopies, cystoscopies and fecal transplants. In addition to visualizing the structures, these exams were also performed with the aim of collecting biopsies or removing foreign bodies. I also collaborated in the elaboration of the endoscopy reports and discussion of the histopathological results.

Weekly, I participated in the journal club, where each one had to prepare a presentation of a recent article and summarized the most important conclusions, followed by a discussion.

During that time, I acquired knowledge about the different areas of internal medicine, such as endocrinology, hematology, respiratory system, digestive system, hepatobiliary system, and urinary system, including how to diagnose and treat various conditions. It also allowed me to refine my clinical reasoning, enabling me to more easily develop a list of differential diagnoses and thus select the most appropriate diagnostic tests. Additionally, it improved my medical skills in managing animals with chronic diseases and overseeing their care throughout their lives. Finally, it enhanced my soft skills through communication with pet owners at various moments.

## 1.2. General Practice

For 140 hours, I had the opportunity to attend first and second opinion consultation, preventive care, follow-up, and emergencies. I actively participated in the consultation process, which involved gathering the animal's medical history and conducting physical examinations. These tasks were supervised or later confirmed by the attending veterinarian, offering a chance to refine diagnostic skills. I also had practical experience with a variety of medical procedures, including blood collection, cystocentesis, placing intravenous catheters, preparing and administering fluids, measuring glycaemia, and conducting diagnostic procedures such as thoracocentesis, abdominocentesis, and electrocardiography. In some cases, I assisted with radiographs and ultrasounds when indicated by the animal's clinical signs. In emergency situations, I was involved in managing urgent cases such as trauma, dyspnea, and seizures, where I contributed to stabilizing the patients. This included placing intravenous catheters, providing oxygen support, monitoring vital signs, and preparing fluid systems. This rotation also provided opportunities to engage in difficult discussions with owners, addressing concerns such as euthanasia and supporting its execution. The diverse clinical experiences helped deepen my understanding of the clinical decision-making process, as well as the importance of quick thinking in critical situations. I also provided support to the nursing team by assisting in animal restraint, removing sutures, and other tasks. This rotation was an important part of my training, providing significant insights into daily clinical practice, which is crucial for my future career as an early graduated veterinarian.

## 1.3. Diagnostic Imaging

### 1.3.1. X-Ray, CT and MRI

Approximately 28 hours were spent in this service. During the diagnostic imaging rotation, I was actively involved in a range of imaging procedures, gaining experience in X-rays, CT, and some contact with MRI.

My responsibilities in the X-ray room included placing the cassette correctly, positioning animals on the table, and performing the radiation exposure. I also helped with patient preparation, including intravenous catheter placement, endotracheal intubation, administering sedation as well as monitoring anesthesia throughout the exam. Following the exams, I often managed post-procedure monitoring, ensuring a calm recovery for each patient. Radiographs covered various regions, from thoracic and abdominal imaging to orthopedic evaluations, post-surgical checks, and assessments following the placement of feeding tubes. Each X-ray examination concluded with a collaborative image review and discussion with the supervising radiologist. In the CT room, my responsibilities were the same as in X-ray room. Additionally, I was engaged in image interpretation and contributed to report writing.

This rotation also provided opportunities to observe MRI exams scheduled during my shifts, where I gained an understanding of this modality's functions, positioning techniques, and variable settings.

### 1.3.2. Ultrasound

About 35 hours were completed in this service. During the ultrasonography rotation, I gained extensive experience by observing and assisting in a variety of ultrasound exams of cats and dogs. My role often included trichotomy and patient positioning, ensuring the animals were ready for the exam. Under supervision, I was able to conduct and interpret some ultrasounds independently, focusing on identifying various structures, including abdominal organs and typical pathologies. Throughout this period, I closely observed and assisted in numerous ultrasound-guided procedures, such as fine-needle aspirations (FNA), cystocentesis, abdominocentesis, cholecystocentesis, exfoliative catheterization, and flushing of Subcutaneous Ureteral Bypass (SUB) systems. I was also involved in advanced guided procedures like gallbladder, liver, and pulmonary Tru-Cut® biopsies. Ocular ultrasounds and cervical scans were also part of my learning experience. After each exam the veterinary did the ultrasound reports, where I involved in discussions on diagnostic findings and possible causes, which helped me develop a stronger grasp of medical terminology specific to ultrasonography.

## 1.4. Cardiology

Around 35 hours were spent in this service. In cardiology, I had the opportunity to observe clinical procedures such as echocardiography, electrocardiograms, Holter monitoring, and even a bubble study. My responsibilities included performing trichotomy and positioning the patient to ensure the animal was properly prepared for the examination. During the procedure, the veterinarian explained the images on the monitor, helping me to become familiar with both normal and pathological findings. After the exam, any cardiac pathology detected was discussed with the owners, and if the animal was already on medication, adjustments were made as necessary. Following the consultation, the veterinarian would review and discuss the case with me, deepening my understanding of the diagnosis and treatment approach.

## 1.5. Exotic Animals

A total of 28 hours were completed in this service. During my time with exotic animal consultations, I had the opportunity to observe a variety of species, including domestic rats, guinea pigs, and rabbits. In addition to assisting and helping with restraint, I observed procedures such as dental care, ovariohysterectomies, and orchiectomies. Throughout these consultations, I also became familiar with common health issues in rodents and reptiles and

learned about the appropriate care and treatments for each case. When necessary, I administered oral medications and participated in discussions of differential diagnoses and treatment approaches, enriching my understanding of exotic animal care and the specific challenges these cases present.

## 1.6. Ophthalmology

During my 35 hours of ophthalmology rotation, I gained experience with several diagnostic tests and examinations to assess ocular health in dogs and cats. In addition to general physical exams, I participated in specialized ophthalmologic evaluations, which included checking palpebral and pupillary reflexes and assessing the menace response. I also had the opportunity to perform the Schirmer tear test and the fluorescein stain test, as well as measure intraocular pressure to evaluate the anterior and posterior segments of the eye.

Throughout this rotation, I became familiar with various common ophthalmologic conditions and treatment options, especially corneal ulcers and keratoconjunctivitis sicca, which were frequently encountered during follow-up appointments. I also observed the use of direct microscopy with a slit lamp to get a detailed view of ocular structures. The rotation included a mix of first-opinion, referral, and follow-up appointments, providing exposure to a range of ophthalmologic cases and an understanding of the most common medications and treatments used in eye care for companion animals. When necessary, blood samples were also taken for further diagnostic tests, allowing for a comprehensive approach to the management of eye diseases. I also assisted a cataract surgery, an electroretinography and an ocular ultrasound.

## 1.7. Dermatology

Approximately 63 hours were spent in this service. During my rotation in dermatology, I had the opportunity to widen my understanding of common skin conditions in veterinary medicine. I participated in follow-up, second opinion, and referral consultations, where I took patient histories and performed physical examinations. Under the supervision of the attending veterinarian, I conducted various diagnostic tests, including skin cytology (via slide or tape preparation), skin scrapings, ear cytology, and trichograms. I was also involved in FNA and skin biopsies, gaining experience in these essential dermatological procedures. In addition, I learned how to collect samples for bacteriological and mycological cultures, particularly from skin, hair, and ear swabs. I had the opportunity to observe deep ear cleanings and video otoscopic procedures, which further enriched my knowledge of dermatological treatments. Throughout the rotation, we discussed differential diagnoses and the best treatment protocols for each case, encouraging clinical reasoning based on current scientific evidence.

## 1.8. Oncology

About 35 hours were completed in this service. During my rotation in the oncology department, I had the opportunity to be involved in a variety of cases, including first-opinion consultations, second opinions, and follow-up appointments. These consultations often focused on the diagnosis, staging, and treatment plans for oncological patients. I participated in physical examinations and FNA biopsies, particularly of cutaneous nodules. The responsible veterinarians also provided valuable insights into chemotherapy, where I was actively involved in patient reception, weighing, catheter placement, and blood sample collection for complete blood counts. I observed the preparation and administration of chemotherapy, assisted by the nursing team, and participated in monitoring the animals during treatment. Throughout the rotation, I learned about the different tumor types commonly found in small animals, including the most appropriate diagnostic tests and treatment protocols. The rotation allowed me to develop my clinical reasoning skills, particularly when it came to diagnosing and determining the best course of treatment for cancer patients. I also gained experience in delivering difficult news to pet owners and dealing with more complex situations, such as those involving poor prognosis, which helped me improve my communication and soft skills.

## 1.9. Surgery

Around 28 hours were spent in this service. During my surgical rotation, I gained experience in the preparation, execution, and aftercare of surgical procedures. My responsibilities included receiving patients, weighing them, and performing pre-anesthetic physical examinations. I assisted in the preparation of scheduled medications, the administration of premedication, and catheter placement. Additionally, I participated in the induction of anesthesia, intubation of patients, and the preparation of the surgical site through trichotomy, cleaning, and disinfection. In the operating room, I had the opportunity to assist the surgeon, particularly during the application of skin sutures to close incisions at the end of procedures. Throughout the rotation, I observed various surgical procedures, including soft tissue surgeries, stomatology surgeries and specialized surgeries such as eye enucleations, the correction of pectus excavatum with a splint, and intraoperative biopsies. I was also given the opportunity to perform orchiectomies under direct supervision. Post-operatively, I was involved in the monitoring of animals to ensure proper recovery until they were discharged. The rotation also encouraged the independent performance of some procedures which contributed significantly to both my technical skills and my understanding of surgical principles and patient management.

## 1.10. Inpatient Care

Nine night shifts of 12 hours were added to the schedule, making a total of 108 hours. During my rotation in the inpatient department, I was involved in a variety of activities that provided experience in patient care and clinical management. Each shift began with a medical round, during which the hospitalized cases were discussed in detail, covering the patient's history, cause of hospitalization, treatment plans, and prognosis. I had the opportunity to follow the evolution of inpatient cases and contribute to their care by performing physical examinations, administering oral and injectable medications, and ensuring that the animals received proper nutrition and hydration. Depending on the animal's condition, I also assisted with walking the patients, cleaning their enclosures, and ensuring their overall well-being. Throughout the rotation, I was involved in a range of procedures, including intravenous catheter placement, fluid system preparation, glycemia measurement, blood pressure assessment, urethral catheterization, and placement of nasogastric feeding tube. Additionally, I assisted in sample collection for analysis and helped with animal restraint when needed. I was exposed to critical, life-threatening situations where I observed and participated in emergency interventions, such as fluid supplementation, blood transfusions, oxygen therapy, and cardiopulmonary resuscitation. The rotation allowed me to develop essential practical skills while under the supervision of experienced professionals. These experiences deepened my clinical knowledge and prepared me for handling urgent and complex situations in veterinary practice.

# 2. Literature Review

## 2.1. Upper Respiratory Tract

The upper respiratory tract refers to the respiratory organs located in the head, namely the nose, the paranasal sinuses, and the nasopharynx (the nasal portion of the pharynx) (König and Liebich 2016).

It is mostly lined by respiratory epithelium (Getty 1975; Hudson and Hamilton 2010; König and Liebich 2016; Junqueira and Carneiro 2017; Aspinall and Cappello 2019), which main function is the protection of the respiratory tract (Getty 1975; Hudson and Hamilton 2010; König and Liebich 2016; Singh 2018; Aspinall and Cappello 2019), In case of respiratory disease, the mucosa becomes hyperaemic and thickened; some areas contain cavernous blood spaces, making them semi-erectile tissues, which exacerbates nasal congestion and difficulty in breathing (Singh 2018).

### 2.1.1. Nose

The nose of cats is composed of the nostrils and the nasal cavity (including the nasal meatuses and nasal conchae) (König and Liebich 2016).

Aggressive nasal illness can harm any of these bone structures and the first suspicion of disease in the nasal cavities is frequently raised by noticeable changes in these bony areas (Batalla et al. 2021). The nostrils correspond to the external openings the nasal vestibules, which continue caudally to the nasal cavities (Hudson and Hamilton 2010; Singh 2018). Internally, the nose is divided into two equal parts (right and left) by the nasal septum (Getty 1975; Hudson and Hamilton 2010; Singh 2018).

### 2.1.2. Nasal Cavity

As mentioned before, there are two nasal cavities: left and right. The interior of the nasal cavities is mostly filled by the nasal conchae which function is to increase the surface of the respiratory area, thus warming the inspired air that goes to the lungs and reducing the temperature of the arterial blood that irrigates the brain. There are 3 nasal conchae - the dorsal, the middle, and the ventral - dividing the nasal cavity into three spaces: the dorsal nasal meatus, the middle nasal meatus, and the ventral nasal meatus (Getty 1975; Hudson and Hamilton 2010; Reece et al. 2015; König and Liebich 2016).

The caudal zone of the nasal cavity is filled by the ethmoidal concha, which is lined by olfactory mucosa (Hudson and Hamilton 2010; König and Liebich 2016; Junqueira and Carneiro 2017; Singh 2018). Because the epithelium is full of nerve endings, whose axons converge and form the olfactory nerve, this is considered the olfactory region of the nasal cavity. The olfactory nerve passes through the cribriform plate of the ethmoid to reach the cerebral olfactory bulb, transporting the information required for olfaction to the brain (Hudson and Hamilton 2010; Aspinall and Cappello 2019).

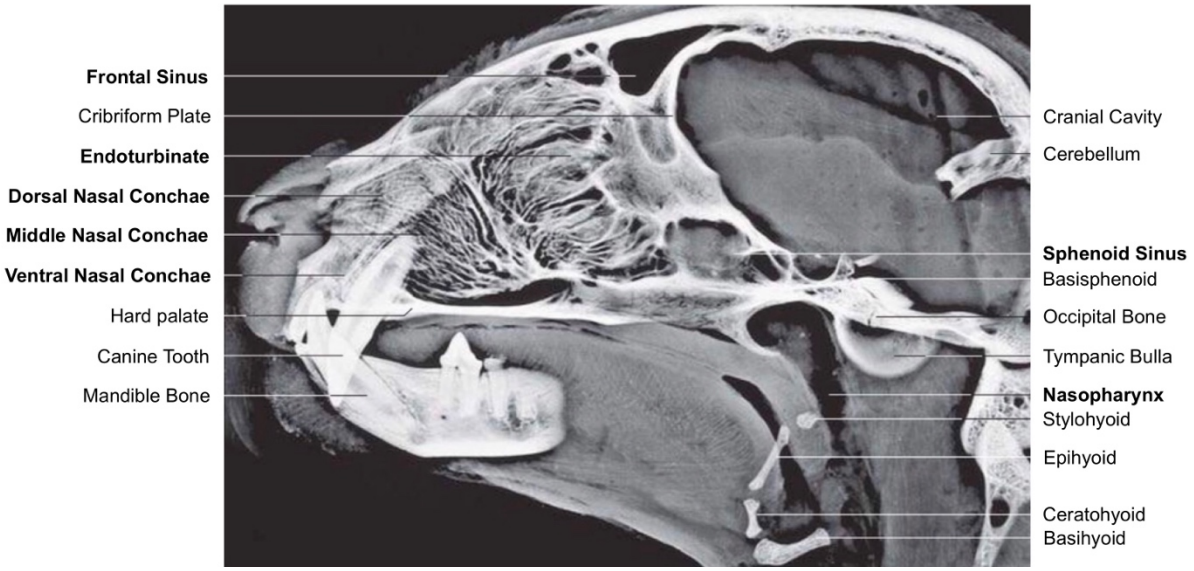



**Figure 1 – X-ray of a cat's head (Adapted from** (König and Liebich 2016)

### 2.1.3. Paranasal Sinuses

The paranasal sinuses are empty spaces incorporated into the bones of the face (Singh 2018). Despite their unknown function, the paranasal sinuses provide protection for the orbits, the nose, and the nasal cavities (Singh 2018). The sinuses are narrow cavities, with openings that are not easily accessible; thus, they have the potential to cause obstructions when the mucosa becomes inflamed and thickened (König and Liebich 2016). Since normal drainage of the frontal sinuses into the ethmoid concha is frequently pathologically obstructed in diseased states, these are the only sinuses that commonly have clinical significance in respiratory diseases (Batalla et al. 2021).

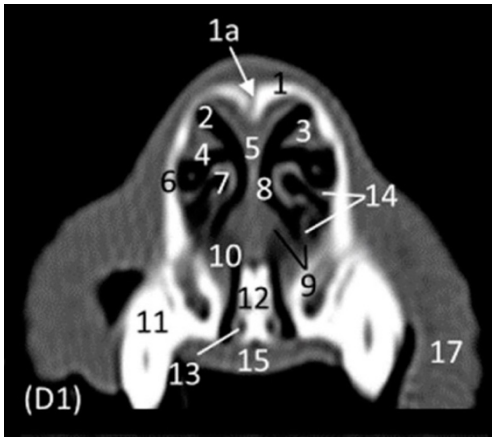



**Figure 2 – Representative transverse CT image of a cat ate the level of the rostral portion of the respiratory part. Transverse image is oriented so that the left side of the head is to the right and the dorsal is at the top. View is rostral. (Adapted from** (Díaz Martínez et al. 2024)**.**

Legend: 1 – Nasal bone; 1a – nasal bone: nasal symphysis (arrow); 2 – dorsal nasal meatus; 3 – dorsal nasal concha; 4 – middle nasal meatus; 5 – nasal septum: cartilage; 6 – 3rd endoturbinate; 7 – maxillary bone: body; 8 – maxillary recess; 9 – common nasal meatus; 10 – ventral nasal concha; 11 – nasal cavernous plexuses; 12 – root of the upper canine tooth; 13 – ventral nasal meatus; 14 – vomer bone; 15 – maxillary bone: palatine process; 17 – hard palate.

## 2.2. Chronic Rhinitis

Rhinitis is the inflammation of the nasal mucosa (Zachary 2022). It usually starts with an insult to the mucosa that leads to a dysbiosis of the commensal microbiota of the nasal cavity. Causes include respiratory viruses, bacteria, fungi, immunosuppression, foreign bodies, trauma (Kuehn 2006; Ettinger et al. 2024), and even prolonged therapy with antibacterials (Zachary 2022) or drug induced rhinitis (e.g., Cyclosporine) (Ettinger et al. 2024). Even when a thorough diagnostic investigation is carried out, the etiology is frequently undetermined (Hawkins 1988; Michiels et al. 2003).

Early treatment of bilateral nasal disease in a young, pet is often empirical, consisting of an antibiotic and an anti-inflammatory drug (Batalla et al. 2021; Ettinger et al. 2024). However, specific signs should warrant additional diagnostics: persistent epistaxis, unilateral discharge that spreads bilaterally, facial asymmetry, pain, or unresponsiveness to prior treatments (Batalla et al. 2021). All causes of acute rhinitis can lead to chronic signs if not diagnosed early (or correctly) or if treated improperly (Cape 1992), since it can be an uncurable disease. Rhinitis is considered chronic when clinical signs are present for four weeks or longer (Cape 1992; Harvey and Tasker 2013).

### 2.2.1. Etiology

Chronic rhinitis (CR) can have several causes (Table 1); however, some are more commonly found in cats, such as: Feline Herpesvirus 1 (FHV-1), Feline Calicivirus (FCV), Feline Immunodeficiency Virus (FIV), Feline Leukemia Virus (FeLV), Chlamydiosis, Mycoplasmosis, Cryptococcosis, and immune-mediated (i.e., idiopathic lymphoplasmacytic rhinitis) (Kuehn 2006; Zachary 2022). FHV-1, FCV, *Chlamydophila felis*, and *Mycoplasma felis* are part of the Feline Upper Respiratory Disease Complex; each can cause disease by itself or in combination with the others. Other significant but less frequent causes are nasopharyngeal polyps, fungal infections, foreign bodies, periodontal disease, and nasopharyngeal stenosis (Kuehn 2006; Ettinger et al. 2024).

**Table 1 – Differential Diagnosis for Chronic Rhinitis in Cats (Adapted from** (Kuehn 2006; Zachary 2022; Ettinger et al. 2024)

| | |
|---|---|
| **Viral Infection**<br>▪ FHV-1<br>▪ FCV<br>▪ FIV<br>▪ FeLV | **Immune-mediated/Inflammatory**<br>▪ Idiopathic chronic rhinitis<br>▪ Lymphoplasmacytic rhinitis<br>▪ Nasopharyngeal polyp |
| **Bacterial Infection (usually secondary to primary conditions)**<br>▪ *Chlamydophila felis*<br>▪ *Mycoplasma felis*<br>▪ *Pasteurella multocida*<br>▪ *Bordetella bronchiseptica*<br>▪ Anaerobic bacteria | **Stenosis**<br>▪ Nasopharyngeal stenosis<br>▪ Stenotic nares<br>**Periodontal disease**<br>**Foreign body**<br>**Neoplasia (nasal, oral, extraocular)** |
| **Fungal Infection**<br>▪ *Cryptococcus spp*<br>▪ *Aspergillus spp* | **Others**<br>▪ Trauma<br>▪ Immunosuppression (e.g. glucocorticoids, cyclosporine)<br>▪ Prolonged antibacterial therapy |

FHV-1 and FCV are two of the most common causes of viral rhinitis and tend to induce moderate to severe upper respiratory signs (Johnson et al. 2005; Zachary 2022). Although FHV-1 and FCV combined are responsible for 80-90% of acute cases (Ford 1997; Michiels et al. 2003; Johnson et al. 2005; Nelson and Couto 2019; Zachary 2022; Ettinger et al. 2024), chronic disease is typically associated with FHV-1 (Johnson et al. 2005). CR is considered a consequence of prior infection with these viruses (Van Pelt and Lappin 1994), as 80% of cats that had an acute infection may become carriers, with or without persistent clinical signs and chronic inflammation (Gaskell and Wardley 1978).

FIV, and FeLV can also cause severe and chronic rhinitis, due to their ability to suppress the immune system, leading to secondary bacterial infections (Johnson et al. 2005; Zachary 2022).

With the exception of *Chlamydophila felis* and *Mycoplasma felis* (which can be primary agents of rhinitis) bacterial infection is a secondary complication of other nasal diseases. Bacterial infection may accompany nasal neoplasia, viral infection, fungal infection, parasitic infection, trauma, foreign bodies, periodontal diseases, or oronasal fistulation. It occurs once the normal microbiome is altered, and mucosal defense barriers are broken. This reinforces the need to search for underlying diseases attempting a diagnosis, especially if clinical signs are chronic (Nelson and Couto 2019; Raskin et al. 2021; Zachary 2022).

Fungal rhinitis is uncommon and can either be a primary or secondary opportunistic cause of disease (Raskin et al. 2021), usually seen in endemic geographic areas and immunosuppressed cats (Kuehn 2006; Ettinger et al. 2024). The most common agents are

*Cryptococcus neoformans* and *Cryptococcus gatti*, while aspergillosis is not so often diagnosed in cats (Kuehn 2006; Zachary 2022).

Allergic rhinitis is considered a type I hypersensitivity reaction to airborne antigens that is not, however, well characterized in small animals (Nelson and Couto 2019; Raskin et al. 2021). Clinical signs can include nasal pruritus, serous or mucopurulent nasal discharge, and sneezing (Nelson and Couto 2019).

Access to medical history is important for diagnosis, since the clinical signs may be seasonal or occur only when the animal is exposed to certain irritants, such as perfumes, cleaning detergents, and fabric, among others. These irritants can be present for weeks or months, meaning signs can be acute or chronic (Nelson and Couto 2019). If the allergen is identified, its removal from the environment is the ultimate treatment. However, identification is not always successful, requiring a more complete diagnostic evaluation and/or administration of antihistamines. If the animal does not respond to these, glucocorticoids may be used, which can be administered through an inhaler, for as long as needed to control signs (Nelson and Couto 2019).

#### 2.2.1.1. Idiopathic Chronic Rhinosinusitis

This category includes Feline Chronic Rhinosinusitis (FCR) and Feline Lymphoplasmacytic Rhinitis (FLR). These are more commonly diagnosed in cats than in dogs, and constitute diagnoses of exclusion, requiring the ruling out of all other differential diagnoses through diagnostic tests (Kuehn 2006).

As these diseases can precede nasal lymphoma, an initial diagnosis of FCR or FLR should be revised by repeating biopsies and immunohistochemical analysis if clinical findings become highly suggestive of neoplasia (Kuehn 2006).

##### 2.2.1.1.1. Feline Chronic Rhinosinusitis

This is one of the two most common causes of sneezing and nasal discharge in cats (Henderson et al. 2003; Michiels et al. 2003; Johnson et al. 2005; Nelson and Couto 2019). The term Chronic Rhinosinusitis is applied to this condition due to its ability to impact both the nasal cavity and the frontal sinuses (Reed 2020).

As many studies have failed to associate various viruses and bacteria as main causes of FCR, the cause remains unknown (Henderson et al. 2003; Johnson et al. 2005; Nelson and Couto 2019). It is possible that infection with FHV-1 or FCV may lead to a damaged mucosa, which is then more vulnerable to bacterial infections or prone to reacting excessively to irritants or to the nasal microbiota, leading to CR (Hawkins 1988; Henderson et al. 2003; Nelson and Couto 2019).

Experimental infection with FHV-1 induced turbinate lysis, which is a common lesion seen in FCR (Henderson et al. 2003). It was also suggested that FHV-1 can remain latent within the nasal epithelium and reactivate during an episode of stress or immunosuppression,

triggering clinical signs; this would support a role of chronic or recurrent infection with FHV-1 as a contributing factor in the development and high incidence of FCR in cats, but no evidence of such an association exists of yet (Johnson et al. 2005; Nelson and Couto 2019).

Clinical signs usually consist of bilateral chronic mucoid or mucopurulent nasal discharge and sneezing. Other nonspecific findings include turbinate erosion, mucosal inflammation and increased mucus accumulation seen in nasal imaging and rhinoscopy. Secondary bacterial or *Mycoplasma felis* infection can also develop (Nelson and Couto 2019). Since no underlying cause is evident, only supportive treatment can be provided, consisting in facilitating drainage of discharge, treatment of possible secondary bacterial infections, control of inflammation via anti-inflammatory drugs, and, in extreme cases due to the difficulty of managing these cases, the last option is turbinectomy and frontal sinus ablation (Nelson and Couto 2019).

#### 2.2.1.1.2. Feline Lymphoplasmacytic Rhinitis

Not so common in cats, FLR is a form of non-infectious CR in cats (Kuehn 2006; Ettinger et al. 2024). While the specific cause in not yet determined, various factors such as disruption of the nasal microbiota, allergies, and immune dysregulation have been proposed as potential contributors (Kuehn 2006; Raskin et al. 2021; Ettinger et al. 2024). Usually, there is no history of acute episodes of upper respiratory tract infection and the signs are initially mild (Kuehn 2006); as such, this disease is thought to be immune mediated rather than an allergic reaction (Day 2011; Raskin et al. 2021). An analysis of cytokine profiles reported that a T-helper type 1 (Th1) response is normally seen in cats with FLR (Johnson et al. 2005).

### 2.2.2. Patient History, Clinical Signs and Physical Examination

Common clinical signs of chronic rhinitis include uni or bilateral nasal discharge, sneezing, stertor, and epistaxis (Hawkins 1988; Michiels et al. 2003; Kuehn 2006; Johnson 2020; Ettinger et al. 2024). Ocular discharge, cough, facial deformity (fungal rhinitis), fever, anorexia and weight loss are less common (Kuehn 2006; Nelson and Couto 2019; Ettinger et al. 2024). Concurrent otitis externa or vestibular disease can occur in cats with nasopharyngeal polyps. Central nervous system signs can be seen in cats with fungal rhinitis due to a compromised cribriform plate and extension of disease to the brain (Kuehn 2006; Ettinger et al. 2024). While there is significant overlap among numerous diseases, a differential diagnosis list can frequently be narrowed down by examining the location and type of nasal discharge. For instance, unilateral discharge usually tends to be caused by foreign bodies, oronasal fistulas or neoplasia, although this is not an absolute rule. Serous discharge also tends to change over time to mucoid or mucopurulent, if the disease is not appropriately treated (Kuehn 2006; Ettinger et al. 2024).

For most patients, chronic clinical signs have been present for four weeks or longer (Cape 1992; Harvey and Tasker 2013). These are usually not pathognomonic and overlap with

other nasal diseases, although some can be helpful in directing suspicion toward a particular diagnosis. For example, unilateral nasal discharge and bleeding are more frequently observed in cats diagnosed with neoplasia compared to rhinitis, although this presentation can also be found in cats with nasal foreign bodies (Hawkins 1988; Henderson et al. 2003).

History is important, since multi-cat households, shelters cats, outdoor cats or cats with a travelling history tend to be more susceptible and exposed to infectious diseases (Ettinger et al. 2024). Even though CR can affect cats of all ages, ranging from 6 months to 20 years (Henderson et al. 2003; Johnson 2020), younger animals tend to suffer congenital or infectious disease, while older cats are more prone to neoplasia or dental disease (Henderson et al. 2003; Kuehn 2006; Ettinger et al. 2024).

Physical examination is an important step in diagnosing nasal disease. The external nose should be examined to look for ulceration/crusting around the nares and depigmentation. Also, structural abnormalities and airflow impairment by palpating the palate and facial bones to detect pain, swelling, asymmetry, and other signs of bone lysis (Johnson 2020; Batalla et al. 2021; Ettinger et al. 2024). Airflow can be visualized by placing a cold glass or a metal utensil close to the nares to observe the condensation from the expired humid air (Ettinger et al. 2024). Gentle percussion on the rhinarium and sinus areas can identify masses, fluid buildup, or mucus. Periodontal disease should be ruled out, since it is a common cause of secondary bacterial rhinitis. Oronasal fistulas are also a contributing factor for nasal discharge or epistaxis. Examining the oropharynx can identify diseases in the posterior nares. This may be done in a conscious patient if they allow it but is more often observed during rhinoscopy (Kuehn 2006; Johnson 2020; Batalla et al. 2021; Ettinger et al. 2024).

### 2.2.3. Diagnosis

A detailed anamnesis and physical examination are not enough for a definitive diagnosis. According to Day et al. (2004), additional diagnostic methods, such as specific laboratory tests, advanced imaging techniques (i.e., CT scans), rhinoscopy, as well as histopathology and immunohistochemistry (IHC), are required for a successful diagnosis in over 90% of cases.

#### 2.2.3.1. Laboratory Analysis

As an initial approach, a complete blood workup [complete blood count (CBC) and chemistry profile] and urinalysis should be performed to rule out extranasal systemic causes. A CBC may reveal certain changes such as neutrophilia in acute inflammatory cases, eosinophilia in cases of allergy, and eosinophilia and basophilia in parasitic or fungal infections. Any signs of epistaxis should alert the clinician to thoroughly investigate systemic disease such as coagulation disorders or systemic hypertension (Batalla et al. 2021). A coagulation profile [platelet counts, coagulation timings (prothrombin time, aPTT, buccal mucosal breeding),

vitamin K] should also be included (Kuehn 2006; Nelson and Couto 2019; Johnson 2020; Batalla et al. 2021; Ettinger et al. 2024).If the cat has traveled to or lives in an endemic area, *Rickettsia*, *Ehrlichia*, *Leishmania*, and *Hepatozoon* should be tested for these pathogens (Ettinger et al. 2024).

Cytology samples of nasal discharge or nasal mucosa are nontraumatic (Raskin et al. 2021), and can be collected through impression smears of nasal biopsies, brushing, swabbing, or flushing of the nasal cavity (Nelson and Couto 2019; Johnson 2020; Batalla et al. 2021; Ettinger et al. 2024). These samples can also be used in bacterial and fungal cultures (Batalla et al. 2021; Ettinger et al. 2024). The results should be interpreted in the context of the animal's history and clinical signs, since, as mentioned, the presence of bacteria and fungi are not always indicative of infection (Nelson and Couto 2019; Johnson 2020; Batalla et al. 2021; Ettinger et al. 2024). In cases of cryptococcosis, the cytology of the exudate can be highly diagnostic, since it contains typical thick-walled PAS-positive round-to-oval yeasts with a thick capsule, corresponding to *Cryptococcus spp.* (Kuehn 2006; Raskin et al. 2021; Zachary 2022). Still, apart from few exceptions, biopsy is required for a definitive diagnosis, since the distinction between inflammatory or neoplastic conditions cannot always be made with cytology alone (Caniatti et al. 1998).

Since the nasal cavity is filled with commensal organisms, culture of superficial nasal mucosa often detects normal intranasal microbiota and is judged unnecessary. Because primary bacterial rhinitis is uncommon and the majority of infections are secondary and opportunistic, it can be difficult to interpret culture and antimicrobial susceptibility testing (AST). Therefore, unless a localized infection site is found, or culture is positive for *Mycoplasma felis* or *Bordetella bronchiseptica*, these tests are usually not recommended (Nelson and Couto 2019; Sykes 2022; Ettinger et al. 2024).

Swabs of the nasal cavity can be used to identify respiratory pathogens, such as FHV-1, *Chlamydophila felis* and *Mycoplasma felis* using PCR as the gold standard, while FCV is detected by rt-PCR (Nelson and Couto 2019; Sykes 2022; Ettinger et al. 2024). Testing for retroviruses can be done by ELISA, the gold standard screening test for FeLV antigen and the FIV antibody (Maggs et al. 1999; Little 2012; Ettinger et al. 2024). If cryptococcosis is suspected, nasal discharge samples should be tested through cytology and cryptococcal serology, especially when cats' lives or had travelled to an endemic area (Nelson and Couto 2019; Johnson 2020; Batalla et al. 2021; Ettinger et al. 2024). Fungal cultures are not recommended since presence can be transient and culture may be positive in clinically normal cats (Duncan et al. 2005).

#### 2.2.3.2. Diagnostic Imaging

Although it is widely available and inexpensive, nasal radiographs are not the ideal imaging technique, because it cannot detect subtle changes in the nasal cavity,

superimposition of structures or fluid may hide other abnormalities, resolution is poor, and overall radiographic appearance can be very similar among almost every nasal disease, since it varies depending on the chronicity and severity of the disease (Russo et al. 2000; Lamb et al. 2003; Holland and Hudson 2020; Batalla et al. 2021; Ettinger et al. 2024).

While research demonstrates that MRI can be used to differentiate between neoplastic and inflammatory nasal diseases, it is not currently considered the preferred advanced imaging technique for this purpose (Drees et al. 2009; Ettinger et al. 2024).

CT provides extremely precise cross-sectional images without overlap, making it possible to clearly identify anatomical limits. This enhances the accuracy of diagnosis by allowing the identification of diseases in regions that would otherwise be inaccessible for direct endoscopic viewing. It can also be helpful in planning biopsies and therapeutic interventions (e.g., to check cribriform plate integrity in cases of fungal rhinitis) (Schoenborn et al. 2003; Batalla et al. 2021; Ettinger et al. 2024). Although the diagnostic precision offered by CT scans often allows for quicker and more precise diagnosis, there are also disadvantages: it requires general anesthesia, accessibility may be restricted based on the location, and cost may be a barrier (Schoenborn et al. 2003; Batalla et al. 2021; Ettinger et al. 2024). When it comes to disease, CT makes it possible to identify different degrees of tissue damage. Inflammatory rhinitis is typically associated with normal to moderate levels of destruction, with or without concomitant soft tissue densities (Schoenborn et al. 2003; Little 2012; Holland and Hudson 2020; Batalla et al. 2021; Ettinger et al. 2024). The use of iodinated contrast can help differentiate vascularized soft tissue from fluid-filled areas, although it cannot identify the type of lesion (Schoenborn et al. 2003; Batalla et al. 2021; Ettinger et al. 2024). CT is mostly used in chronic inflammatory/infectious and fungal rhinitis (Holland and Hudson 2020).

#### 2.2.3.2.1. Rhinoscopy

In veterinary medicine, endoscopy of the respiratory tract is a crucial diagnostic and treatment technique. This procedure allows an easier detailed examination of the airways, including the nasal passages, nasopharynx, pharynx, larynx, trachea, and bronchi, as well as an evaluation of laryngeal function. The gold standard for diagnosing intranasal disease is rhinoscopy, which offers minimally invasive access for examining nose lesions as well as collecting biopsy and fluid samples for culture, cytological, histological and immunohistochemical tests, which are essential for a definitive diagnosis and long-term monitoring of chronic changes or airway damage (Batalla et al. 2021; Ettinger et al. 2024).

Rhinoscopy is typically chosen over surgical techniques, such as dorsal or ventral rhinotomy, due to its lower morbidity and capacity to directly visualize lesions and nasal tumors, remove foreign bodies, and even treat conditions like nasal cryptococcosis by debriding fungal plaques and administering antifungal treatments, or removing obstructions and/or secretions (Batalla et al. 2021; Ettinger et al. 2024).

Both caudal and rostral rhinoscopy approaches are performed in a complete examination of the nasal cavity. Caudal rhinoscopy is usually performed first because it provides clean biopsy sampling by allowing access to the nasopharynx and choanae without the need for fluid irrigation. It is performed *per os* (PO) by retroflexing a flexible endoscope. During rostral rhinoscopy, fluid irrigation is typically necessary for a clear view. However, if using cold saline, the mucosa may be a bit more blanched than usual. The operator must begin with the normal or less affected side to not contaminate a healthy side. With rostral rhinoscopy, the nasal meatus, the frontal sinuses with their scrolls of turbinates and the cribriform plate can be visualized. The endoscope should enter the nasopharynx to search for the Eustachian tubes. The normal mucosa appears pink/red with no discharge at the pharyngeal and retropharyngeal level. However, when the animal has nasal disease, it often becomes hyperemic, irregular, or friable, and may show visible masses along with mucopurulent or hemorrhagic discharge. In animals that have been sneezing for long periods of time or in cases where chronic inflammation is present, it is common to observe raised nodules of benign lymphoid hyperplasia. Hemorrhage in the presence of little or no trauma from the endoscope, as well as narrowing of the meatuses are signs of inflammation. Enlarged airways, due to the destruction of the turbinates or other bone structures or ulcerative lesions can be seen in bacterial or fungal rhinitis (Johnson 2020; Batalla et al. 2021).

Although hemorrhage is a frequent side effect of rhinoscopy, it usually resolves in a few minutes to hours. Some cases may require postoperative hospitalization in order to monitor and control bleeding. Owners should be advised of the possibility of nasal discharge and temporary breathing difficulties caused by mucosal swelling. Additionally, if bleeding persists, nasal packing, cold saline, or irrigants with adrenaline may help control the bleeding (Batalla et al. 2021; Ettinger et al. 2024).

#### 2.2.3.3. Nasal Biopsy

Biopsies can be obtained through various methods: brushing, nasal flushing, blind biopsies, and rhinoscopy-guided biopsies. Each approach offers different levels of detail and should be chosen depending on case requirements and equipment available.

Several biopsy samples from each nasal cavity should be taken, even if clinical signs are unilateral or obvious (e.g., when there is a foreign body or no visible lesion is observed), and any masses or abnormal tissues should be sampled (Batalla et al. 2021; Ettinger et al. 2024).

Brushes and swabs may be collected from the airway surfaces for bacterial culture or cytology. Also, flush samples can be collected using thin aspiration catheters inserted in the endoscope's instrument channel, to collect sterile fluid samples. It is important to note that before starting a rostral rhinoscopy, swabs from the nasopharynx, choanae, and nares must

be obtained, since the irrigation fluid used during the procedure may contaminate the sampling field by removing infectious material or debris (Batalla et al. 2021; Ettinger et al. 2024).

For some lesions, especially those in retropharyngeal regions, alternative approaches, like vigorous flushing procedures, may be applied. Any tissue that becomes dislodged can be collected from the oral cavity, the nares, or the gauze packing after sterile saline is manually pushed through the catheter at high pressure using a syringe (Batalla et al. 2021; Ettinger et al. 2024).

Biopsy samples should be taken after the observation part of the rhinoscopy is finished, since hemorrhage will difficult viewing. To obtain representative biopsy samples, these should be collected from various locations, preferably from both normal and abnormal tissue and polyps or masses (Johnson et al. 2005; Batalla et al. 2021; Ettinger et al. 2024), as correlation for the presence or absence of rhinoscopy abnormalities and histologic evidence of inflammation is weak (Johnson et al. 2004; Johnson et al. 2005). A flexible biopsy forceps is introduced via the instrument channel of the endoscope; if the animal is too small, samples should be collected blindly. In this case it is necessary to measure, on the outside, the distance from the rhinarium to the medial canthus of the eyes, to prevent trauma of the cribriform plate and frontal lobes. A CT scan can also help determine the precise depth at which the biopsy equipment should be placed to reach the affected area (Johnson et al. 2005; Batalla et al. 2021; Ettinger et al. 2024). While the biopsy site may not be directly visualized while collecting, blind biopsies are larger because bigger biopsy/grasping forceps can be used (Johnson et al. 2005; Batalla et al. 2021). When collecting the samples, the operator must be assertive in order to take deeper tissue samples, because superficial biopsies usually are inconclusive or reveal only inflammation (Johnson 2020; Batalla et al. 2021).

Samples may be used for aerobic and anaerobic bacterial cultures, PCR for molecular identification, cytology for cellular analysis, histopathology for detailed tissue examination, and IHC to detect specific cellular markers. Each of these tests contributes with valuable information for an accurate diagnosis, helping to clarify the underlying conditions (Kuehn 2006; Batalla et al. 2021; Ettinger et al. 2024).

#### 2.2.3.4. Histopathology and Immunohistochemistry

When paired with imaging, clinical data, and patient history, histopathologic analysis of nasal biopsies provides important diagnostic information that helps achieve a definitive diagnosis and guide toward the right treatment. Careful analysis of high-quality samples is necessary since the underlying cellular structure and function have a major role in the reported histopathologic changes (Raskin et al. 2021).

Normal architecture of the nasal cavity consists in a ciliated, pseudostratified columnar epithelium, with serous, mucous, and mixed tubuloalveolar glands, rostrally, and olfactory glands caudally. In the submucosa, MALT and lymphoid follicles can be found (Raskin et al.

2021). Histological examination of nasal tissue may occasionally reveal moderate to severe inflammation, even when the tissue looks generally normal on visual inspection during rhinoscopy (Johnson et al. 2004).

When chronic inflammation is present, various adaptive responses are activated, and histological features such as epithelial erosion, turbinate lysis or remodeling, fibrosis, and necrosis may be seen (Harvey and Tasker 2013; Raskin et al. 2021).

In CR cases, some reversible changes of the structure and integrity and function of normal cells may occur, such as hyperplasia, dysplasia, or metaplasia. Hyperplasia, an increase in cell number, is often accompanied by dysplasia, which is a disorganization of tissue architecture, making it difficult to evaluate the tissue histologically. Clusters and layers of epithelial cells with a higher nucleus:cytoplasm ratio and cytoplasmic basophilia, mild to moderate anisocytosis, and normally structured mitotic figures, are more common in these cases. Metaplasia is another adaptive cellular mechanism to survive a chronic insult; though some specialized functions can be lost in the process, such as the ability to produce mucus. It consists in a transformation of the normal respiratory epithelium into a squamous type. Histologically, basal cells are usually organized in clusters, while more keratinized squamous cells tend to appear individually; these typically present angular borders, and a hyalinized or basophilic cytoplasm. Nuclei are small and sometimes pyknotic or karyorrhectic, because the cells are damaged and under distress (Raskin et al. 2021).

Different inflammatory infiltrates can be present on histopathology of CR cases, like primarily neutrophilic, lymphoplasmacytic, or mixed infiltrates (Harvey and Tasker 2013). Other inflammatory cells, such as macrophages, mast cells and globular leukocytes can also be present (Johnson et al. 2005). A normal mucosa usually presents no more than 20 inflammatory cells in each slide, yet inflammatory infiltration is categorized as mild when a small number is diffusely distributed; moderate when a prominent number is present, although diffusely distributed as well; and severe when the tissue is completely infiltrated by inflammatory cells (Johnson et al. 2005).

Due to the various etiologies of CR, some cases cannot be diagnosed by histopathology only. However, some findings may help narrow down the list of differential diagnoses. In the case of foreign bodies, suppurative to pyogranulomatous inflammation is present, accompanied by hemorrhage and secondary bacterial infection. Sometimes pieces of foreign material can also be seen. In viral rhinitis, nonspecific inflammation with various numbers and types of cells is present, and rarely viral inclusions can be observed within the epithelial cells. In bacterial rhinitis, suppurative inflammation is extremely evident, that is accompanied by a high number of bacteria individualized or phagocytized; however, due to abundant mucus, the identification of bacteria can be difficult. In fungal rhinitis, eosinophilic inflammation it typically present, though it can range from a few to no inflammatory cells to robust pyogranulomatous inflammation. In allergic rhinitis, eosinophils are predominant since

this is a type I hypersensitivity reaction. Hyperplasia of goblet cells, a few neutrophils and occasional mast cells and plasma cells, may be seen as well (Raskin et al. 2021), when chronic, a mixed inflammatory response can occur (Nelson and Couto 2019).

In FCR, moderate to severe neutrophilic (acute), lymphocytic (chronic), or pleiocellular inflammation is expected (Kuehn 2006; Johnson 2020), as well as epithelial hyperplasia and ulceration, turbinate destruction and remodeling, fibrosis, necrosis, and glandular hyperplasia. Although it can be on both sides, it usually is a unilateral finding (Kuehn 2006). In FLR, there is evident infiltration of lymphocytes and plasma cells in the lamina propria, and neutrophilic inflammation on the epithelium, that is usually degenerated (Kuehn 2006; Day 2011). The absence of pathogens is characteristic of this condition (Kuehn 2006).

IHC is also a diagnostic method that can be applied in cases of CR, as will be discussed further ahead in Chapter 2.4. Immunohistochemistry.

### 2.2.4. Treatment

Although treatment is often empirical, based on clinical signs and/or cytology or histopathology results (Hawkins 1988; Michiels et al. 2003), it should be chosen according to the etiology and clinical signs presented by the patient. Due to the chronicity of this disease, lifelong management is usually necessary (Van Pelt and Lappin 1994; Nelson and Couto 2019; Ettinget al. 2024).

Supportive care is crucial, since these animals can be dehydrated and anorexic, especially severely affected cats. Warmed portions of strong-smelling foods should be given to these patients, due to the lack of a sense of smell, as well as appetite stimulants (e.g., mirtazapine) when necessary, and subcutaneous (SC) fluids in dehydrated cats (Van Pelt and Lappin 1994; Nelson and Couto 2019; Ettinger et al. 2024). Smoke (e.g., tobacco, fireplace) and perfumed products should be avoided to reduce irritants, and owners should be motivated to improve air quality in their homes (e.g., using an air cleaner) (Ettinger et al. 2024).

Even though they do not treat the cause, nasal flushing, mucolytics and decongestants are useful to facilitate drainage of discharge, while cleaning the nasal cavity and improving breathing. Nasal flushing can be done during rhinoscopy or at home with the aid of a syringe and saline solution (Kuehn 2006). Nasal flushing at home can lead to distress, so shower steam inhalation or nebulization with a saline solution for 10-15 minutes twice a day, may be a good option to help reduce the viscosity of secretions (Van Pelt and Lappin 1994; Ettinger et al. 2024). In cats with thick and occlusive nasal discharge, mucolytics can be an option, such as nebulization with acetylcysteine (Van Pelt and Lappin 1994). Topical decongestants, such as 0.25% phenylephrine or 0.025% oxymetazoline, can improve nasal discharge and congestion, however not all cats allow application of intranasal medication (Van Pelt and Lappin 1994; Nelson and Couto 2019).

For viral rhinitis, treatment is mainly supportive care and resolution of secondary bacterial infections (Van Pelt and Lappin 1994). In cats with FCR, antiviral therapy is not routinely recommended, since the role of active viral infection is not yet established. Lysine (500mg, PO, q12h) may be beneficial for herpes-viral conjunctivitis and may reduce reactivated shedding by latently infected cats (Sykes 2022); however, some studies had also shown that this amino acid can have the opposite effect and increase viral replication (Maggs et al. 2000; Bol and Bunnik 2015). Immune stimulators, like interferon, can be used, although with varying efficacy (Johnson 2003; Nelson and Couto 2019). Some studies have shown improvement of clinical signs and prolonged survival times in cats treated with human interferon-α (10 IU/kg) and feline recombinant interferon [$10^6$ U/kg, SC, q24h; for 5 days in three series (starting on days 0, 14, and 60)] (Mari et al. 2004; Pedretti et al. 2006). It can also be used in cats infected with FIV and FeLV due to their immunomodulatory effect (Nelson and Couto 2019). BRMs, such as Acemannan, can also be used in FIV-positive cats (August 1991; Cotter 1992; Macy 2020). Famciclovir was shown to be effective in cats with active FHV-1 infection (Nelson and Couto 2019). According to Thomasy et al. (2016) a dosage of 90mg/kg q8h significantly reduced clinical signs.

Although primary bacterial rhinitis is uncommon, antibiotics are usually used to treat secondary infections, which may improve clinical signs (Ettinger et al. 2024). Antibiotic selection should ideally be based on culture and AST results from nasal biopsy samples or specimens obtained through deep nasal flush or biopsy (Kuehn 2006). International Society for Companion Animal Infectious Diseases (ISCAID) guidelines recommend that the first-line antimicrobial option should be doxycycline (5mg/kg, PO, q12h; or 10 mg/kg, PO, q24h) - especially if the cat is positive for *Chlamydophila felis, Mycoplasma felis* and/or *Bordetella bronchiseptica* - or amoxicillin-clavulanate (12.5mg/kg, PO, q12h) (Lappin et al. 2017). Other options include azithromycin (5-10 mg/kg, PO, q12h for 1 day and then q3 days), fluoroquinolones, such as enrofloxacin (5mg/kg, PO, q24h) and marbofloxacin (2.7-5.5mg/kg, PO, q24h), and clindamycin (10-15mg/kg, PO, SC, q24h) (Kuehn 2006; Lappin et al. 2017). Along with their antibacterial effect, azithromycin (Nelson and Couto 2019) and doxycycline (Gabler and Creamer 1991)also have immunomodulatory effects (Kuehn 2006; Nelson and Couto 2019). The chosen antimicrobial should be administered for at least seven days, for as long as there is clinical improvement and for at least one week past resolution of clinical signs or plateau in response to treatment (Lappin et al. 2017). In cats with FCR, if clinical signs minimize within the first week of antibiotics, it should be continued at least for 4 to 6 weeks (Kuehn 2006; Nelson and Couto 2019). Otherwise, it must be ceased and a new antibiotic started, or underlying causes should be explored (Nelson and Couto 2019). If the cat relapses shortly after discontinuation, a continuous long-term treatment must be equated, for at least 7-10 days, usually with the previously effective antimicrobial agent (Lappin et al. 2017; Nelson and Couto 2019). In cats with conjunctivitis due to *Chlamydophila felis*, chloramphenicol or

tetracycline ophthalmic ointment should be applied for a minimum of 14 days after signs have resolved, at least three times a day (Nelson and Couto 2019).

For fungal rhinitis, the combination of amphotericin B and flucytosine has been recommended. Other agents, such as fluconazole (50mg, PO, q12h), ketoconazole (10mg/kg, q12), itraconazole (10mg/kg, q24), were also reported as successful (Van Pelt and Lappin 1994). Intranasal infusion with a topical antifungal agent (e.g. clotrimazole), prior to the debridement of fungal plaques, can be done with the animal anesthetized (Ettinger et al. 2024). Treatment should be continued for at least 1 to 2 months past resolution of clinical signs (Nelson and Couto 2019).

Although discussable, as mentioned before, antihistamines can be used in feline allergic rhinitis. Some successful cases of cats with FCR treated with cetirizine have been reported (Nelson and Couto 2019).

From corticosteroids to nonsteroidal anti-inflammatory drugs (NSAIDs), anti-inflammatory drugs play a major role in treating cats with CR, used to alleviate clinical signs while reducing chronic inflammation. Corticosteroids can even be used chronically due to the nature of these illnesses, like in FCR, FLR and allergic rhinitis. They can be administered orally, inhaled (Nelson and Couto 2019), or applied topically, to improve nasal discharge and swelling, and therefore improve olfaction and appetite (Van Pelt and Lappin 1994). They are usually prescribed in allergic rhinitis, when antihistamines do not work (Nelson and Couto 2019). In viral rhinitis, their usage is controversial, since they can increase viral shedding and predispose to bacterial infections (Van Pelt and Lappin 1994; Kuehn 2006; Nelson and Couto 2019); however, they significantly reduce nasal inflammation and, consequently, clinical signs (Van Pelt and Lappin 1994). Corticosteroid therapy during fungal infections worsens the symptoms as well as disease progression (Sykes 2022). NSAIDs, like Piroxicam (0,3mg/kg, PO, q24-48h) can be an alternative to corticosteroids; however, prolonged use is not recommended (Kuehn 2006; Nelson and Couto 2019). Oher drugs like leukotriene inhibitors, and supplementation with omega-3 fatty acid can also help reduce inflammation (Nelson and Couto 2019).

#### 2.2.4.1. Vaccines

Some of the pathogens referred earlier can be prevented with the use of vaccines, although it does not prevent infection it decreases the severity of the disease (Van Pelt and Lappin 1994).

Respiratory illnesses brought on by FHV1 and FCV continue to be a major clinical concern even with the extensive use of vaccines (Ettinger et al. 2024). A killed vaccine containing immunogens from two FIV isolates is licensed for use in some countries, although its efficacy is questioned (Nelson and Couto 2019). Three FeLV vaccines are available across the world, with equal efficacy, and should be given to cats that are considered at high risk (e.g.,

outdoor cats, multi-cat household), and were not yet exposed to FeLV, since cats with persistent viremia and latent infections do not benefit from vaccination. The efficacy of this vaccine ranges from 0% to 100% (Legendre et al. 1991; Nelson and Couto 2019; Macy 2020). FeLV vaccine is also associated with the development of fibrosarcoma in some cats (Nelson and Couto 2019). *Chlamydophila felis* and *Bordetella* vaccination is only recommended for cats in catteries, shelters or multi-cat household (Nelson and Couto 2019).

#### 2.2.4.2. Surgery

Besides medical treatment, surgery like turbinectomy and frontal sinus ablation can be an option in cats with severe or deteriorating signs and with FCR that are difficult to manage medically, although results are usually poor. Clinical signs are not expected to disappear completely, since sneezing and nasal discharge will not be resolved with these procedures, but to be more easily managed (Johnson 2003; Nelson and Couto 2019). It is necessary to steer clear of major blood vessels and the cranial vault, and to ensure that no tissue remnants are left behind. Esophagostomy or gastrostomy tube placement must be considered since anorexia can surge in the postoperative (Nelson and Couto 2019) and become persistent due to the definitive loss of olfaction (Johnson 2003).

### 2.2.5. Prognosis

The underlying cause of CR influences the severity of disease, and therefore its response to treatment and prognosis.

As for viral CR, most cats become chronic carriers of these viruses and lifelong management is required. Cats with FHV-1 and FCV are persistently infected and may have intermittent and chronic signs, that can be managed with treatment. FIV-positive cats can survive for years after diagnosis, and according to Liem et al. (2013), there is no difference in the survival time between cats infected with FIV and those not infected. However, FeLV co-infection showed a significant shorter life span (Spada et al. 2018). FeLV-positive cats with persistent viremia have a guarded prognosis, and the majority die within 2 to 3 years after diagnosis (Nelson and Couto 2019).

In bacterial CR, prognosis is usually good if response to antibiotic therapy is positive, but the outcome depends on identification and resolution of the underlying disease (Nelson and Couto 2019).

As for fungal CR, cure is usually achieved with treatment, unless CNS or ocular disease is present, in which case the animals are less likely to respond (Nelson and Couto 2019). Still, one study showed that 32% of cats with CNS cryptococcosis, survived for more than 6 months with treatment (Sykes et al. 2010).

Curative treatment of allergic rhinitis is questionable, but there is a positive outlook for achieving control with long-term medication (Nelson and Couto 2019).

Due to its difficult management, FCR prognosis is extremely guarded. Clinical signs and secondary bacterial infections can be controlled; however, it tends to be temporary since there is a high probability of recurrence (Kuehn 2006).

## 2.3. Nasal Lymphoma

Lymphoma is one of the most common tumors diagnosed in cats (Vail et al. 2020), representing more than 50% of all feline tumors (Jacobs et al. 2016). Lymphoma can be classified as multicentric, thymic, mediastinal, digestive, splenic, cutaneous and subcutaneous, renal, tonsillar, cardiac, nervous, ocular, nasal and nasopharyngeal, and osseous (Jacobs et al. 2002; Vail and Thamm 2005). Although they can appear in almost any tissue, they usually develop in lymphoid tissues such as the spleen, and lymph nodes, and originate from lymphoid cells (Vail et al. 2020).

A heterogeneous tumor has a higher risk of metastasis and therapy failure. Tumor development, and at times, inhibition of immune cell function, are often led by the dysregulated network of cytokines and growth factors that come from cancer and stromal tumor microenvironment cells. These result from phosphorylation and activation of signal transducers and activators of transcription (STAT) proteins, that have the potential to induce changes in gene expression specific to the cell type (Mroz et al. 2013; Polak et al. 2019).

Both B and T cells can suffer neoplastic transformation at nearly any stage in their maturation process. It is possible to distinguish cells within a lymphoid tumor based on their phenotype, defined by the surface antigens; for example, presence of CD20 identifies B-cells, while CD3 indicates T-cells (Tizard 2017).

Nasal lymphoma (NL), which includes nasopharyngeal and sinonasal regions, is categorized as an extranodal form of lymphoma, and is the second most common type after gastrointestinal lymphoma (Taylor et al. 2009). Older cats are particularly affected (median age 10.3 years; range 1–16 years) (Santagostino et al. 2015), a preponderance of B-cell immunophenotypes is seen in roughly 75% of cases, and a 2:1 male-to-female ratio has also been noted, probably because of its behavioral characteristics which makes transmission of FeLV more efficient (Vail et al. 1998; Kuehn 2006; Little et al. 2007; Haney et al. 2009; Taylor et al. 2009; Santagostino et al. 2015; Vail and Pinkerton 2020).

Environmental factors, such as exposure to tobacco smoke, influences the development of lymphoma (Denson 2003; Vail and Pinkerton 2020), alongside immunosuppression and FIV-associated-immunosuppression (Shelton et al. 1990; Hutson et al. 1991; Endo et al. 1997; Beatty et al. 2002; Vail and Pinkerton 2020). Chronic inflammation has also been found to be an important contributory factor, especially for intestine and nasal lymphomas (Carreras et al. 2003; Louwerens et al. 2005; Vail and Pinkerton 2020). In human medicine, it is known that bacterial and viral infection are related to the development of MALT B-cell lymphomas (Ferreri et al. 2009; Grivennikov et al. 2010; Sadrzadeh et al. 2012), and

that chronic inflammation is also considered a risk factor for developing B-cell lymphoma (Copie-Bergman et al. 1997; Petitjean et al. 2002; Swerdlow et al. 2016). In a clinical study, it was noted that inflammation and FeLV tissue antigen positivity was present in 31% cats (Santagostino et al. 2015), which corroborates the hypothesis that CR can lead for expansion of virally transformed B-lymphoid cells, explaining why B-cell phenotype has a much higher frequency among upper respiratory tract lymphomas (Mukaratirwa et al. 2001; Day et al. 2004; Little et al. 2007).

FeLV and FIV are two of the many contributing factors to the multifactorial etiology of lymphoma in cats (Macy 2020; Vail and Pinkerton 2020). These viruses may have a tumorigenic potential at the early phase of lymphoid differentiation (Chino et al. 2013), especially FeLV, which is prone to infect dividing cells (Fujino et al. 2008).

Clinical signs are similar to those observed in CR, including nasal discharge (60%–85%), usually mucopurulent, sneezing (20%–70%), upper respiratory noises (including stridor, stertor, and wheezing; 20%–60%), facial deformities (0%–20%), hyporexia (10%–60%), and ocular discharge (10%–30%). Less common clinical signs include ulceration and depigmentation of nasal planum, dyspnea, epistaxis, cough, and regional lymphadenopathy (Little et al. 2007; Haney et al. 2009; Taylor et al. 2009; Santagostino et al. 2015; Vail and Pinkerton 2020; Ettinger et al. 2024). Central nervous system dysfunction can occur due to cerebral invasion by nasal tumors (Ettinger et al. 2024). The time between development of clinical signs and diagnosis is 1 to 1800 days, with a median of about 2 months (Little et al. 2007; Haney et al. 2009; Taylor et al. 2009; Santagostino et al. 2015; Vail and Pinkerton 2020). Affected cats may seem clinically healthy when the disease is limited to the primary site (stage I), while nonspecific systemic symptoms like anorexia, weight loss, lethargy, or depression can also be observed (Savary et al. 2000; Vail and Pinkerton 2020). Although uncommon, secondary bone marrow infiltration can cause anemia and symptoms of paraneoplastic hypercalcemia, such as polyuria or polydipsia, may occur as well (Savary et al. 2000; Vail and Pinkerton 2020).

Due to the similarity of presentation between CR and NL, diagnostic procedures must follow the same protocol as for CR to obtain a definitive diagnosis. It is necessary to systematically exclude all possible causes to accurately identify the underlying condition. Therefore, it is important to do a comprehensive physical exam and a complete blood work (Gerou-Ferriani et al. 2011; Vail and Pinkerton 2020), as detailed previously in Chapter 2.2.2. Patient History, Clinical Signs and Physical Examination.

Advanced imaging methods like CT scan, in addition to rhinoscopy and biopsy (flush, blind or rhinoscopy-guided), are generally required. In addition to determining the extent of the disease, CT helps in choosing the appropriate treatment, like radiation therapy or chemotherapy. When performed previously to rhinoscopy, they help planning biopsy. Unilateral or bilateral nasal/sinus mass or fluid, bulla effusion, areas of soft tissue density,

septal deviation, and lysis of surrounding bone structures and nasal turbinates are the usual CT findings in NL cases (Detweiler et al. 2006; Tromblee et al. 2006; Nemanic et al. 2015; Santagostino et al. 2015; Holland and Hudson 2020; Vail and Pinkerton 2020; Ettinger et al. 2024). In felines, distinguishing between nasal neoplasia and fungal rhinitis can be challenging due to overlapping radiographic features, since in fungal rhinitis there is also turbinate destruction and hyperlucency of the nasal passages (Schoenborn et al. 2003; Karnik et al. 2009; Ettinger et al. 2024).

As previously mentioned, radiographs are not the preferred imaging method. However, when performed, it is necessary to take into account that destruction of conchae can be a finding of both CR and NL, but, when associated with soft tissue swelling, invasion of the surrounding bone structures and ipsilateral sinus opacity it is more likely to be a nasal neoplasia (Russo et al. 2000; Lamb et al. 2003; Ettinger et al. 2024). MRI is not currently considered the preferred diagnostic imaging method, as highlighted earlier. More than being an expensive technique, it is not as accessible as CT (Drees et al. 2009; Ettinger et al. 2024).

Rhinoscopy findings are similar to those reported in CR, like hyperemic, irregular, or friable mucosa, visible masses, mucopurulent or hemorrhagic discharge and destruction of bone structures (Johnson 2020; Batalla et al. 2021). During rhinoscopy, Santagostino et al. (2015) describes NL as space-occupying exophytic lesions, pale pink to whitish, with cerebroid appearance, and friable; in 28% of cases there were no masses visible, although the mucosa was thickened (Santagostino et al. 2015).

For a conclusive diagnosis, cytopathologic or histopathologic evaluation of lymph node(s) or affected tissue by fine-needle aspiration (FNA), or by surgical or endoscopic biopsy is necessary. Incisional or excisional biopsy may be required to provide guidance and information regarding invasiveness and architectural abnormalities, since cytology alone may not always be adequate (Caniatti et al. 1998; Moore et al. 2005; Werner et al. 2005; Henrich et al. 2009; Weiss et al. 2011; Hammer et al. 2017; Martini et al. 2018; Vail and Pinkerton 2020). According to Santagostino et al. (2015), lymphoplasmacytic rhinitis can be misdiagnosed as NL by cytology, and the likelihood of diagnosis NL correctly on cytology is low. This occurs probably because most lymphomas develop in mid to deep lamina propria, and cytology samples are collected with swabs or brushes of the superficial mucosa (Santagostino et al. 2015).

To further characterize the disease and improve diagnosis in cases that are unclear, biopsies can also be submitted to histochemical and immunohistochemical analyses (including immunophenotypic evaluations), flow cytometry, and molecular techniques (e.g., PCR for Antigen Receptor Rearrangement [PARR]) (Moore et al. 2005; Werner et al. 2005; Henrich et al. 2009; Weiss et al. 2011; Hammer et al. 2017; Martini et al. 2018; Vail and Pinkerton 2020).

Histologically, NL is characterized by a monomorphic population of medium to large-sized immature lymphoid cells. These have scarce, deeply basophilic cytoplasm and a large

round nucleus with finely granular chromatin and one or multiple nucleoli (Raskin et al. 2021). NL can be mistaken for other diseases, like lymphoid hyperplasia or an inflammatory polyp. In lymphoid hyperplasia, there is a heterogeneous population of lymphocytes and plasma cells, with fewer intermediate and large lymphoid cells and a higher proportion of small, mature lymphocytes. A preponderance of intermediate-sized lymphocytes with more cytoplasm and smooth chromatin devoid of nucleoli can also be a sign of NL. Cases where the neoplastic population is composed of small, well-differentiated lymphocytes are even more challenging (Raskin et al. 2021). The nasal mucosa can present hyperplasia and dysplasia, suggesting early neoplastic changes (Santagostino et al. 2015; Raskin et al. 2021), and NL growth can be diffuse or follicular (Santagostino et al. 2015). Other histopathological findings include multifocal mucosa ulceration, secondary neutrophilic and catarrhal inflammation, cystic mucous glands, edema, fibroplasia, and bone remodeling (Santagostino et al. 2015).

As mentioned, IHC is also a diagnostic method that can be applied in cases of NL, as will be discussed further ahead. Many cases of severe lymphoplasmacytic rhinitis can progress to NL; it is likely that at the time of the diagnosis, these cases are either in a preneoplastic or in an early neoplastic stage of disease (Kuehn 2006; Santagostino et al. 2015). So, for cats exhibiting severe lymphoplasmacytic inflammation, immunohistochemical staining targeting B-cell and T-cell markers is recommended to effectively rule out NL (Kuehn 2006). A phenotypic evaluation has a significant prognostic role for NL, since T-cell phenotype as poor response to chemotherapy and higher relapse rates (Lei et al. 1999).

Staging (Table 2 and 3) is required when it comes to deciding which treatment is more appropriate and provides prognostic information. It includes bone marrow aspiration or biopsy, peripheral lymph node aspiration (clinically normal or abnormal nodes), and thoracic and/or abdominal imaging.

**Table 2 – Classification of Lymphoma in Domestic Animals According to the World Health Organization's Clinical Staging System (adapted from** (Vail and Pinkerton 2020)**.**

| World Health Organization's Clinical Staging System for Lymphoma in Domestic Animals |
|---|
| 1. Anatomic site |
| A. Generalized |
| B. Digestive |
| C. Thymic |
| D. Skin |
| E. Others (including solitary renal) |
| 2. Stage (to include anatomic site) |
| I. Involvement limited to a single node or lymphoid tissue in a single organ[1] |
| II. Involvement of many lymph nodes in a regional area (± tonsils) |
| III. Generalizes lymph node involvement |
| IV. Liver and/or spleen involvement (± Stage III) |
| V. Manifestations in the blood and involvement of bone marrow and/or other organ systems (± Stage I-IV) |
| Each stage is subclassified into: |
| a. Without systemic signs |
| b. With systemic signs |
| [1] – Excluding bone marrow. |

**Table 3 – Clinical Staging System for Feline Lymphoma Developed to Address the High Rate of Visceral/Extranodal Involvement in Cats, Providing a More Tailored Approach than the WHO System (adapted from** (Mooney and Hayes 1986)**.**

| Clinical Staging System for Feline Lymphoma |
|---|
| **Stage 1** |
| ▪ A single tumor (extranodal) or a single anatomic area (nodal) |
| ▪ Includes primary intrathoracic tumors |
| **Stage 2** |
| ▪ A single tumor (extranodal) with regional lymph nodes involvement |
| ▪ Two or more nodal areas on the same side of the diaphragm |
| ▪ Two single (extranidal) tumors with or without regional lymph node involvement of the same side of the diaphragm |
| ▪ A resectable primary gastrointestinal tract tumor, usually in the ileocecal area, with or without involvement of associated mesenteric nodes only |
| **Stage 3** |
| ▪ Two single tumors (extranodal) on opposite sides of the diaphragm |
| ▪ Two or more nodal areas above and below the diaphragm |
| ▪ All extensive primary unresectable intraabdominal disease |
| ▪ All paraspinal or epidural tumors, regardless of the other tumor site or sites |
| **Stage 4** |
| ▪ Stages 1-3 with liver and/or spleen involvement |
| **Stage 5** |
| ▪ Stages 1-4 with the involvement of CNS or bone marrow or both |

When it comes to treatment, staging guides the decision of whether to do locoregional therapy (e.g., surgery and/or radiotherapy) - when solitary site disease is suspected (stage I) - or systemic therapy (e.g., chemotherapy) (Mooney and Hayes 1986; Vail and Pinkerton 2020). Stage I usually requires local radiotherapy without the need for systemic chemotherapy (Nagata et al. 2014; Vail and Pinkerton 2020), with complete remission rates of 75% to 95% and median survival time of 1.5 to 3 years (Elmslie et al. 1991; Haney et al. 2009; Vail and Pinkerton 2020), and 4.5 months for those whose complete remission was not achieved (Elmslie et al. 1991; Sfiligoi et al. 2007; Fujiwara-Igarashi et al. 2014; Vail and Pinkerton 2020). However, reports indicate that untreated cats only survived for 53 days (ranging from 0 to 301 days) (Santagostino et al. 2015; Vail and Pinkerton 2020). Chemotherapy protocols, such as COP (Cyclophosphamide, Oncovin, Prednisone) or CHOP (Cyclophosphamide, Hydroxydaunorubicin, Oncovin, Prednisone), are alternatives to radiotherapy, with 75% of cases achieving complete remission and 2 years of survival rate. Chemotherapy is usually used for situations in which the disease is not confined to the nasal cavity, when radiotherapy is not available or declined, or in circumstances where the disease recurs after radiotherapy (Taylor et al. 2009; Vail and Pinkerton 2020).

Other therapeutic options are being studied. Immunotherapy studies have been performed in canine lymphoma, using monoclonal antibodies and lymphoma anti-CD20 vaccines, and small molecules (e.g., idelalisib), which may be an option in the future. However, further studies must be performed, applied to feline lymphoma (Thamm 2019).

With a good to fair prognosis, and an indolent biological behavior (Fujino et al. 2009; Ahmad and Levy 2010; Fujino et al. 2010; Weiss et al. 2010; Stützer et al. 2011; Vail and Pinkerton 2020), this type of lymphoma usually manifests as a localized disease that is not likely to metastasize, since only 20% of patients present local extension or distant metastasis at necropsy (Zwahlen et al. 1998; Vail and Pinkerton 2020). According to Santagostino et al. (2015), there are some prognostic factors to consider, such as epitheliotropism, that is a positive prognostic factor and usually associated with B-cell NL; younger cats or cats older than 10 years usually have a negative prognostic.

## 2.4. Immunohistochemistry

IHC is a very useful diagnostic method in veterinary medicine, enabling characterization of tissue samples through antigen-antibody reactions. It serves multiple purposes, ranging from diagnosing diseases to guiding treatment strategies (therapeutic responsiveness), as well as providing prognosis (biologic aggressiveness) (Cirstoiu-Hapca et al. 2007; Vail et al. 2020; Kieslinger et al. 2021).

It is a staining technique that uses commercial antibodies to identify specific cellular and extracellular molecules, like cytoplasmatic intermediate filaments, cell surface markers, and secretory substances (Vail et al. 2020). It can also detect pathogens in tissue samples,

such as viruses (e.g., p27 and gp70 for FeLV; FIV) and bacteria (Nelson and Couto 2019; Sykes 2022).(Nelson and Couto 2019; Sykes 2022). For more reliable results, it is recommended to use a panel of IHC stains with specific immunopositive and immunonegative markers for each type of marker (Vail et al. 2020).

Lymphocytes may appear morphologically similar; however, there are various subpopulations distinguishable by their phenotype, according to molecules present on the cell surface, and biological behavior (Tizard 2017). The type of cells present in NL can be determined by immunophenotyping, using IHC (Vail et al. 2020), and surface markers can be used to distinguish diseases from one another (e.g. severe lymphoplasmacytic rhinitis versus NL, T-cell versus B-cell lymphoma) (Kuehn 2006; Chino et al. 2013), as well as to classify some tumors (e.g. CD20/Pax5 for B-cell lymphomas, CD3 for T-cell lymphomas). A lymphoplasmacytic heterogenous population usually supports the diagnosis of inflammation, while in lymphoma the lymphoid population is usually monomorphic. With IHC, immunophenotyping can be done in order to identify expression of molecules specific of B-cells (e.g., CD20, Pax5, CD79a) and T-cells (e.g., CD3, CD4, CD8) (Vail et al. 2020). The phenotypic characterization of a tumor can also lead to the development of targeted treatments, like rituximab, an anti-CD20 monoclonal antibody used in human medicine (Thamm 2019). This diagnostic technique allows the study of the tumor microenvironment and the expression of immune checkpoint molecules, such as PD-L1, and DNA mismatch repair proteins like MLH1 and MSH6, which not only support diagnosis but also contribute to prognostic evaluation and therapeutic planning (Maekawa et al. 2023).

### 2.4.1. Biomarkers

#### 2.4.1.1. CD3

The CD3 complex is present on the surface of all T-cells. It is first expressed in the cytoplasm of developing T-cells and migrates to the membrane as the cell matures (Kuhns et al. 2006). It refers to a group of signal transducer proteins in the T-cell antigen receptor (TCR), that associate with the TCR's antigen-binding chains, sending a signal to prompt the activation of the T-cell response (Tizard 2017).

It is a commonly used marker to identify T cells, with cytoplasmic positivity in immature T-cells, and membranous surface positivity in mature cells (Dukers et al. 2000).

In NL cases, it is used to determine the lymphoid lineage and distinguish a T-cell lymphoma from a B-cell one (Chino et al. 2013), while in CR it is used to determine the ratio of B:T cells (Vail et al. 2020).

#### 2.4.1.2. CD20 and Pax5

CD20 is a tetraspan receptor found exclusively on the surface of B cells (Cirstoiu-Hapca et al. 2007; Male et al. 2012). It is expressed in increasing concentrations from late pro-B to

activated B-cells; however, it is not present in plasma cells (Male et al. 2012; Vail et al. 2020). It does not exist in bone marrow cells (Cirstoiu-Hapca et al. 2007), but it is present in mature normal, as well as in neoplastic, B-cells (Dworzak et al. 2008). It is overexpressed in 70% of B-cell lymphomas (Tizard 2017), making this antigen a compelling target for cancer treatment with chemotherapy (Cirstoiu-Hapca et al. 2007; Tizard 2017). If further studies are performed in veterinary medicine, it could be used in immunotherapy, like rituximab (chimeric murine/human anti-CD20 antibody to treat B-cell lymphomas) is in human medicine (Thamm 2019).

Pax5 is also used to identify B cells. It is a transcription factor specific of the B-cell lineage, present in the nuclei, that it is expressed in B-cell precursors and in all stages of development of B cells, from early pro-B to plasma cells (Male et al. 2012; Vail et al. 2020). It guarantees the correct development of B-cell lineages by limiting the transcription of inappropriate genes and by promoting the expression of specific signaling molecules (Male et al. 2012).

#### 2.4.1.3. STAT5

STAT5, part of the Signal Transducer and Activator of Transcription (STAT) family, includes STAT5a and STAT5b, which are cell-specific and can have synergetic or antagonistic functions (Meinke et al. 1996; Bromberg 2002; Polak et al. 2019). These transduce signals from the cell membrane to the nucleus and are phosphorylated by Janus Kinases or Tyrosine Kinase 2 (Bromberg 2002; Polak et al. 2019). The STAT signaling pathway has been preserved throughout evolution and regulates cell division, apoptosis, metabolism, and cell fate (Stark and Darnell 2012; Kieslinger et al. 2021), and is activated in nearly 70% of solid and hematological human tumors, contributing to hematological malignancies (Frank 2007; Kieslinger et al. 2019).

STAT5 promotes tumorigenesis and the progression of tumors by cooperating with other pathways within cells and triggering further mechanisms that accelerate the progression of disease. It suppresses anti-tumor immune responses by promoting the expansion of $CD4^+/CD25^+$ regulatory T cells, substantially reducing the function of cytotoxic ($CD8^+$) and helper ($CD4^+$) T cells (Morcinek et al. 2002; Burchill et al. 2007; Kieslinger et al. 2019; Polak et al. 2019; Kieslinger et al. 2021). It also plays a role in metabolism, promoting aerobic glycolysis (Warburg Effect) in tumor cells, enhancing survival (Chueh et al. 2010; Creamer et al. 2010; Schmidt et al. 2014; Polak et al. 2019). Blocking STAT5 delays tumor growth in murines and humans, with minimal effects on normal differentiated cells. STAT5 knock-out mice have a high prevalence of perinatal mortality and show inhibition of the correct development of B and T cells, as well as reduced function of hematopoietic stem cells (Yao et al. 2005; Polak et al. 2019). These observations make it an interesting target for inhibition of tumor cells, as suppressing it has been shown to pause proliferation and induce apoptosis

(Burke et al. 2001; Frank 2007; Kieslinger et al. 2019; Polak et al. 2019). There are currently new inhibitors being developed that target STAT5, which should allow for selective interference with the corresponding proteins (Page et al. 2012; Pham et al. 2018). This protein also provides prognostic indications since its activity is frequently elevated in aggressive subtypes of cancer (Burke et al. 2001; Frank 2007; Kieslinger et al. 2019; Polak et al. 2019).

#### 2.4.1.4. Mismatch Repair Proteins: MLH1 and MSH6

These are part of the highly conserved mismatch repair (MMR) system, which has an essential role in post-replication DNA mismatch repair during cell division. The MMR system is needed for cell cycle arrest and/or programmed cell death when DNA damage naturally occurs during DNA replication (Li 2008; Aberdein et al. 2012). Mutation in one or more of the four genes involved in MMR – MSH2, MLH1, PMS2 and MSH6 – predisposes to a range of tumorigenic conditions (Martin et al. 2010). Although all viable cells express MMR proteins, expression is increased within replicating cells (Marra et al. 1996; Wei et al. 1997; Aberdein et al. 2012).

Although the presence of MMR dysfunction in feline tumors has not been investigated, Aberdein et al. (2012) confirmed the cross-reactivity of antibodies specific for human MLH1 and MSH6 with the corresponding proteins in the cat, which was predictable since the MMR system is conserved across multiple mammalian and other species (Buermeyer et al. 1999; Weaver 2011). In the same study, MLH1 and MSH6 were confirmed immunohistochemically to be present in feline lymphomas (Aberdein et al. 2012). In human medicine, MMR protein overexpression is associated with adverse survival outcomes in a variety of tumors (Zhou et al. 2024)

In neoplastic cells, MLH1 staining is characterized as a diffuse nuclear reaction; staining intensity can be similar or higher than in a normal epithelium or lymphocytes, which can be used as controls (Torlakovic et al. 2015) and MSH6 staining is characterized as a diffuse intranuclear reaction, while cytoplasmic staining is considered abnormal (Aberdein et al. 2012). The increased mitotic activity of the higher-grade tumors is probably the reason for the higher intensity of MLH1 labeling in neoplastic cells (Aberdein et al. 2012). Genetic changes in MSH6 contribute to DNA mismatch repair deficiency that leads to microsatellite instability and an increased risk of cancer. MMR protein expression has been demonstrated to correlate with proliferation rate as determined by expression of Ki67 (Cuilliere-Dartigues et al. 2007). Since a high mitotic rate is a common characteristic of high-grade tumors, indicative of the tumor proliferation rate, it makes sense that these rapidly dividing tumor cells would have stronger, more prevalent MMR protein expression. However, in one study, this was not demonstrated for MSH6 (Aberdein et al. 2012).

# 3. Feline Chronic Rhinitis versus Feline Nasal Lymphoma: diagnostic issues and pitfalls

## 3.1. Introduction and Objectives

Chronic Rhinitis and Nasal Lymphoma in cats present similar clinical signs, and imaging is not pathognomonic of one or the other. They are both very common in daily practice and the only way to differentiate them is through nasal biopsy and histopathology, and in some cases, by IHC.

Distinction between CR and NL is particularly challenging in daily practice, mainly due to the similarities observed in cell population on biopsy specimens. In CR, lymphocytes and plasma cells are the most abundant cell population, while in NL there are frequently infiltrating inflammatory lymphocytes and plasma cells (tumor-infiltrating lymphocytes) among the neoplastic cells. Consequently, differentiating the cell population present is an important step for a more accurate diagnosis; IHC is important in this differentiation in nasal biopsy specimens, especially when the diagnosis is lymphoplasmacytic CR.

Another reason that justifies the difficulty on the diagnosis is the fact that there are no pathognomonic features in CT images and many of them associated with NL overlap with those found in CR.

Even though histopathology and diagnostic imaging are relevant for a prompt diagnosis, some pitfalls are commonly recognized, impairing a rigorous medical approach.

The current study consisted in two parts: a retrospective study about the population and the evaluation of the of IHC using new biomarkers. It aims to retrospectively characterize cases of feline with chronic nasal disease diagnosed at the Veterinary Teaching Hospital and Pathology Laboratory (Faculty of Veterinary Medicine, University of Lisbon), between 2017 and 2022, regarding general data, signalment, age and clinically relevant information. As well as reviewing imaging and histopathology findings and correlate the latter with clinical outcome, when possible. Finally, it aims to retrospectively revise histopathological diagnosis by correlating it with biomarker (CD3, CD20, Pax5, STAT5, MLH1, MSH6) expression on IHC, and with clinical outcome.

## 3.2. Materials and Methods

### 3.2.1. Sample Population

A retrospective study was conducted and focused on cats diagnosed with CR and NL, examined at the Veterinary Teaching Hospital – Faculty of Veterinary Medicine - University of Lisbon – between January 2017 and December 2022. All information regarding age, sex, reproductive status, clinical signs and their duration, viral status (FIV, FeLV, Herpesvirus, and Calicivirus), bacterial cultures (*Chlamydophila felis*, *Mycoplasma felis*), biopsy sampling method, rhinoscopy reports, CT reports, histopathology reports, IHC results, final diagnosis,

medical treatment, follow-up, outcome and medical history was retrieved from the veterinary hospital management software *GuruVet*®. Also, when available, FIV, FeLV, FHV-1, *Chlamydophila felis*, *Mycoplasma felis* and FCV testing methodology was detailed.

As per the inclusion criteria, cats should have been submitted to nasal biopsies collected during the study period, with histopathological analysis performed at the Pathology Laboratory, and have samples preserved in the laboratory's repository. Histopathological diagnosis was revised by a veterinary pathologist, and IHC was performed on samples in which it had not been previously conducted. The diagnosis of either CR or NL was based on the histological and immunohistochemical findings from the corresponding biopsies.

Exclusion criteria included the presence of foreign bodies and other nasal diseases, such as nasal carcinomas, nasal polyps, oronasal fistulas and nasopharyngeal stenosis.

All selected samples were reexamined and subjected to IHC for the biomarkers CD3, Pax5, STAT5, MLH1, and MSH6. The last three biomarkers were added to supplement the immunohistochemical analyses that had already been conducted. Following discontinuation of commercial production of anti-CD20 antibodies that cross-react with feline samples, in all cases where IHC for CD20 had not been performed IHC was conducted using Pax5, as it is currently the standard replacement in such cases.

### 3.2.2. Computed Tomography

CT scans were always performed at the Veterinary Teaching Hospital. The equipment utilized was the Toshiba Astelion® TSX-034[a], Toshiba Medical Systems, Tochigi, Japan, and the acquired images were processed using the Vitrea® LT 4.1.52, Vital Image Inc., Minnesota, E.U.A. software.

While anesthetized, the animals were positioned in sternal recumbency with the head extended. Cranial CT scans were performed, including the nasal plane until the C2 vertebra and local lymph nodes. The scans were made with a slice thickness of 3/0.5 mm.

An iodinated non-ionic contrast agent (Optiray® 350mg I/ml, Laboratoire Guerbet, Auhay sous Bois, France) was used in all patients, with a dosage of 2mL/kg, up to a maximum of 80mL.

### 3.2.3. Nasal Biopsy

The biopsies were collected using two methods: a blind technique using biopsy/grasping forceps, and a rhinoscopy-guided approach, as described below. Regardless of the method used, the procedure was always performed with the animal under anesthesia, intubated with the oropharynx packed with gauze and positioned in sternal recumbency with the head and neck extended.

All biopsy samples were placed in a container with saline solution, which was subsequently replaced with 10% formalin to preserve the samples, for at least 24-48 hours, until they were processed by the pathology laboratory.

### 3.2.4. Rhinoscopy

Endoscopy of the upper respiratory tract consisted in two parts: caudal (retropharyngeal posterior) rhinoscopy and anterior (rostral) rhinoscopy. All the structures and changes can be seen in the monitor connected to the camera of the endoscope.

As mentioned before, in order to not contaminate the nasal cavity, the caudal rhinoscopy was systematically performed first. With a flexible endoscope (Choledocho-Fiberscope, 7.5Fr.; Karl Storz – Endoskope; Refª 11292ADK1) with instrument channel and fibre optic light guide the operator examined the nasopharynx, the pharynx and the larynx. Once the structures were visualized, if lesions were observed the operator collected multiple samples before proceeding to the anterior rhinoscopy. Then the operator changed to a rigid endoscope (Hopkins Telescope 30º, Karl Storz – Endoskope, Refª 26008BA) with instrument channel and fibre optic light guide and proceeds to the anterior rhinoscopy. Once again, after visualizing the structures, the operator obtained multiple samples (4-12). All anterior biopsies were collected with biopsy forceps introduced via the instrument channel of the endoscope. A nasal cavity was judged normal if there was smooth and pink mucosa as well as a mild serous discharge. Changes such as hyperemic, irregular or friable tissue, the presence of masses or nodules and the presence of non-serous discharge were classified as pathological.

At the end of the examination, all findings, along with photographs taken during the procedure, were reported and included in the endoscopy report.

### 3.2.5. Histopathology

Histopathological processing begins with documenting the number and size of samples submitted. Samples are then dehydrated through a series of graded alcohol baths, diaphanized by immersion in xylene for 3 hours, and then embedded in paraffin wax for 4 hours. Samples were then placed in a metallic mold and embedded in a paraffin block for subsequent sectioning. Sections 3µm in thickness, using Minot Microtome (Leica, Refª RM2125), were adhered to microscopy slides for subsequent staining with Hematoxylin and Eosin.

Finally, the slides were analyzed under an optical microscope, and tissues were classified based on their morphology, type of inflammatory/neoplastic infiltration and degree of inflammation (Table 4).

**Table 4 – Histopathological Classification of Samples: Morphology, Inflammatory Infiltrate Type, and Inflammation Degree**

| Histopathology Classification |
|---|
| **Morphology Classification** |
| ▪ CR: distortion or preservation of the epithelium and lamina propria |
| ▪ NL: distortion or preservation of the mucosa |
| ▪ Both: absence or presence of lamina propria glands, fibrosis, mucosa erosion, atypical cells and angiogenesis |
| **Inflammatory Infiltration** |
| ▪ CR: lymphoplasmacytic infiltration |
| ▪ NL: lymphocytic and plasmocytic infiltration |
| ▪ Both: neutrophilic and eosinophilic infiltration, presence of macrophages and infectious agents |
| **Degree of inflammation** |
| ▪ Mild, moderate, and severe |

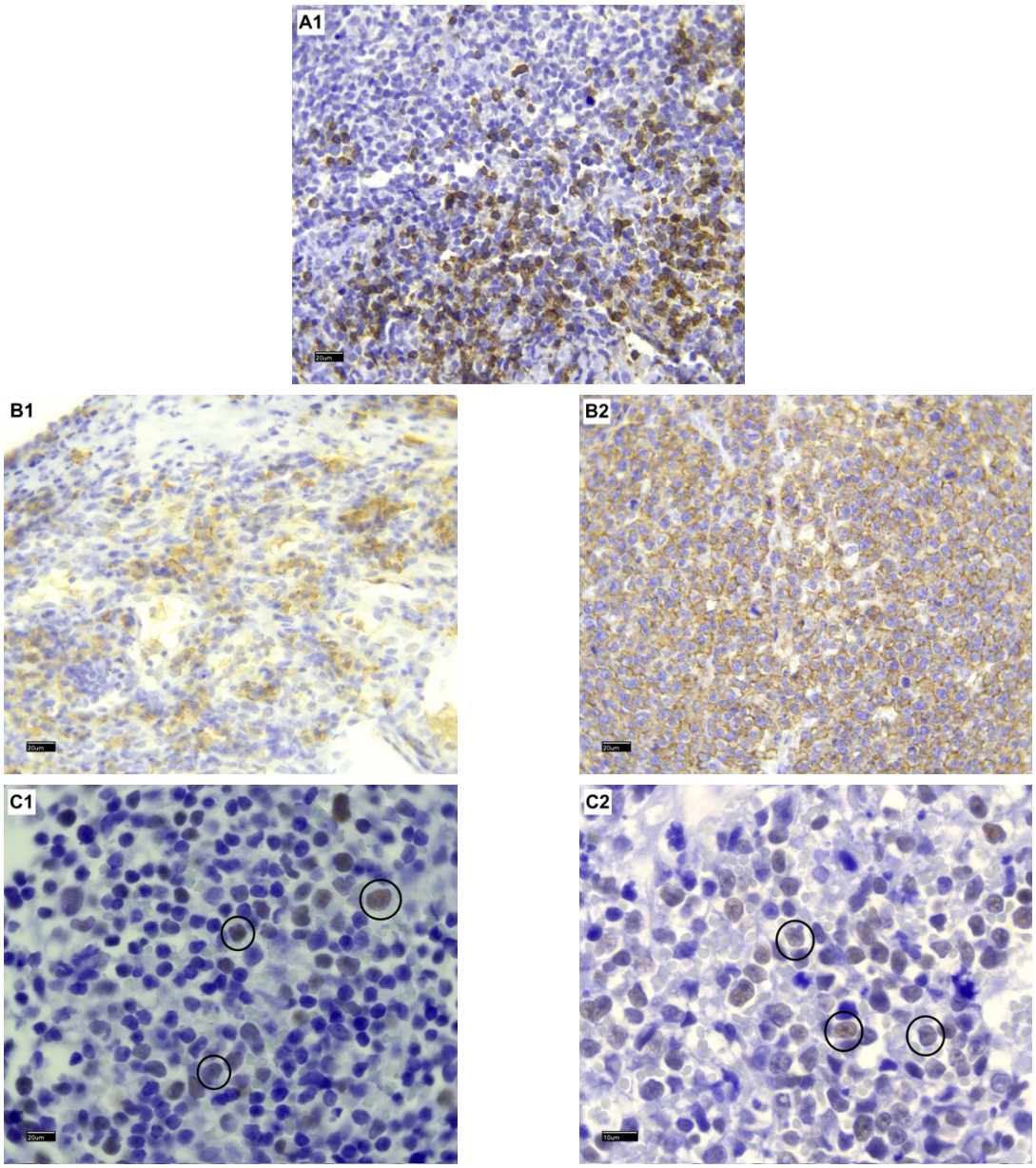
### 3.2.6. Immunohistochemistry

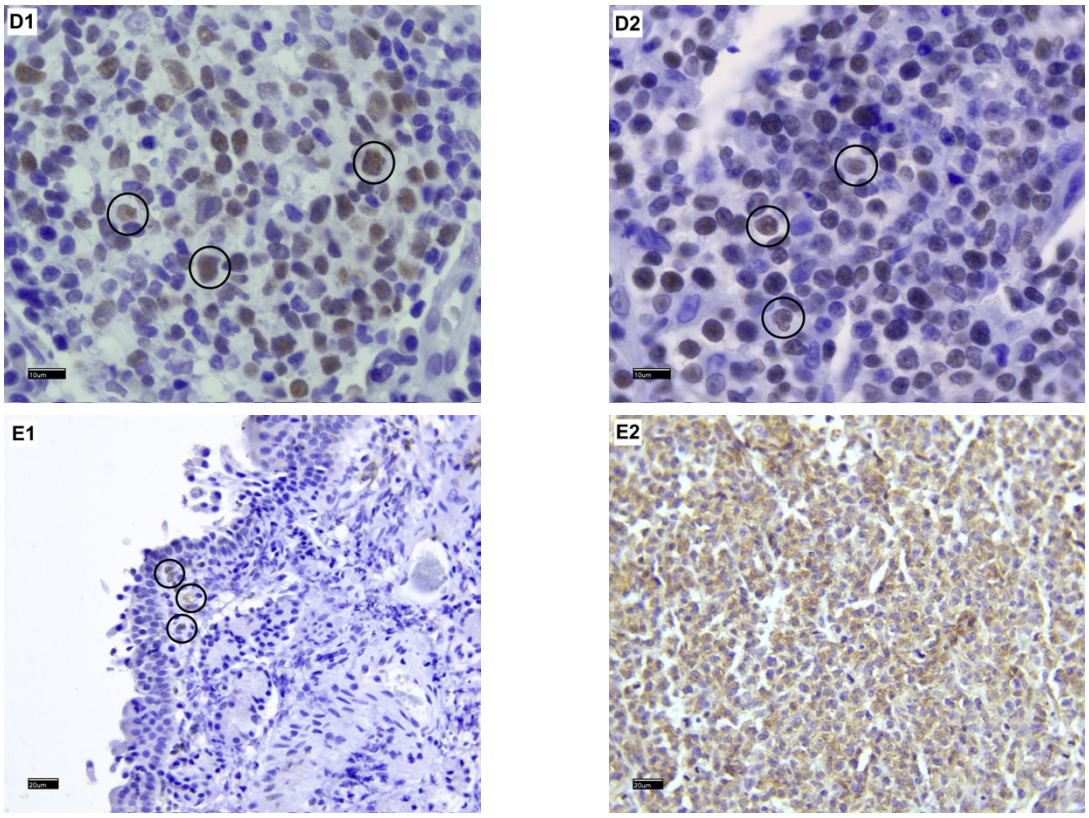
Sections 3µm in thickness were adhered to charged microscopy slides for immunohistochemistry, along with the appropriate positive control tissue for each antibody. After drying at 60ºC for an hour, followed by incubation at 37ºC overnight, the sections were processed in the Agilent Dako PT Link, Pre-Treatment Module for Tissue Specimens, where deparaffinization, hydration, and antigen retrieval were performed in antigen retrieval buffers, depending on the antibody used (Annex 5).

Next, the slides were washed in distilled water twice (5 + 5 minutes) and incubated in EnVision FLEX Peroxidase-Blocking Reagent (Agilent Dako kit) for 15 minutes, followed by washing in phosphate buffered saline (PBS) twice (5 + 5 minutes). Then, they were incubated in the primary antibody (CD3, CD20, Pax5, STAT5, MLH1 or MSH6) for the corresponding time (Annex 5), diluted in EnVision FLEX Antibody Diluent (Agilent Dako kit) at the appropriate concentration for each antibody, followed by another wash in PBS. Afterwards, the slides were incubated in EnVision/HRP polymer (Agilent Dako kit) for 30 minutes, washed in PBS, and incubated for 5 minutes in EnVision FLEX DAB + Chromogen (Agilent Dako kit), diluted in EnVision FLEX Substrate Buffer (Agilent Dako kit), as recommended by the supplier. Subsequently, the slides were rinsed in running water for 2 minutes, stained with Harris' Hematoxylin for 5 minutes and then rinsed again for 2 minutes in running water. Finally, they were dehydrated in ethanol and mounted.

The slides were then examined under a microscope to quantify the percentage of cells marked with each antibody, reflected by a brownish color (Figure 3), scoring them as follows: 0 (0% cells stained ), 1 (1 to 25% cells stained), 2 (26 to 50% cells stained), 3 (51 to 75% lymphoid cells stained), and 4 (>75% cells stained). Only lymphoid cells (normal or neoplastic) were considered in the analysis.

**Figure 3 – A1: Example of Positive Result in Immunohistochemistry for CD3 in a CR sample (40x objective; 10x eyepiece); B1: Example of Positive Result in Immunohistochemistry for CD20 in a CR sample (40x objective; 10x eyepiece); B2: Example of Positive Result in Immunohistochemistry for CD20 in a NL sample (40x objective; 10x eyepiece); C1: Circled example of Positive Result in Immunohistochemistry for MLH1 in a CR sample (100x objective; 10x eyepiece); C2: Circled example of Positive Result in Immunohistochemistry for MLH1 in a NL sample (100x objective; 10x eyepiece); D1: Circled example of Positive Result in Immunohistochemistry for MSH6 in a CR sample (100x objective; 10x eyepiece); D2: Circled example of Positive Result in Immunohistochemistry for MSH6 in a NL sample (100x objective; 10x eyepiece); E1: Circled example of Positive Result in Immunohistochemistry for STAT5 in a CR sample (40x objective; 10x eyepiece); E2: Example of Positive Result in Immunohistochemistry for STAT5 in a NL sample (40x objective; 10x eyepiece).**






### 3.2.7. Statistical Analysis

All collected data was entered into Microsoft® Excel version 16.91 (24111020).

Descriptive statistics and statistical analyses were conducted using IBM SPSS Statistics for Windows, version 29.0.0.0. To infer about the normality of the distribution of continuous quantitative variables (age and duration of clinical signs), the Shapiro-Wilk statistical test and graphical methods (boxplot) were used. Descriptive statistics were used and when applied, median was presented followed by the interquartile range (Q1-Q3: $25^{th}$-$75^{th}$) or standard deviation. In the course of the statistical analysis, when suitable, tests including the Mann-Whitney U test, Fisher's Exact Test, Binary and Multinomial Logistic Regression, Spearman Correlation coefficient, Kaplan-Meier, McNemar Test, Cohen's Kappa coefficient, Kruskal-Wallis Test, and Proportions Z-Test were implemented to assess possible correlations among the variables. For all tests, *p*-values <0.05 were considered significant for a 95% confidence interval. Examined relationships included age versus diagnosis, sex versus diagnostic, duration of clinical signs versus diagnosis, CT scan conclusion versus diagnosis, IHC versus diagnosis, treatment versus outcome, diagnosis versus outcome and survival time versus diagnosis. Mann-Whitney U test was used to analyze the statistical significance between age versus diagnosis, duration of clinical signs versus diagnosis, IHC versus diagnosis, and survival time versus diagnosis. Fisher's Exact test was used to analyze the statistical significance between gender versus diagnosis, CT scan conclusion versus diagnosis, IHC versus diagnosis, treatment versus diagnosis, and diagnosis versus outcome. Cohen's Kappa was used to assess the agreement between the CT scan conclusion and

diagnosis in classifying cases as either “correct”, “incorrect” or “inconclusive” (Table 12). Binary Logistic Regression and Proportions Z-Test were used to analyze the association between immunohistochemical markers (CD3, CD20, Pax5, STAT5, MLH1, MSH6) and the diagnosis of NL or CR (Table 13). Multinomial logistic regression analysis was used to analyze the statistical significance between treatment versus outcome. The likelihood ratio test was used to assess the strength of association between the treatment and the outcome (Table 16) and diagnosis versus outcome. Kruskal-Wallis’s test was used to compare the distribution of outcomes across diagnoses. Kaplan-Meier analysis (graph 3) was performed to estimate the survival function and compare the survival time between the different diagnosis (CR or NL).

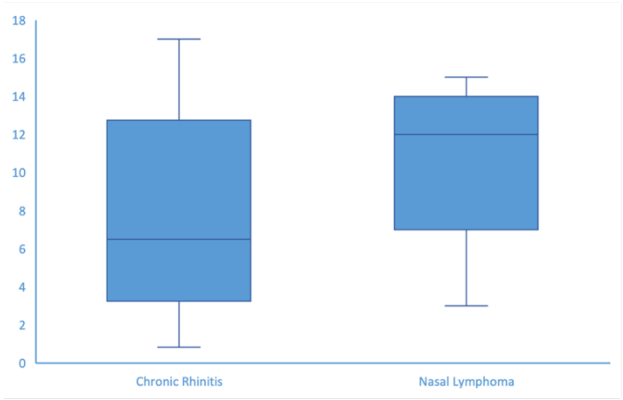
## 3.3. Results

### 3.3.1. Characterization of the population

A total of 35 cases were recruited. Of them, 18/35 (51.4%) were female and, of these, 17/18 (94.4%) were spayed. A total of 17/35 (48.6%) were male, with 14/17 (82.4%) being neutered. Median duration of clinical signs was 82 days (30.5-220.5), ranging from 4 to 1095 days.

The median age was 7 years (4-13), ranging from 10 months to 17 years. Statistically there is no significant difference in ages between the NL and CR groups ($p$=0.154); however, it is important to note that the majority of animals diagnosed with NL were older than those in the CR group (Graph 2).

**Graph 2 – Age Distribution of Animals with Chronic Rhinitis and Nasal Lymphoma**




As for referral status, twenty-four (24/35; 68.6%) were referred to the hospital by the assistant veterinarian, and eleven (11/35; 31.4%) were regular (intern) patients. Twenty-four (24/35; 68.6%) cats underwent a CT scan before biopsy.

Regarding biopsy sampling methods, twenty-nine (29/35; 82.9%) animals were submitted for rhinoscopy while six (6/35; 17.1%) had blind biopsies taken. Of those who underwent rhinoscopy, all (29/29; 100%) had visible changes, which variably included erythematous mucosa (25/29; 86.2%), thickened turbinates (22/29; 75.9%), increased mucosal friability (15/29; 51.7%), and presence of secretions ranging from mucous to

mucopurulent to, occasionally, bloody. Eight (8/29; 27.6%) cats had masses in the nasal cavity seen during physical exam or rhinoscopy.

In terms of final diagnosis, based on histopathology, twenty-eight (28/35; 80%) animals were diagnosed with CR and seven (7/35; 20%) with NL.

### 3.3.2. Chronic Rhinitis

The median age at diagnosis for CR was 6.5 years (3.75-12.25), ranging from 10 months to 17 years.

Nasal discharge was the most reported clinical finding (19/28; 67.9%) - with mucopurulent/purulent discharge being the most frequent (7/19; 36.8%) -, followed by sneezing in seventeen (17/28; 60.7%) animals. The absolute and relative frequencies of each clinical sign is shown in table 5. Duration of symptoms from onset to hospital presentation, averaged 72 days (33.3-208.25), ranging from 28 to 728 days.

**Table 5 – Clinical Signs Reported in Chronic Rhinitis Cases**

| Clinical Signs | Absolute Frequency | Relative Frequency (%) |
|---|---|---|
| Nasal Discharge | 19 | 67.9 |
| Mucopurulent | 7 | 36.8 |
| Purulent | 7 | 36.8 |
| Serous | 4 | 21.1 |
| Sanguineous | 1 | 5.2 |
| Mucous | 0 | 0 |
| Sneeze | 17 | 60.7 |
| Stertor | 10 | 35.7 |
| Dyspnea | 8 | 28.6 |
| Cough | 5 | 17.9 |
| Epistaxis | 5 | 17.9 |
| Ocular Discharge | 3 | 10,7 |
| Facial deformity | 2 | 7,1 |
| Stridor | 2 | 7,1 |
| Nasal obstruction | 1 | 3,6 |

Nineteen cats (19/28; 67.9%) had a known FIV and FeLV status, all screening with ELISA-based tests. Two cats (2/19; 10.5%) were FeLV-positive, and one cat (1/19; 5.3%) was FIV-positive. Ten (5/14; 35.7%) cats were tested for other pathogens, with two (1/14; 7.1%) testing positive for FHV-1, one (1/28; 3.6%) for FCV, one (1/28; 3.6%) for *Chlamydophila felis*, and one (1/28; 3.6%) for *Mycoplasma felis*. All samples collected for testing FHV-1, FCV, *Chlamydophila felis* and *Mycoplasma felis* were oronasal swabs and tested with the gold standard test for each agent (PCR for FHV-1, *Chlamydophila felis* and *Mycoplasma felis*; and RT-PCR for FCV).

Seventeen (17/28; 60.7%) cats had a CT scan, of which fourteen (14/28; 50%) had it performed before rhinoscopy and the other three (3/28; 10.7%) before performing blind biopsies. Fourteen (14/17; 82.4%) cats had an image compatible with a mass. Nasal conchae alterations, including atrophy and/or lysis, were categorized based on severity: mild changes were observed in three (3/17; 17.6%) cases, moderate in four (4/17; 23.5%) cases, and severe in four (4/17; 4/17%) cases. Additionally, nasal septum deviation was noted in five (5/17; 29.4%) cases, while asymmetry of the paranasal sinuses was present in six (6/17; 35.3%) cases. In twelve (12/17; 70.5%) lymph node enlargement was likely due to reactive lymph nodes, and of those three (3/17; 70.6%) had enlarged lymph nodes compatible with either reactive hyperplasia or metastasis.

Twenty-five out of twenty-eight animals (25/28; 89.3%) underwent rhinoscopy, while three (3/28; 10.7%) had blind biopsies taken. All twenty-five (25/25; 100%) cats that underwent rhinoscopy had an erythematous nasal mucosa. Friability of the nasal mucosa was considered high in six (6/25; 24%), moderate in five (5/25; 20%), and low in two (2/25; 8%) cases. Regarding the turbinates, two (2/25; 8%) cats had normal turbinates on both sides; five (5/25; 20%) had bilateral turbinate thickening and four (4/25; 16%) had unilateral thickening; one (1/25; 4%) cat had bilateral atrophy and another (1/25; 4%) had unilateral atrophy; seven (7/25; 28%) had bilateral turbinate lysis, and four (4/25; 16%) had unilateral lysis. Mucopurulent discharge was present in sixty percent (15/25) of cats.

Additionally, the mucosa of the nasopharynx was erythematous in nineteen (19/25; 76%) cats; friability was moderate in eight (8/25; 32%) cases and high in four (4/25; 16%); five cases had nasopharyngeal nodules/masses (5/25; 20%), and mucopurulent discharge was present in eight (8/25; 32%).

Information regarding medical treatment was available for twenty-four (24/28; 85.7%) CR cases. Of these, twenty-two were treated with steroids (22/24; 91.67%) - fourteen (7/12; 58.3%) in combination with antibiotics and eight (1/3; 33.3%) treated exclusively steroids (oral ± inhaled) -, one (1/24; 4.2%) was treated with an AINE combined with an antibiotic, and one (1/24; 4.2%) was only treated with antibiotic drugs. Among those receiving antibiotics (16/24; 66.7%), doxycycline was prescribed in 8/16 (50%), marbofloxacin in 3/16 (18.8%), potentiated amoxicillin in 2/16 (12.5%), combined doxycycline and azithromycin in 1/16 (6.3%), and cefovecin in 1/16 (6.3%). One cat (1/24; 4.2%) was treated with a course of interferon-Ω. Besides medical treatment, one (1/24; 4.2%) cat had a ventral rhinotomy for polyp removal.

Eleven (11/28; 39.3%) of the twenty-eight cats were successfully controlled with medication, four (4/28; 14.3%) resolved with medication, and one (1/28; 3.6%) died. The remaining twelve (12/28; 42.9%) were lost to follow-up. Two of the eleven cats (18.2%) controlled with medication were submitted to euthanasia, and one (1/11; 9.1%) died from unknown causes.

### 3.3.3. Nasal Lymphoma

A total of 7 cases were identified as having NL. The age at diagnosis of NL typically ranged from 3 to 15 years, with a median age of 10.6 years (± 4.3).

The most frequently reported clinical finding was nasal discharge, present in six (6/7; 85.7%) animals with purulent discharge being the most frequent (4/6; 66.7%). Sneezing and ocular discharge (4/7; 57.1%) were the second most reported clinical finding. The absolute and relative frequencies of each clinical sign in these animals is shown in table 6. The mean duration of clinical signs was 82 days (27.5-224.5), spanning from 4 to 1095 days.

**Table 6 – Clinical Signs Reported in Nasal Lymphoma Cases**

| Clinical Signs | Absolute Frequency | Relative Frequency (%) |
|---|---|---|
| Nasal Discharge | 6 | 85.7 |
| Purulent | 4 | 66.7 |
| Sanguineous | 1 | 16.7 |
| Serous | 1 | 16.7 |
| Mucopurulent | 0 | 0 |
| Mucous | 0 | 0 |
| Ocular Discharge | 4 | 57.1 |
| Sneeze | 4 | 57.1 |
| Cough | 2 | 28.6 |
| Dyspnea | 2 | 28.6 |
| Stertor | 2 | 28.6 |
| Epistaxis | 1 | 14.3 |
| Lymphadenomegaly | 1 | 14.3 |
| Stridor | 1 | 14.3 |
| Weight Loss | 1 | 14.3 |

FIV and FeLV status was known for 4/7 (57.1%) cats. One (1/4; 25%) was positive for both FIV and FeLV and another cat (1/4; 25%) was positive for FIV. None of the NL cats were tested for other pathogens.

All cats had a CT scan, of which four (4/7; 57.1%) had it performed before rhinoscopy. A mass effect was identified in all cases. Nasal conchae alterations, including atrophy and/or lysis, were categorized based on severity: moderate in two (2/7; 28.6%) cases, and severe in four (4/7; 57.1%) cases. Additionally, nasal septum deviation was noted in one (1/7; 14.3%) cases, while asymmetry of the paranasal sinuses was present in one (1/7; 14.3%) cases. Six (6/7; 85.1%) had changes compatible with reactive lymph nodes, of those two (2/7; 28.6%) had changes compatible with either reactive hyperplasia or metastasis.

Four (4/7; 57.1%) animals had biopsies taken during rhinoscopy while three (3/7; 42.9%) had blind biopsies. Three (3/4; 75%) cats that underwent rhinoscopy had erythematous nasal mucosa, and one (1/4; 25%) had thickened turbinates and moderate friability of the nasal mucosa. Mucopurulent discharge was present in 3/4 (75%) cats. Erythema and moderate friability of the nasopharyngeal mucosa was found in three (3/4; 75%) cats, two (2/4; 50%) had visible masses, and one (1/4; 25%) had mucopurulent discharge.

Data about medical treatment was available for six (6/7; 85.7%) cases. All six were treated with steroids, of which four (4/6; 66.7%) were combined with a chemotherapy protocol, two (2/4; 50%) with Chlorambucil and the other two (2/4; 50%) with CHOP protocol; and one (1/6; 16.7%) was combined with doxycycline.

Only one (1/7; 14.3%) cat was controlled with medication, one (1/7; 14.3%) died of unknown causes, and one (1/7; 14.3%) was euthanized. The remaining four (4/7; 57.1%) cats were lost to follow-up, although it is known that treatment was not being effective in two (1/2; 50%) of those cats.

### 3.3.4. Histopathological Evaluation

In terms of nasal biopsy methods, twenty-nine (29/35; 82.9%) underwent rhinoscopy, while six (6/35; 17.1%) were blind biopsies. Of those who's biopsies were collected by rhinoscopy, twenty-five (25/29; 86.2%) had a diagnosis of CR, and three (3/29; 10.3%) of NL. In relation to the blind biopsies, 50% (1/2) had CR, and the other half (1/2; 50%) had NL.

Through these nasal biopsy methods, 35 slides containing nasal samples were collected.

#### 3.3.4.1. Chronic Rhinitis

##### 3.3.4.1.1. Morphology Classification

Epithelial changes were seen in more than 50% (17/28; 60.7%) of cases, five (5/17; 26.4%) of which had epithelial metaplasia, one (1/17; 5.9%) had hyperplasia, and one (1/17; 5.9%) had hypertrophy. In four (4/28; 14.3%) cases, the epithelium was devoid of cilia, and epithelial erosion was present in twelve (12/28; 42.9%).

Only six (6/28; 21.4%) cases had distortion of the lamina propria, of which two (2/6; 33.3%) had hyperplasia. Lamina propria glands were normal in four (4/24; 14.3%) cases. However, these glands were not found or not present in most (6/7; 85.7%) cases. Fibrosis was present in only two (1/14; 7.1%) cases.

##### 3.3.4.1.2. Inflammatory Classification

Regarding the type of inflammatory cells in the epithelium (Table 7), only lymphoplasmacytic (11/28; 39.3%) and neutrophilic (14/28; 50%) infiltration were present. Eosinophilic infiltration was absent in all cases. As for lymphoplasmacytic infiltration, seven (7/28; 25%) were classified as mild, moderate in three (3/28; 10.7%), and severe in only one

case (1/28; 3.6%). Neutrophilic infiltration was classified as mild in six (3/14; 21.4%) cases, moderate in five (5/28; 17.9%), and severe in three (3/28; 10.7%) cases.

In the lamina propria (Table 7), lymphoplasmacytic, neutrophilic and eosinophilic infiltration was observed in twenty-four (24/28; 85.7%), nineteen (19/28; 67.9%), and three (3/28; 10.7%) cases, respectively. Lymphoplasmacytic infiltration was classified as mild in five (5/28; 17.9%) cases, moderate in eight (8/28; 28.6%), and severe in eleven (11/28; 39.3%) cases. Plasma cells predominated in six (6/28; 25%) of these cases. Neutrophilic infiltration was classified as mild in eight (8/28; 28.6%) cases, moderate in seven (7/28; 25%), and severe in four (4/28; 14.3%) cases. Macrophages were present in the majority (17/28; 60.7%) of cases. In the epithelium, macrophagic infiltration was classified as mild in two (1/14; 7.1%) samples. As for the lamina propria, eleven (11/28; 39.3%) cases were considered mild, five (5/28; 17.9%) moderate, and one (1/28; 3.6%) severe.

Infectious agents were absent in nearly all samples (27/28; 96.4%). Mast cells were identified in one (1/28; 3.6%) case.

**Table 7 – Type of Infiltration in the Epithelium and Lamina Propria**

| Type of infiltration | | AF | RF (%) | Mild (%) | Moderate (%) | Severe (%) |
|---|---|---|---|---|---|---|
| Lymphoplasmacytic | Ep | 11 | 39.3 | 63.6 | 27.3 | 9.1 |
| | LP | 24 | 85.7 | 20.8 | 33.3 | 45.8 |
| Neutrophilic | Ep | 14 | 50 | 42.8 | 35.7 | 21.4 |
| | LP | 19 | 67.9 | 42.1 | 36.8 | 21.1 |
| Eosinophilic | Ep | 0 | 0 | 0 | 0 | 0 |
| | LP | 3 | 10.7 | 100 | 0 | 0 |
| Macrophagic | Ep | 2 | 7.1 | 7.1 | 0 | 0 |
| | LP | 17 | 60.7 | 39.3 | 17.9 | 3.6 |

**Legend: Ep – Epithelium; LP – Lamina Propria; AF – Absolute Frequency; RF – Relative Frequency**

### 3.3.4.2. Nasal Lymphoma

#### 3.3.4.2.1. Morphology Classification

In six (6/7; 85.7%) cases, the mucosal epithelium was altered, and one (1/7; 14.3%) cat only had distortion of the lamina propria. Six (6/7; 85.7%) samples were devoid of lamina propria glands, which were only seen in one (1/7; 14.3%) case. Four (4/7; 57.1%) cases had epithelial erosion, and four (4/7; 57.1%) others had epithelial atypia. None of the cases exhibited fibrosis or angiogenesis.

#### 3.3.4.2.2. Inflammatory Classification

Lymphocytic, plasmacytic, and neutrophilic infiltration were present in seven (7/7; 100%), four (4/7; 57.1%), and four (4/7; 57.1%) cases, respectively.

Mucosal lymphocytic infiltration was mild in six (6/7; 85.7%) cases and moderate in one (1/7; 14.3%). One (1/7; 14.3%) case had mild blastic lymphoid infiltration. Plasma cell infiltration was classified as mild in three (3/7; 42.9%) cases, and as moderate in one (1/7; 14.3%).

Finally, neutrophilic infiltration of the mucosa was mild in one case (1/7; 14.3%), moderate in one case (1/7; 14.3%), and severe in two cases (2/7; 28.6%). Of these, one (1/2; 50%) case only had severe neutrophilic infiltration in the epithelium.

Macrophages were observed in three (3/7; 42.9%) cases and was considered mild. No infectious agents were observed.

The type of cells in the mucosa in these animals are shown in table 8.

**Table 8 – Type of Cells in the Mucosa**

| Type of cells | AF | RF (%) | Mild (%) | Moderate (%) | Severe (%) |
|---|---|---|---|---|---|
| Lymphocytes | 7 | 100 | 85.7 | 14.3 | 0 |
| Blastic Lymphoid Cells | 1 | 14.3 | 100 | 0 | 0 |
| Plasmacytes | 4 | 57.1 | 75 | 25 | 0 |
| Neutrophiles | 4 | 57.1 | 25 | 25 | 50 |
| Macrophages | 3 | 42.9 | 100 | 0 | 0 |

**Legend: AF – Absolute Frequency; RF – Relative Frequency**

### 3.3.5. Immunohistochemistry

The biomarkers used in this study were CD3, CD20, Pax5, STAT5, MLH1, and MSH6. As mentioned, in all cases with previous IHC CD3 and CD20 results, these were supplemented with biomarkers STAT5, MLH1, and MSH6. Pax5 was used in cases where CD20 had not been applied. Hereafter, these two markers will be considered equivalent for the purposes of this study, as they serve the same diagnostic role.

Due to technical limitations, such as reduced sample size or unavailable samples, IHC was only performed on thirty-three (33/35; 94.3%) cases. Of these, twenty-six (26/33; 78.8%) belonged to CR cases, and seven (7/33; 21.2%) to NL.

#### 3.3.5.1. Chronic Rhinitis

The absolute and relative frequencies of each IHC staining result is shown in table 9.

Twenty-five (25/26; 96.2%) cases had CD3-positive cells. The staining score was 1 (1 to 25% cells stained) in twelve (12/26; 46.2%) cases, 2 (26 to 50% cells stained) in nine (9/26; 34.6%) cases, and 3 (51 to 75% cells stained) in four (4/26; 15.4%) cases.

All five samples tested for CD20 expression had a staining score of 3. Of those tested for Pax5 expression, ten (10/21; 47.6%) had a staining score of 1, while one (1/21; 4.8%) had a staining score of 2.

Concerning CD20/Pax5, ten (10/26; 38.5%) had 1 to 25% inflammatory lymphoid cells marked, one (1/26; 3.8%) had 26 to 50%, and five (5/26; 19.2%) cases had 51 to 75% of inflammatory cells marked.

Regarding STAT5, sixteen (16/26; 61.5%) cases had a staining score of 1 in inflammatory cells. STAT5 expression in inflammatory lymphoid cells was exclusively nuclear.

Finally, for MLH1 and MSH6, five (5/26; 19.2%) and eight (8/26; 30.8%), had a staining score of 1, respectively, while the remaining cases had a staining score of 0.

**Table 9 – Immunohistochemistry Results for Chronic Rhinitis Cases**

| Marker | Type of cell | N | RF (%) | 0 (%) | 1 (%) | 2 (%) | 3 (%) |
|---|---|---|---|---|---|---|---|
| CD3 | INF | 26 | 96.2 | 3.8 | 46.2 | 34.6 | 15.4 |
| CD20 | INF | 5 | 100 | 0 | 0 | 0 | 100 |
| Pax5 | INF | 21 | 52.4 | 47.6 | 47.6 | 4.8 | 0 |
| CD20/Pax5 | INF | 26 | 61.5 | 38.5 | 38.5 | 3.8 | 19.2 |
| STAT5 | INF | 26 | 57.7 | 42.3 | 57.7 | 0 | 0 |
| MLH1 | INF | 26 | 19.2 | 80.8 | 19.2 | 0 | 0 |
| MSH6 | INF | 26 | 30.8 | 69.2 | 30.8 | 0 | 0 |

**Legend: INF – Inflammatory cells; NEO – Neoplastic cells; N – Cases tested; RF – Relative Frequency of positive staining; 0 – 0% marked cells; 1 – 1 to 25% marked cells; 2 – 26 to 50% marked cells; 3 – 51 to 75% marked cells**

### 3.3.5.2. Nasal Lymphoma

The absolute and relative frequencies of each IHC result is shown in table 10.

None of the cases showed CD3 expression in neoplastic cells. When looking at tumor-infiltrating lymphoid cells, four (4/7; 57.1%) cases had a staining score of 1, one (1/7; 14.3%) had a staining score of 2, and two (2/7; 28.6%) had a staining score of 3.

Five (5/7; 71.4%) samples were stained using CD20. CD20 expression in neoplastic cells was scored as 3 in one (1/5; 20%) case, and as 4 in four (4/5; 80%) cases. As for tumor-infiltrating lymphoid cells, all five cases had a staining score of 1.

Pax5 was only used in two (2/7; 28.6%) cases, of which none showed Pax5 expression in neoplastic cells. Tumor-infiltrating lymphoid cells had a staining score of 1 in both cases.

Concerning CD20/Pax5, all samples (7/7; 100%) had 1 to 25% inflammatory lymphoid cells marked.

Six (6/7; 85.7%) cases showed STAT5 expression in neoplastic cells. Staining score was 4 in three (3/6; 50%) of these cases, while the other three were equally distributed in the

other three scores (1/6; 14.3% for each score). As for tumor-infiltrating lymphoid cells, five (5/7; 71.4%) cases had a staining score of 1, while the remaining cases were negative. STAT5 expression in neoplastic cells was exclusively cytoplasmic, while in inflammatory lymphoid cells it was exclusively nuclear.

MLH1 was only expressed in neoplastic cells, with six (6/7; 85.7%) cases showing positive staining. Of these, one (1/6; 16.67%) had a staining score of 1, two (2/6; 33.3%) had a staining score of 2, another two (2/6; 33.3%) had a staining score of 3, and the last one (1/6; 16.67%) had a staining score of 4.

Finally, five (5/7; 85.7%) cases showed MSH6 expression in neoplastic cells. Of these, one (1/5; 20%) case had a staining score of 2, two (2/5; 40%) had a staining score of 3, and two (2/5; 40%) had a staining score of 4. As for tumor-infiltrating lymphoid cells, three (3/7; 42.9%) cases had a MSH6 staining score of 1, while the remaining cases were negative.

**Table 10 – Immunohistochemistry Results for Nasal Lymphoma Cases**

| Marker | Type of cell | N | RF (%) | 0 (%) | 1 (%) | 2 (%) | 3 (%) | 4 (%) |
|---|---|---|---|---|---|---|---|---|
| CD3 | INF | 7 | 100 | 0 | 57.1 | 14.3 | 28.6 | 0 |
| CD20 | INF | 5 | 100 | 0 | 100 | 0 | 0 | 0 |
| | NEO | | 100 | 0 | 0 | 0 | 20 | 80 |
| Pax5 | INF | 2 | 100 | 0 | 100 | 0 | 0 | 0 |
| CD20/Pax5 | INF | 7 | 100 | 0 | 100 | 0 | 0 | 0 |
| STAT5 | INF | 7 | 71.4 | 28.6 | 71.4 | 0 | 0 | 0 |
| | NEO | | 85.7 | 14.3 | 14.3 | 14.3 | 14.3 | 42.9 |
| MLH1 | NEO | 7 | 85.7 | 14.3 | 14.3 | 28.6 | 28.6 | 14.3 |
| MSH6 | INF | 7 | 42.9 | 57.1 | 42.9 | 0 | 0 | 0 |
| | NEO | | 71.4 | 28.6 | 0 | 14.3 | 28.6 | 28.6 |

**Legend: INF – Inflammatory cells; NEO – Neoplastic cells; N – Cases tested; RF – Relative Frequency of positive staining; 0 – 0% marked cells; 1 – 1 to 25% marked cells; 2 – 26 to 50% marked cells; 3 – 51 to 75% marked cells; 4 – >75% marked cells**

### 3.3.6. Statistical Comparison Between Groups

#### 3.3.6.1. Gender versus Diagnosis

All NL cases were female, while 61% of CR cases were male (Table 11), with a significant association between sex and diagnosis ($p$=.008). Therefore, there is a significant relationship between gender and the likelihood of a diagnosis of NL versus CR in the studied sample.

**Table 11 – Distribution of Cases by Gender and Diagnosis Category**

| | Chronic Rhinitis | Nasal Lymphoma |
|---|---|---|
| Female | 11 | 7 |
| Male | 17 | 0 |

### 3.3.6.2. Duration of clinical signs versus Diagnosis

There was no statistically significant difference in the duration of clinical signs between the groups ($p$=0.853), suggesting that the length of clinical signs is not influenced by the diagnosis.

### 3.3.6.3. CT Scan Conclusion versus Diagnosis

A fair level of agreement (0.375) was found between the CT scan conclusion and diagnosis, which is consistent with a $p$-value of 0.402, suggesting a low probability of a significant relationship between the two.

**Table 12 – Summary of CT scan Conclusion Accuracy and Final Outcomes for Inconclusive Cases**

| CT Scan Conclusion | AF | RF (%) | Final Diagnosis | |
|---|---|---|---|---|
| | | | Chronic Rhinitis | Nasal Lymphoma |
| Correct | 11 | 45.8% | 7 | 4 |
| Incorrect | 5 | 20.8% | 4 | 1 |
| Inconclusive | 8 | 33.3% | 6 | 2 |

**Legend: AF – Absolute Frequency; RF – Relative Frequency**

### 3.3.6.4. Immunohistochemistry versus Diagnosis

Statical results are listed in Table 13. Expression of STAT5, MSH6 and CD3 did not exhibit statistically significant correlation with diagnosis.

CD20/Pax5 showed a significant association with diagnosis, suggesting an important difference in CD20/Pax5 expression between the two diseases. Compared to CR, NL cases had noticeably larger numbers of CD20/Pax5-positive cells, whereas in CR, the degree of positivity varied across scores (0-4), with some cases showing a much higher staining score (Table 10 and 11).

MLH1 and CD20/Pax5 stood out among the examined markers as having a high link with diagnosis, with a $p$-value of 0.001 and 0.024, respectively, and a statistically significant difference between the two diagnostic groups. Although the $p$-value was just below the significance threshold, MSH6, like STAT5 and CD3, failed to demonstrate any significant differences.

**Table 13 – *p*-value of Statistical Tests for Biomarker Expression**

| | | STAT5 | MLH1 | MSH6 | CD3 | CD20/Pax5 |
|---|---|---|---|---|---|---|
| Fisher's Exact Test | | 0.676 | 0.559 | 0.661 | 1.000 | 0.067 |
| Mann-Whitney U Test | | 0.516 | 0.215 | 0.553 | 0.604 | 0.038 |
| Binary Logistic Regression | B | -0.606 | 20.104 | -0.523 | -19.930 | -20.441 |
| | Exp(B) | 0.545 | 538492339.459 | 0.593 | 0.000 | 0.000 |
| | *p*-value | 0.513 | 0.999 | 0.549 | 1.000 | 0.999 |
| Proportions Z-Test | Z | -0.660 | -3.312 | -1.954 | -0.527 | -2.253 |
| | *p*-value | 0.509 | 0.001 | 0.051 | 0.598 | 0.024 |

### 3.3.6.5. Treatment versus Outcome

No statistically significant association was found between treatment and outcome ($p$=0.207), as well as no strong evidence of a relationship between these variables ($p$=0.219).

The following tables present the absolute and relative frequencies, as well as the distribution of outcomes for medical treatment in cases of CR (Table 14) and NL (Table 15).

**Table 14 – Medical Treatment of Cats with Chronic Rhinitis and their Respective Outcomes**

| Medical Treatment | AF | RF (%) | Resolved | Controlled | Ineffective treatment | Euthanasia | Deceased |
|---|---|---|---|---|---|---|---|
| AMC | 1 | 6.3 | 1 | 0 | 0 | 0 | 0 |
| PRED | 3 | 18.8 | 0 | 2 | 0 | 0 | 1 |
| PRED + FLT | 3 | 18.8 | 0 | 3 | 0 | 0 | 0 |
| PRED + AMC | 1 | 6.3 | 0 | 1 | 0 | 0 | 0 |
| PRED + AZT | 1 | 6.3 | 1 | 0 | 0 | 0 | 0 |
| PRED + DOXY | 3 | 18.8 | 0 | 3 | 0 | 0 | 0 |
| PRED + DOXY + FLT | 1 | 6.3 | 0 | 1 | 0 | 0 | 0 |
| PRED + DOXY + AZT | 1 | 6.3 | 1 | 0 | 0 | 0 | 0 |
| PRED + MARBO | 2 | 12.5 | 1 | 1 | 0 | 0 | 0 |

**Legend: AF – Absolute Frequency; RF – Relative Frequency; AMC – Potentiated Amoxicillin; PRED – Prednisolone; FLT – Fluticasone; AZT – Azithromycin; DOXY – Doxycycline; MARBO – Marbofloxacin**

**Table 15 – Medical Treatment of Cats with Nasal Lymphoma and their Respective Outcomes**

| Medical Treatment | AF | RF (%) | Resolved | Controlled | Ineffective Treatment | Euthanasia | Deceased |
|---|---|---|---|---|---|---|---|
| PRED + DOXY | 1 | 16.7 | 0 | 0 | 1 | 0 | 0 |
| PRED + Chlorambucil | 2 | 33.3 | 0 | 1 | 1 | 0 | 0 |
| CHOP Protocol | 2 | 33.3 | 0 | 0 | 0 | 1 | 1 |

**Legend: AF – Absolute Frequency; RF – Relative Frequency; PRED – Prednisolone; DOXY – Doxycycline**

Most treatment outcomes did not show statistically significant differences compared to the category-controlled category, indicating a lack of evidence for treatment effectiveness.

**Table 16 – Multinomial Logistic Regression: Treatment Outcomes**

| Outcome | B | Exp(B) | *p*-value[1] |
|---|---|---|---|
| Resolved | 0.077 | 1.080 | 0.694 |
| Ineffective Treatment | 0.351 | 1.420 | 0.242 |
| Euthanasia | 15.75 | 6955561.495 | <0.001 |
| Deceased | 0.162 | 1.176 | 0.535 |

**[1] - Multinomial Logistic Regression**

### 3.3.6.6. Diagnosis versus Outcome

There was a significant association between diagnosis (CR or NL) and disease outcome. Detailing, statistical analysis indicates a significant difference in outcomes across diagnoses ($p$=0.010), with no strong association ($p$=0.008) and an incoherent distribution of outcomes across diagnoses ($p$=0.004).

Table 17 illustrates the absolute and relative frequencies, along with the distribution of outcomes based on the diagnosis.

**Table 17 – Distribution of Outcomes Based on Diagnosis**

| | AF | RF (%) | Chronic Rhinitis | Nasal Lymphoma |
|---|---|---|---|---|
| Resolved | 4 | 19.0 | 4 | 0 |
| Controlled | 12 | 57.1 | 11 | 1 |
| Ineffective treatment | 2 | 9.5 | 0 | 2 |
| Euthanasia | 1 | 4.8 | 0 | 1 |
| Deceased | 2 | 9.5 | 1 | 1 |

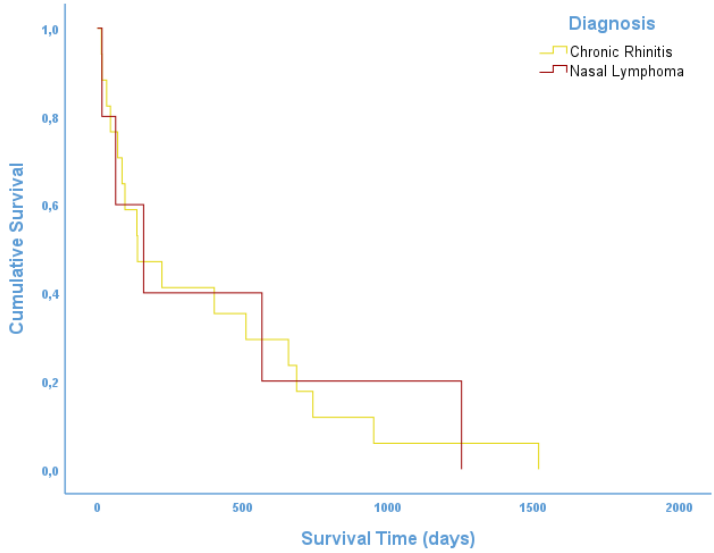
**Legend: AF – Absolute Frequency; RF – Relative Frequency**

### 3.3.6.7. Survival Time versus Diagnosis

Statistical analysis ($p$=0.945) indicates no statistically significant difference in survival times between animals diagnosed with NL and those diagnosed with CR. There was no significant difference in the survival time between the two groups.

The differences in survival times between the two groups, are illustrated in the survival curves presented in graph 3.

**Graph 3 – Kaplan-Meier Survival Curves for Chronic Rhinitis and Nasal Lymphoma Cases**




## 3.4. Discussion

This study aimed to clarify challenges in diagnosing CR and NL by exploring and correlating specific findings observed in each disease during the diagnostic process, to facilitate a more accurate and efficient diagnosis. The CR and NL cases were described in detail for this purpose. A total of 35 cases examined in the Veterinary Teaching Hospital during 2017 and 2022 constituted the sample.

The median age (6.5 years) and range (10 months to 17 years) observed in this study for cats developing CR were consistent with the age range reported by Henderson et al. (2003)

and Johnson (2020), which spanned between 6 months and 20 years. In NL cases, animals were usually older (median age 10.6 ± 4.3 years, range 3-15 years) which is also consistent with research showing that NL typically develops between the ages of 9 and 10 (range 1-16 years) (Santagostino et al. 2015; Vail and Pinkerton 2020). This observation implies that age may play a factor in the diagnosis; however, no statistically significant differences were observed, perhaps due to the small sample size of the NL group may have limited our capacity to identify this difference, and it is important to note that the majority of animals diagnosed with NL were of older ages (Graph 2).

Two young adult cats, one 3 and the other 7 years old, developed NL. This may be explained by the fact that these cats were infected with retroviruses, which are linked to the development of NL at an early age (Shelton et al. 1990; Chino et al. 2013; Macy 2020); both FIV and FeLV cause immunosuppression, which compromises immunosurveillance (Macy 2020), while FeLV has proto-oncogenes (Hartmann et al. 2001; Macy 2020). The 3-year-old cat was co-infected with both FIV and FeLV and, according to Shelton et al. (1990) and Macy (2020), these cats have a 77% higher chance of developing lymphoma, when compared to healthy cats, and 5.6 times more prone to lymphoma than those infected by one virus alone. The 7-year-old cat was FeLV-positive, which increases risk of developing lymphoma by 62 times in comparison to healthy cats (Shelton et al. 1990; Macy 2020).

In this study, 100% of cats with NL were female and 61% of CR cases were male, which demonstrates a relationship (although not a statistically significant one) between sex and the likelihood of a diagnosis of NL versus CR in the sample in study. These results are not in agreement with the available literature, in which no sex predisposition is reported for CR; as for NL, a 2:1 male-to-female ratio has been noted (Santagostino et al. 2015).

In both CR and NL, the most common clinical sign reported is nasal discharge, primarily mucopurulent, characteristic of chronic conditions (Kuehn 2006; Santagostino et al. 2015; Vail and Pinkerton 2020; Ettinger et al. 2024). This was the case for this study, with the second most reported clinical sign being sneezing. Visually, only one cat exhibited lymphadenomegaly, and was diagnosed with both NL and FIV. Previous studies reported that enlargement of lymph nodes can occur either in NL or FIV infection (Little et al. 2007; Nelson and Couto 2019). Although more commonly associated with fungal rhinitis or NL (Van Pelt and Lappin 1994; Santagostino et al. 2015), two cats with CR presented with facial deformities.

There was no statistically significant difference or correlation between the duration of clinical signs in CR cases when compared with NL cases. The duration of clinical signs is often influenced by the owners, as they are responsible for seeking veterinary care, and tends to be shorter in cases with more severe and evident clinical signs, as owners are more likely to recognize the urgency and act promptly. Conversely, when clinical signs are mild, they may take longer to seek medical help. Given that the signs of both diseases may be similar in terms of manifestation and severity, it can be inferred that the specific disease does not significantly

affect the time taken to seek veterinary assistance. This observation is further supported by the mean duration of clinical signs, which is remarkably similar in both cases. The average symptom duration for CR cases was 72 days, qualifying it as chronic disease (Cape 1992; Harvey and Tasker 2013), and 82 days for NL cases, spanning from 4 to 1095 days, which once again, is in agreement with previous studies (median 2 months; range 1-1800 days) (Little et al. 2007; Santagostino et al. 2015; Vail and Pinkerton 2020). Although the duration of clinical signs in NL cases exceeds the reported mean in previous studies, it is noteworthy that animals with stage I NL may appear clinically normal (Savary et al. 2000; Vail and Pinkerton 2020).

Two cats had longer clinical signs (1095 days for one and 365 days for the other), presented sneezing and mucopurulent nasal and ocular discharge for almost 3 years and 1 year, respectively. This suggests that CR may have evolved to NL, which can be justified by the fact that chronic inflammation in an important contributor for the development of NL, especially B-cell lymphoma (Mukaratirwa et al. 2001; Day et al. 2004; Vail and Pinkerton 2020).

As for potential viral and bacterial respiratory pathogens, some CR cases were tested for both viruses and bacteria unlike the NL cases, which were only tested for FIV and FeLV. In the CR group, only eight cats were tested for other pathogens, with two testing positive for FHV-1 and one positive for FCV. Although FHV-1 and FCV are common viruses, they usually have a higher prevalence in catteries, shelters, and multi-cat households (Van Pelt and Lappin 1994; Sykes 2022) and almost every cat of this study was indoor-only and lived alone. Another reason can be that these viruses are known to enter latency and are often undetectable after the early infection stages are over (Nelson and Couto 2019; Sykes 2022; Ettinger et al. 2024). Testing for these two viruses was performed through PCR for FHV-1 and rt-PCR for FCV, on superficial oronasal swabs. These may have a low sensitivity due to brief shedding or low-level shedding in chronic infections. For a more accurate diagnosis, testing samples from multiple anatomical sites or combining them with other diagnostic tests (e.g., virus isolation, serology) can increase sensitivity. For FCV, assays may not detect all virus strains, and attenuated live vaccine virus can be detected after vaccination; thus, sensitivity and specificity may vary depending on assay design and laboratory (Sykes 2022). Only one cat was positive for *Mycoplasma felis*, in co-infection with FHV-1; this corroborates the fact that viral infection can lead to secondary bacterial infection (Henderson et al. 2003; Johnson et al. 2005; Zachary 2022).

For FIV and FeLV, only two CR cats were FeLV-positive and one was FIV-positive, despite over 50% of the cats having a known FIV/FeLV status. As mentioned before, FIV and FeLV can lead to CR due to their immunosuppressive effect and to infection with opportunistic pathogens (Nelson and Couto 2019; Bezerra et al. 2024). This was observed in one case, in which the animal was co-infected with FHV-1 and *Mycoplasma felis*. As for NL cases, only four cats were tested, and only one was positive for FIV, and one for both FIV and FeLV. This

screening was performed by ELISA, which is the gold standard assay for these viruses (Maggs et al. 1999; Little 2012; Ettinger et al. 2024), validating accuracy of the results.

Approximately 46% of cats who underwent CT scanning had a final diagnosis consistent with the imaging assessment. Although not all animals underwent CT scanning during their clinical investigation, analysis of the relationship between CT conclusions and the final diagnosis using Cohen's Kappa coefficient indicated a fair level of agreement between the two variables. This suggests that while there is some consistency between CT scan interpretation and the definitive diagnosis, the agreement is not strong and may be influenced by other factors, such as interpretative variability or overlapping features between diseases. Approximately 30% of cases had an inconclusive CT scan diagnosis, which justifies the need for other exams and tests in order to achieve a definitive diagnosis. These findings highlight the limitations of relying solely on CT imaging for diagnostic decision-making in this context. They also underline the need for a comprehensive diagnostic approach that integrates imaging findings with clinical history, physical examination, and additional diagnostic tests, to enhance diagnostic accuracy.

Regarding CT scan, a mass was identified in 83.3% (20/24) of cats, which was a much higher incidence than the seven (7/35; 20%) cases in which a mass was identified during rhinoscopy. Of the animals with both a CT scan and rhinoscopy, nine (9/18; 50%) had a mass reported in CT that was not found during rhinoscopy. This is supported by a previous study that demonstrated that CT scanning can be helpful when done prior to rhinoscopy, as all cases that had CT done first, a mass was identified, compared to the 41% of cases where rhinoscopy alone was performed (Harris et al. 2014). As for lymph nodes, only one (1/35; 2.9%) case reported lymphadenomegaly during rhinoscopy, while enlarged lymph nodes were found in sixteen (16/24; 66.7%) animals in the CT scan.

Rhinoscopy findings for both diseases include hyperemic, irregular or friable mucosa, visible masses, mucopurulent and/or hemorrhagic discharge, lymphoid nodule hypertrophy, destruction of turbinates or other bone structures, and ulcerative lesions (Johnson 2020; Batalla et al. 2021). This corroborates the observations in this study, since all cats had an erythematous mucosa, mucosal friability, thickened or lysed turbinates, mucopurulent discharge, while a mass was identified in five cats with CR and two cats with NL. Rhinoscopy findings are not diagnostic and different nasal diseases can have similar findings (Batalla et al. 2021), as demonstrated by this study; and even if the mucosa has a normal appearance during rhinoscopy, histologically it can reveal moderate to severe inflammation (Johnson et al. 2004).

In every case, histopathology revealed both morphological and inflammatory criteria consistent with some degree of chronic inflammation, especially in CR cases, which were predominantly (85.7%) chronic lymphoplasmacytic rhinitis cases.

Cases of nasal lymphoma exhibited more noticeable structural alterations, as previously reported (Santagostino et al. 2015). Most cases displayed epithelial erosion and

distortion throughout the mucosa. On the other hand, in cases with CR, these changes were only seen at a superficial level, specifically in the epithelium and overall tissue integrity was unaffected. In CR, changes frequently attributed to chronic insult to the mucosa, like hyperplasia, metaplasia and erosion (Raskin et al. 2021), were also seen, although at a superficial level. This implies that while CR mostly affects the superficial layers of the mucosa, preserving underlying tissue architecture, NL has a more broad and invasive structural influence. Atypical cells were only found in NL cases, as previously reported by Raskin et al. (2021).

Regarding inflammation, in cases of CR it made sense to evaluate the epithelium and lamina propria separately, as the integrity of the tissue was preserved in most samples, unlike in cases of NL. Notably, in CR, most inflammatory cells were concentrated in the lamina propria, perhaps due their natural presence there, along with nasal MALT and lymphoid follicles, as described in available literature (Junqueira and Carneiro 2017; Raskin et al. 2021). In both diseases, lymphocytes and plasma cells were the most prevalent inflammatory cells, which can be justified by the fact that lymphoplasmacytic inflammation is more common in chronic cases (Kuehn 2006). This kind of inflammation can be seen accompanying NL, and it is severe in most cases (Kuehn 2006; Santagostino et al. 2015). However, in this study, CR cases showed more severe inflammation, contrary the NL cases, where lymphoplasmacytic inflammation was mostly mild. Although NL is often characterized by a monomorphic population of medium to large-sized immature lymphoid cells, some lymphoma variants can be composed of small, well-differentiated lymphocytes (Raskin et al. 2021) and, in these cases, immune phenotyping can be useful, as well as clonality assays (Vail et al. 2020). Eosinophils were observed exclusively in CR cases, which could suggest an allergic component to the disease in these cases; one of these samples also contained mast cells, which is also suggestive of allergy (Raskin et al. 2021). Neutrophils were present in most samples from both groups; however, 50% of NL cases exhibited more severe inflammation. This can indicate bacterial infection; however, bacterial pathogens were not tested for, nor seen in histopathology of NL cases. This type of inflammation mostly affected the epithelium, which aligns with the fact that this type of cell is usually recruited to the surface to help eradicate possible pathogens (Kuehn 2006; Day 2011). These findings suggest that the inflammatory and structural changes in CR are less severe and more variable, while NL involves more aggressive tissue changes.

Regarding bacteria, they were only occasionally observed, and only in CR cases. Macrophages were present at a higher number in CR cases. Given the degree of inflammation and loss of tissue integrity, one might have expected a higher occurrence of infectious agents in both CR and NL (Nelson and Couto 2019; Raskin et al. 2021; Zachary 2022). However, it is important to note that most animals had already undergone at least one course of antibiotics, which could have reduced pathogen load.

Immune phenotyping of the samples revealed distinct patterns of lymphocyte distribution between cases of CR and NL, shedding light on the immune landscape and cellular dynamics of these diseases (Vail et al. 2020). In CR, 96.2% of the samples were positive for T lymphocytes while 61.5% of the samples were positive for B lymphocytes. This indicates a predominant role of T cells in the inflammatory process, with varying levels of infiltration reflecting differences in immune activation and chronicity; T cells are more predominant in chronic inflammation and in the presence of pathogens, while B cells are usually more common in acute infections that lead to production of antibodies (Murphy and Weaver 2016). The high number of T cells in CR cases may also be associated with the abundance of macrophages, as these cells are crucial for T-cell activation through antigen presentation and were more prevalent in CR cases (Murphy and Weaver 2016). Although few bacteria were observed, it is possible that they had already been phagocytosed by the macrophages.

The distribution across staining score for both T and B lymphocytes highlights a potential gradient in immune activity, where milder cases (grade 1) demonstrate a relatively higher proportion of B cells, possibly reflecting an early or active adaptive immune response. Conversely, more severe cases (grade 2 and 3) show a dominance of T cells, consistent with prolonged or chronic inflammatory processes, where cellular immunity plays a central role. In contrast, all NL cases exhibited immunoreactivity for both T and B lymphocytes, mostly within grade 1. This uniformity suggests a distinct and likely neoplastic process, where both lymphocyte subsets are present. Malignant transformation of cells of the lymphoid lineage can be an explication to this finding (Tizard 2017); a clonality assay should be performed in those cases to distinguish between normal or neoplastic lymphocytes (Vail et al. 2020).

Statistically, CD20/Pax5-positive inflammatory lymphocytes were more prevalent in NL than in CR, which may suggest that the presence of this marker is more indicative of this condition.

The findings regarding NL samples demonstrate a monomorphic B-cell neoplastic population, typical of B-cell lymphomas. Among these, 71.4% of the samples were positive for neoplastic B lymphocytes, distributed between staining scores 3 (14.3%) and 4 (57.1%). This aligns with what has already been reported, that B-cell immune phenotypes predominate in about 75% of cases (Vail et al. 1998; Kuehn 2006; Little et al. 2007; Haney et al. 2009; Taylor et al. 2009; Santagostino et al. 2015; Vail and Pinkerton 2020); B-cell lymphomas have a better prognosis than T-cell lymphomas, which have a poor response to chemotherapy and higher relapse rates (Lei et al. 1999).

Statical analysis showed that MLH1 may play a crucial role in differentiating between CR and NL, since MLH1 expression had a notable association with the diagnosis. NL had a substantially more frequent (85.7%) MLH1 expression than CR (19.2%). This supports the potential of MLH1 as a marker to differentiate between CR and NL, as a higher staining score of MLH1 in a sample is significantly more suggestive of the latter than of the former. During

mitosis, this MMR protein plays a crucial part in post-replication DNA mismatch repair since some DNA damage might naturally occur during DNA replication (Li 2008; Aberdein et al. 2012) and, while, it is expressed in normal tissues undergoing replication, expression may be stronger and more prevalent in tumors (especially in high-grade ones), due to increased cell replication (Aberdein et al. 2012; Torlakovic et al. 2015).

MSH6 was positive in similar percentages in inflammatory lymphoid cells of both NL (42.9%) and CR (30.8%) cases. In neoplastic cells, a high percentage of cases was MSH6-positive (71.4%), with high staining scores (3 and 4), which again is consistent with what is reported for MMR proteins and their stronger expression in high-grade tumors, making these markers prognostic factors as well (Aberdein et al. 2012; Torlakovic et al. 2015).

STAT5 expression was not statistically different in inflammatory cells in CR (57.7%) and NL (71.4%) cases. However, its presence was significantly higher in neoplastic cells, with approximately 86% of samples expressing STAT5 and approximately 43% with a staining score of 4. This supports what was already reported for other tumors; STAT5 encourages carcinogenesis and tumor growth by interacting with other cellular pathways and setting off additional processes that accelerate the course of disease (Morcinek et al. 2002; Burchill et al. 2007; Kieslinger et al. 2019; Polak et al. 2019; Kieslinger et al. 2021). Inhibiting STAT5 could represent an interesting mechanism for treatment of animals with NL, given its high prevalence in these cases and its association with more aggressive tumors when highly expressed (Burke et al. 2001; Yao et al. 2005; Polak et al. 2019). Another finding in this study, was that STAT5 expression in neoplastic cells was exclusively cytoplasmic, while in inflammatory lymphoid cells it was exclusively nuclear, making this marker useful to distinguish between normal lymphoid cells and well-differentiated neoplastic ones.

In this study, one animal was initially diagnosed with CR through histopathology but, 21 days later, was diagnosed with NL via both histopathology and IHC. IHC was performed on both samples, revealing a transition to malignancy through strong STAT5, MLH1 and MSH6 expression in neoplastic cells. This case highlights the critical importance of IHC in diagnostics, especially in cases where uncertainty exists or the distinction between CR and NL is particularly subtle. Two scenarios could explain this finding: either there was a progression from CR to NL due to chronic inflammation, as the animal had been exhibiting severe clinical signs for at least 84 days, or errors may have occurred during the processing of the sample for histopathology.

Treatment approach was different depending on the disease. For CR cases, treatment involved corticosteroids or NSAIDs, and antibiotics; this aligns with previously reported approaches for CR (Nelson and Couto 2019; Ettinger et al. 2024). Even though only two cases of CR were positive for bacterial infection, 66.7% did a course of antibiotic. This may be explained by the presence of purulent or mucopurulent nasal discharge in almost every case, as documented in previous cases of bacterial infection (Van Pelt and Lappin 1994; Nelson and

Couto 2019; Ettinger et al. 2024). Doxycycline was the most prescribed antimicrobial and usually the first-line option, as suggested by the ISCAID guidelines (Lappin et al. 2017). Due to their potent anti-inflammatory effect (Van Pelt and Lappin 1994; Nelson and Couto 2019; Ettinger et al. 2024), corticosteroids were used in more than 90% of CR cases, and eleven cats took them chronically (orally and/or inhaled) to manage clinical signs. One cat, FHV-1 and FeLV-positive, had a course of interferon followed by improvement of clinical signs (Mari et al. 2004; Pedretti et al. 2006).

All cases of NL with available treatment information were treated with prednisolone. This was administered alone, as part of a chemotherapy protocol or combined with a chemotherapy drug, which is in agreement with what the literature recommends (Taylor et al. 2009; Vail and Pinkerton 2020). Only one cat was administered steroids combined with an antibiotic; treatment was not being effective at the time that follow-up was lost.

Although radiotherapy showed to be more efficient and the first approach choice (Nagata et al. 2014; Vail and Pinkerton 2020), it is not available in our country and was not recommended by the clinicians, probably due to its high cost. However, in the present study, statical analysis did not indicate an association between the treatment and the outcome. The main objective of exploring a possible association was to identify a possible combination of medication that would lead to a better outcome. However, the majority of cats (80%) with NL were treated with corticosteroids (oral and/or inhaled), which is probably by the fact that this are a potent anti-inflammatory drug that reduces the chronic inflammation while alleviating the clinical signs and can be used chronically in low doses (Nelson and Couto 2019; Ettinger et al. 2024), as well as induces killing of hematopoietic cancer cells (Vail et al. 2020).

Diagnosis also impacts the outcome, since in this study positive outcomes (resolved, controlled with treatment) were mostly seen in CR cases. As mentioned before, chronic management can be required in most cases of CR (Nelson and Couto 2019; Ettinger et al. 2024), and this study showed that 68% achieved symptom control with medication. A quarter (25%) of cases were fully recovered, which is not common; however this can be due to antibiotic efficacy against secondary bacterial infections, which can improve clinical signs (Ettinger et al. 2024), or may only represent a temporary improvement in clinical signs and may eventually result in future relapse; the vast majority of animals experience phases of exacerbation of the chronic disease, which is then treated with medication, waxing and waning in a continuous cycle (Kuehn 2006). One of the cats that was apparently cured was FHV-1-positive and may have entered the latent stage of infection, becoming asymptomatic as a result (Nelson and Couto 2019; Sykes 2022; Ettinger et al. 2024). Due to chronic inflammation, masses or polyps can emerge from the mucosa, obstructing air flow; in those cases, surgery may be necessary (Batalla et al. 2021). In the present study, besides medical treatment, one cat had a ventral rhinotomy with polyp removal, resulting in complete recovery.

Although cats who received chemotherapy have a 75% chance of achieving complete remission (Taylor et al. 2009; Vail and Pinkerton 2020), in the present study, only one cat was alive 1418 days later (last contact), controlled with treatment; two other cats that were receiving chemotherapy were euthanized or died of unknown causes; another two cats showed poor response to treatment, although follow-up was lost. This can be due to individual variability, as different animals can respond differently to the same chemotherapy protocol, because of tumor characteristics that may lead to resistance or sensitivity to certain drugs (Vail et al. 2020).

Statical analysis suggested that the diagnosis significantly influenced disease outcome underscoring the importance of an accurate diagnosis, as it can directly impact treatment decisions and prognosis. Thus, even though CR management can sometimes be a challenge, it is possible to manage the disease effectively throughout the animal's lifetime with appropriate treatment, either by increasing or adding new drugs to control inflammation or by treating secondary infections with the use of different antibiotics (Nelson and Couto 2019; Ettinger et al. 2024). In contrast, NL typically offers a survival time of approximately two years following diagnosis, though the probability of metastasis is low (20%), even when achieving complete remission with either radiotherapy or chemotherapy (Zwahlen et al. 1998; Taylor et al. 2009; Vail and Pinkerton 2020); however, individual animals may respond differently to the same chemotherapy protocol, even when it is the most effective one studied. This variability is attributed to the unique characteristics of each tumor, which may render it resistant to certain drugs and increased sensitivity to others (Vail et al. 2020).

The statical analysis results underscores the importance of accurate diagnosis, as it can directly impact treatment decisions and prognoses, since it is different according to the disease. CR can often be managed effectively throughout the animal's lifetime with appropriate treatment (Nelson and Couto 2019; Ettinger et al. 2024).

In CR cases, prognosis was generally good, as most responded well to therapy, with only one death reported (from unknown causes). In this study, prognosis for NL cases was poor to fair with a survival time of lower than 1.5 years (median 423.4 days; range 16-1313 days), compared to the 2-year survival rate reported for cats with NL treated with chemotherapy (Taylor et al. 2009; Vail and Pinkerton 2020). Some prognostic factors for NL can also influence the outcome, such as: epitheliotropism, which is a positive prognostic factor and usually associated with B-cell lymphomas; the age at diagnosis, as younger cats or cats older than 10 years usually have a negative prognosis (Santagostino et al. 2015); other co-morbidities like infection with viruses (FIV, FeLV, FHV-1, FCV) and opportunistic bacteria (*Chlamydophila felis*, *Mycoplasma felis*), that increase the chances of developing NL at an earlier age (FIV and/or FeLV) due to immunosuppression and/or chronic inflammation (Rojko and Hardy Jr 1994; Nelson and Couto 2019; Macy 2020), or of having chronic clinical signs (Nelson and Couto 2019); and a T-cell phenotype NL, which has a poor response to chemotherapy and higher relapse rates (Lei et al. 1999). It is worth noting that the cat with NL

that is still alive today has B-cell NL and is 15 years old. Statistical analysis suggests that, according to the available data, the diagnosis of CR or NL does not have a substantial impact on survival times in these groups. However, the survival time was defined as the period between the date of diagnosis and the date of the last recorded status of the animal, indicating whether it was cured, managed with treatment, had died, or was euthanized. In this case, the mean survival time for CR may have been underestimated due to the fact that some animals were either cured or had their condition controlled with medication within a short period. Conversely, for NL, this value may have been overestimated due to the small sample size of the population.

## 3.5. Limitations

The primary limitation of this study was the reduced sample size, as well as the discrepancy between the two groups.

Since all data collection and clinical history are based on records written sometimes by different veterinarians of the Veterinary Teaching Hospital, information was not standardized nor consistently written, as different individuals may record varying observations, which can be subjective. Several animals were followed by the Internal Medicine Service, which minimizes the loss of information, as a detailed report was always made in every consultation and/or exam. However, these animals had routine consultations and sometimes were admitted to emergency rooms due to respiratory symptoms, or even needed to be hospitalized, which means that the evaluation of clinical signs was often done by different clinicians. This resulted in information loss or a more subjective assessment of the severity of clinical signs and response to treatment displayed by various animals. Some animals were referred for rhinoscopy only, so information regarding medication, follow-up and outcome was not available.

## 3.6. Conclusions

In conclusion, this study provides a comprehensive analysis of the challenges associated with diagnosing and managing CR and NL in cats, emphasizing the importance of accurate diagnostic methods to guide treatment strategies.

In addition to the clinical signs of CR and NL being similar, the results of rhinoscopy, imaging, and patient history also overlap in both diseases and the need for additional diagnostic methods or markers often arises. This study emphasized that age is related to diagnosis, as young animals tend to be diagnosed with inflammatory disease, such as CR, while older cats tend to present with NL. However, this study also showed that younger animals can develop NL in case of concomitant infection with FIV and/or FeLV, making this an important screening test in all cats with upper respiratory tract clinical signs. It also reported

the lack of screening for other respiratory pathogens, such as FHV-1, FCV, *Chalmydophila felis* and *Mycoplasma felis*, which have an important role in the management of CR.

The study also underscores the limitations of standalone diagnostic tools such as CT scans and the need for a multimodal diagnostic approach integrating imaging, histopathology, and molecular analyses, as more than 50% of cases were either incorrectly diagnosed or inconclusive on CT. However, 50% identified a mass that was not seen during rhinoscopy, emphasizing the importance of CT scanning prior to rhinoscopy in order to guide biopsies.

As for rhinoscopy, it was confirmed that both diseases can look the same, and that it does not always reflect the severity of the disease, highlighting the need for further tests, such as nasal biopsies histopathology and IHC.

Histopathological examination was revealed to be very useful in differentiating these conditions, since CR cases demonstrated predominantly chronic inflammatory processes with milder structural alterations, while NL was characterized by more aggressive tissue destruction and a distinct lymphoid neoplastic profile. Immunophenotyping and molecular markers, particularly CD20/Pax5 and MLH1, emerged as valuable tools for differentiating these conditions, underscoring their potential for enhancing diagnostic accuracy. STAT5 and MSH6, although not statistically significant, showed potential in diagnosing NL as well. The type of cells observed can help to determine the etiology of the disease, however this study showed that there is not always an heterogenous population in CR nor a homogenous one in NL, which underlines the need for IHC. It was also confirmed that most NL are of a B-cell phenotype.

Treatment outcomes varied significantly, with CR cases generally benefiting from anti-inflammatory therapies and secondary infection control, whereas NL cases exhibited variable responses to chemotherapy, influenced by individual tumor characteristics. These results reinforce the critical role of precise diagnosis in tailoring therapeutic interventions to improve patient outcomes, while also acknowledging the challenges posed by individual variability and disease chronicity. It is important to note that none of the other measures to improve quality of life, such as nebulization or warmed food, were implemented.

Future research should focus on expanding sample sizes and exploring advanced diagnostic and therapeutic modalities to further optimize care for feline patients with these complex nasal diseases.

## 4. Bibliography


Aberdein D, Munday JS, Howe L, French AF, Gibson IR. 2012. Widespread Mismatch Repair Expression in Feline Small Intestinal Lymphomas. J Comp Pathol. 147(1):24–30. doi:10.1016/j.jcpa.2011.08.009.

Ahmad S, Levy LS. 2010. The frequency of occurrence and nature of recombinant feline leukemia viruses in the induction of multicentric lymphoma by infection of the domestic cat with FeLV-945. Virology. 403(2):103–10. doi:10.1016/j.virol.2010.04.011.

Aspinall V, Cappello M. 2019. Respiratory system. In: Introduction to Animal and Veterinary Anatomy and Physiology. 4th ed. Wallingford: CABI. p. 93–101.

August JR. 1991. Husbandry practices for cats infected with feline leukemia virus or feline immunodeficiency virus. J Am Vet Med Assoc. 199(10):1474–7.

Batalla M, Brandão F, Storz K, Byron JK, Chamness CJ, Chernov A, De Lucia M, England GCW, Hall EJ, Innes JF, et al. 2021. BSAVA Manual of Canine and Feline Endoscopy and Endosurgery. 2nd ed. Lhermette P, Sobel D, Robertson E, editors. BSAVA.

Beatty J, Terry A, MacDonald J, Gault E, Cevario S, O'Brien SJ, Cameron E, Neil JC. 2002. Feline immunodeficiency virus integration in B-cell lymphoma identifies a candidate tumor suppressor gene on human chromosome 15q15. Cancer Res. 62(24):7175–80.

Bezerra JAB, Limeira CH, Maranhão ACP de M, Antunes JMA de P, Azevedo SS de. 2024. Global seroprevalence and factors associated with seropositivity for feline immunodeficiency virus (FIV) in cats: A systematic review and meta-analysis. Prev Vet Med. 231:106315. doi:10.1016/j.prevetmed.2024.106315.

Bol S, Bunnik EM. 2015. Lysine supplementation is not effective for the prevention or treatment of feline herpesvirus 1 infection in cats: a systematic review. BMC Vet Res. 11:284. doi:10.1186/s12917-015-0594-3.

Bromberg J. 2002. Stat proteins and oncogenesis. Journal of Clinical Investigation. 109(9):1139–42. doi:10.1172/jci200215617.

Buermeyer AB, Deschênes SM, Baker SM, Liskay RM. 1999. Mammalian DNA Mismatch Repair. Annu Rev Genet.(33):533–64.

Burchill MA, Yang J, Vogtenhuber C, Blazar BR, Farrar MA. 2007. IL-2 Receptor β-Dependent STAT5 Activation Is Required for the Development of Foxp3+ Regulatory T Cells. The Journal of Immunology. 178(1):280–90. doi:10.4049/jimmunol.178.1.280.

Burke WM, Jin X, Lin H-J, Huang M, Liu R, Reynolds RK, Lin J. 2001. Inhibition of constitutively active Stat3 suppresses growth of human ovarian and breast cancer cells. Oncogene. 20(55):7925–34. www.nature.com/onc.

Caniatti M, Roccabianca P, Ghisleni G, Mortellaro CM, Romussi S, Mandelli G. 1998. Evaluation of brush cytology in the diagnosis of chronic intranasal disease in cats. Journal of Small Animal Practice. 39(2):73–7. doi:10.1111/j.1748-5827.1998.tb03597.x.

Cape L. 1992. Feline idiopathic chronic rhinosinusitis: a retrospective study of 30 cases. American Animal Hospital Association. 28(2):149–155.

Carreras JK, Goldschmidt M, Lamb M, McLear RC, Drobatz KJ, Sørenmo KU. 2003. Feline epitheliotropic intestinal malignant lymphoma: 10 cases (1997-2000). J Vet Intern Med. 17(3):326–31. doi:10.1111/j.1939-1676.2003.tb02456.x.

Chino J, Fujino Y, Kobayashi T, Kariya K, Goto-Koshino Y, Ohno K, Nakayama H, Tsujimoto H. 2013. Cytomorphological and immunological classification of feline lymphomas: clinicopathological features of 76 cases. Journal of Veterinary Medical Science. 75(6):701–707. doi:10.1292/jvms.12-0246.

Chueh FY, Leong KF, Yu CL. 2010. Mitochondrial translocation of signal transducer and activator of transcription 5 (STAT5) in leukemic T cells and cytokine-stimulated cells. Biochem Biophys Res Commun. 402(4):778–83. doi:10.1016/j.bbrc.2010.10.112.

Cirstoiu-Hapca A, Bossy-Nobs L, Buchegger F, Gurny R, Delie F. 2007. Differential tumor cell targeting of anti-HER2 (Herceptin®) and anti-CD20 (Mabthera®) coupled nanoparticles. Int J Pharm. 331(2):190–6. doi:10.1016/j.ijpharm.2006.12.002.

Copie-Bergman C, Niedobitek G, Mangham DC, Selves J, Baloch K, Diss TC, Knowles DN, Delsol G, Isaacson PG. 1997. Epstein-Barr virus in B-cell lymphomas associated with chronic suppurative inflammation. J Pathol. 183(3):287–92. doi:10.1002/(SICI)1096-9896(199711)183:3<287::AID-PATH932>3.0.CO;2-Q.

Cotter SM. 1992. Feline leukemia virus: pathophysiology, prevention, and treatment. Cancer Invest. 10(2):173–81. doi:10.3109/07357909209032778.

Creamer BA, Sakamoto K, Schmidt JW, Triplett AA, Moriggl R, Wagner K-U. 2010. Stat5 promotes survival of mammary epithelial cells through transcriptional activation of a distinct promoter in Akt1. Mol Cell Biol. 30(12):2957–70. doi:10.1128/mcb.00851-09.

Cuilliere-Dartigues P, Fabiani B, Dumont S, Copie-Bergman C, Couvelard A, Molina T, Duval A, Flejou JF. 2007. Absence of mismatch repair deficiency in gastric lymphoma: an immunohistochemical study of mlh1 and msh2 protein expression. Virchows Archiv. 451(5):983–4. doi:10.1007/s00428-007-0500-x.

Day MJ. 2011. Clinical Immunology of the Dog and Cat. 2nd ed. Day MJ, editor. Manson Publishing Ltd. www.mansonpublishing.com.

Day MJ, Henderson SM, Belshaw Z, Bacon NJ. 2004. An immunohistochemical investigation of 18 cases of feline nasal lymphoma. J Comp Pathol. 130(2–3):152–61. doi:10.1016/j.jcpa.2003.10.004.

Denson KWE. 2003. Re: Environmental tobacco smoke and risk of malignant lymphoma in pet cats. Am J Epidemiol. 158(12):1227–8. doi:10.1093/aje/kwg269.

Detweiler DA, Johnson LR, Kass PH, Wisner ER. 2006. Computed tomographic evidence of bulla effusion in cats with sinonasal disease: 2001-2004. J Vet Intern Med. 20(5):1080–4. doi:10.1892/0891-6640(2006)20[1080:cteobe]2.0.co;2.

Díaz Martínez E, Arencibia Espinosa A, Soler Laguía M, Kilroy D, Martínez Gomariz F, Casas García DL, Sánchez Collado C, Gil Cano F, Jaber JR, Ramírez Zarzosa G. 2024. An Anatomical Study Using Computed Tomography, Magnetic Resonance Imaging, and Rhinoscopy of the Nasal Cavity of Domestic Cat (Felis silvestris catus L.) and Big Cats: Lion (Panthera leo leo L.), Leopard (Panthera pardus kotiya L.), and Cheetah (Acinonyx jubatus jubatus S.). Animals. 14(8):1172. doi:10.3390/ani14081172.

Drees R, Forrest LJ, Chappell R. 2009. Comparison of computed tomography and magnetic resonance imaging for the evaluation of canine intranasal neoplasia. J Small Anim Pract. 50(7):334–40. doi:10.1111/j.1748-5827.2009.00729.x.

Dukers DF, Oudejans JJ, Jaspars EH, Gras M, Vos W, Middeldorp JM, Meijer CJ, Bloemena E. 2000. All infiltrating T-lymphocytes in Hodgkin's disease express immunohistochemically detectable T-cell receptor zeta-chains in situ. Histopathology. 36(6):544–50. doi:10.1046/j.1365-2559.2000.00909.x.

Duncan C, Stephen C, Lester S, Bartlett K. H. 2005. Sub-clinical infection and asymptomatic carriage of Cryptococcus gattii in dogs and cats during an outbreak of cryptococcosis. Med Mycol. 43(6):511–6. doi:10.1080/13693780500036019.

Dworzak MN, Schumich A, Printz D, Pötschger U, Husak Z, Attarbaschi A, Basso G, Gaipa G, Ratei R, Mann G, et al. 2008. CD20 up-regulation in pediatric B-cell precursor acute lymphoblastic leukemia during induction treatment: setting the stage for anti-CD20 directed immunotherapy. Blood. 112(10):3982–8. doi:10.1182/blood-2008-06-164129.

Elmslie RE, Ogilvie GK, Gillette EL, McChesney-Gillette S. 1991. Radiotherapy with and without chemotherapy for localized lymphoma in 10 Cats. Veterinary Radiology. 32(6):277–80. doi:10.1111/j.1740-8261.1991.tb00121.x.

Endo Y, Cho KW, Nishigaki K, Momoi Y, Nishimura Y, Mizuno T, Goto Y, Watari T, Tsujimoto H, Hasegawa A. 1997. Molecular characteristics of malignant lymphomas in cats naturally infected with feline immunodeficiency virus. Vet Immunol Immunopathol. 57(3–4):153–67. doi:10.1016/s0165-2427(97)00004-4.

Ettinger Stephen J, Feldman EC, Côté E. 2024. Ettinger's Textbook of Veterinary Internal Medicine. 9th ed. Elsevier Health Sciences.

Ettinger Stephen J., Feldman EC, Côté E. 2024. Ettinger's Textbook of Veterinary Internal Medicine. 9th ed. Elsevier Health Sciences.

Ferreri AJM, Ernberg I, Copie-Bergman C. 2009. Infectious agents and lymphoma development: molecular and clinical aspects. J Intern Med. 265(4):421–38. doi:10.1111/j.1365-2796.2009.02083.x.

Ford RB. 1997. Feline viral upper respiratory infection. Supplement to Compendium of Continuing Education Practice. 19(3):16–20.

Frank DA. 2007. STAT3 as a central mediator of neoplastic cellular transformation. Cancer Lett. 251(2):199–210. doi:10.1016/j.canlet.2006.10.017.

Fujino Y, Liao C-P, Zhao YS, Pan J, Mathes LE, Hayes KA, Ohno K, Tsujimoto H, Roy-Burman P. 2009. Identification of a novel common proviral integration site, flit-1, in feline leukemia virus induced thymic lymphoma. Virology. 386(1):16–22. doi:10.1016/j.virol.2009.01.021.

Fujino Y, Ohno K, Tsujimoto H. 2008. Molecular pathogenesis of feline leukemia virus-induced malignancies: insertional mutagenesis. Vet Immunol Immunopathol. 123(1–2):138–43. doi:10.1016/j.vetimm.2008.01.019.

Fujino Y, Satoh H, Ohno K, Tsujimoto H. 2010. Molecular cytogenetic analysis of feline leukemia virus insertions in cat lymphoid tumor cells. J Virol Methods. 163(2):344–52. doi:10.1016/j.jviromet.2009.10.021.

Fujiwara-Igarashi A, Fujimori T, Oka M, Nishimura Y, Hamamoto Y, Kazato Y, Sawada H, Yayoshi N, Hasegawa D, Fujita M. 2014. Evaluation of outcomes and radiation complications in 65 cats with nasal tumours treated with palliative hypofractionated radiotherapy. Veterinary Journal. 202(3):455–61. doi:10.1016/j.tvjl.2014.09.016.

Gabler WL, Creamer HR. 1991. Suppression of human neutrophil functions by tetracyclines. J Periodontal Res. 26(1):52–8. doi:10.1111/j.1600-0765.1991.tb01626.x.

Gaskell RM, Wardley RC. 1978. Feline viral respiratory disease: a review with particular reference to its epizootiology and control. J Small Anim Pract. 19(1):1–16. doi:10.1111/j.1748-5827.1978.tb05452.x.

Gerou-Ferriani M, McBrearty AR, Burchmore RJ, Jayawardena KGI, Eckersall PD, Morris JS. 2011. Agarose gel serum protein electrophoresis in cats with and without lymphoma and preliminary results of tandem mass fingerprinting analysis. Vet Clin Pathol. 40(2):159–73. doi:10.1111/j.1939-165X.2011.00310.x.

Getty R. 1975. Sisson and Grossman's The Anatomy of the Domestic Animals: Volume II. 5th ed. W. B. Saunders.

Grivennikov SI, Greten FR, Karin M. 2010. Immunity, inflammation, and cancer. Cell. 140(6):883–99. doi:10.1016/j.cell.2010.01.025.

Hammer SE, Groiss S, Fuchs-Baumgartinger A, Nedorost N, Gress V, Luckschander-Zeller N, Saalmüller A, Schwendenwein I, Rütgen BC. 2017. Characterization of a PCR-based lymphocyte clonality assay as a complementary tool for the diagnosis of feline lymphoma. Vet Comp Oncol. 15(4):1354–69. doi:10.1111/vco.12277.

Haney SM, Beaver L, Turrel J, Clifford CA, Klein MK, Crawford S, Poulson JM, Azuma C. 2009. Survival analysis of 97 cats with nasal lymphoma: a multi-institutional retrospective study (1986-2006). J Vet Intern Med. 23(2):287–294. doi:10.1111/j.1939-1676.2008.0243.x.

Harris BJ, Lourenço BN, Dobson JM, Herrtage ME. 2014. Diagnostic accuracy of three biopsy techniques in 117 dogs with intra-nasal neoplasia. J Small Anim Pract. 55(4):219–24. doi:10.1111/jsap.12187.

Hartmann K, Werner RM, Egberink H, Jarrett O. 2001. Comparison of six in-house tests for the rapid diagnosis of feline immunodeficiency and feline leukaemia virus infections. Vet Rec. 149(11):317–20. doi:10.1136/vr.149.11.317.

Harvey A, Tasker S. 2013. BSAVA Manual of Feline Practice: A Foundation Manual. 1st ed. British Small Animal Veterinary Association.

Hawkins EC. 1988. Chronic viral upper respiratory disease in cats: differential diagnosis and management. The Compendium on continuing education for the practicing veterinarian. 10(9):1003–1012.

Henderson SM, Bradley K, Day MJ, Tasker S, Caney SMA, Hotston Moore A, Gruffydd-Jones TJ. 2003. Investigation of nasal disease in the cat - a retrospective study of 77 cases. J Feline Med Surg. 6(4):245–57. doi:10.1016/j.jfms.2003.08.005.

Henrich M, Hecht W, Weiss AT, Reinacher M. 2009. A new subgroup of immunoglobulin heavy chain variable region genes for the assessment of clonality in feline B-cell lymphomas. Vet Immunol Immunopathol. 130(1–2):59–69. doi:10.1016/j.vetimm.2009.01.006.

Holland M, Hudson J, editors. 2020. Feline Diagnostic Imaging. John Wiley & Sons, Inc.

Hudson L, Hamilton W. 2010. Atlas of Feline Anatomy for Veterinarians. 2nd ed. Teton NewMedia.

Hutson CA, Rideout BA, Pedersen NC. 1991. Neoplasia associated with feline immunodeficiency virus infection in cats of southern California. J Am Vet Med Assoc. 199(10):1357–62.

Jacobs RM, Messick JB, Valli VE. 2016. Tumors of the hemolymphatic system: lymphoid tumors. In: Meuten DJ, editor. Tumors in Domestic Animals. 5th ed. John Wiley & Sons. p. 203–321.

Johnson LR. 2003. Update on feline rhinosinusitis. In: Proceedings 21st Annual ACVIM Forum, American College of Veterinary Internal Medicine, Lakewood, CO.

Johnson LR. 2020. Canine and Feline Respiratory Medicine. 2nd ed. John Wiley & Sons, Inc.

Johnson LR, Clarke HE, Bannasch MJ, De Cock HE V. 2004. Correlation of rhinoscopic signs of inflammation with histologic findings in nasal biopsy specimens of cats with or without upper respiratory tract disease. J Am Vet Med Assoc. 225(3):395–400. doi:10.2460/javma.2004.225.395.

Johnson Lynelle R, Foley JE, De Cock HE V, Clarke HE, Maggs DJ. 2005. Assessment of infectious organisms associated with chronic rhinosinusitis in cats. J Am Vet Med Assoc. 227(4):579–585.

Johnson Lynelle R., Hilde E, Jane E, Philip H, David J, Christian M. 2005. Cytokine gene transcription in feline nasal tissue with histologic evidence of inflammation. Am J Vet Res. 66(6):996–1001. doi:10.2460/ajvr.2005.66.996.

Junqueira LC, Carneiro J. 2017. Histologia básica: texto e atlas. 13th ed. Guanabara Koogan Lda.

Karnik K, Reichle JK, Fischetti AJ, Goggin JM. 2009. Computed tomographic findings of fungal rhinitis and sinusitis in cats. Vet Radiol Ultrasound. 50(1):65–8. doi:10.1111/j.1740-8261.2008.01491.x.

Kieslinger M, Swoboda A, Kramer N, Freund P, Pratscher B, Neubauer HA, Steinborn R, Wolfesberger B, Fuchs-Baumgartinger A, Moriggl R, et al. 2021. A Recurrent STAT5BN642H Driver Mutation in Feline Alimentary T Cell Lymphoma. Cancers (Basel). 13(20):5238. doi:10.3390/cancers13205238.

Kieslinger M, Swoboda A, Kramer N, Pratscher B, Wolfesberger B, Burgener IA. 2019. Companion animals as models for inhibition of STAT3 and STAT5. Cancers (Basel). 11(12). doi:10.3390/cancers11122035.

König HE, Liebich H-G. 2016. Anatomia dos Animais Domésticos. 6th ed. Stuttgart: Schattauer GmbH.

Kuehn NF. 2006. Chronic Rhinitis in Cats. Clin Tech Small Anim Pract. 21(2):69–75. doi:10.1053/j.ctsap.2005.12.013.

Kuhns MS, Davis MM, Garcia KC. 2006. Deconstructing the form and function of the TCR/CD3 complex. Immunity. 24(2):133–9. doi:10.1016/j.immuni.2006.01.006.

Lamb CR, Richbell S, Mantis P. 2003. Radiographic signs in cats with nasal disease. J Feline Med Surg. 5(4):227–35. doi:10.1016/S1098-612X(03)00023-8.

Lappin MR, Blondeau J, Boothe D, Breitschwerdt EB, Guardabassi L, Lloyd DH, Papich MG, Rankin SC, Sykes JE, Turnidge J, et al. 2017. Antimicrobial use Guidelines for Treatment of Respiratory Tract Disease in Dogs and Cats: Antimicrobial Guidelines Working Group of the International Society for Companion Animal Infectious Diseases. J Vet Intern Med. 31(2):279–294. doi:10.1111/jvim.14627.

Legendre AM, Hawks DM, Sebring R, Rohrbach B, Chavez L, Chu HJ, Acree WM. 1991. Comparison of the efficacy of three commercial feline leukemia virus vaccines in a natural challenge exposure. J Am Vet Med Assoc. 199(10):1456–1462.

Lei KI, Suen JJ, Hui P, Tong M, Li W, Yau SH. 1999. Primary nasal and nasopharyngeal lymphomas: a comparative study of clinical presentation and treatment outcome. Clin Oncol (R Coll Radiol). 11(6):379–87. doi:10.1053/clon.1999.9088.

Li GM. 2008. Mechanisms and functions of DNA mismatch repair. Cell Res. 18(1):85–98. doi:10.1038/cr.2007.115.

Liem BP, Dhand NK, Pepper AE, Barrs VR, Beatty JA. 2013. Clinical findings and survival in cats naturally infected with feline immunodeficiency virus. J Vet Intern Med. 27(4):798–805. doi:10.1111/jvim.12120.

Little L, Patel R, Goldschmidt M. 2007. Nasal and Nasopharyngeal Lymphoma in Cats: 50 Cases (1989-2005). Vet Pathol. 44(6):885–892. doi:10.1354/vp.44-6-885. [accessed 2022 May 14]. https://journals.sagepub.com/doi/10.1354/vp.44-6-885?url_ver=Z39.88-2003&rfr_id=ori:rid:crossref.org&rfr_dat=cr_pub%20%200pubmed.

Little SE. 2012. The Cat: Clinical Medicine and Management. 1st ed. Saunders.

Louwerens M, London CA, Pedersen NC, Lyons LA. 2005. Feline lymphoma in the post-feline leukemia virus era. J Vet Intern Med. 19(3):329–35. doi:10.1892/0891-6640(2005)19[329:flitpl]2.0.co;2.

Macy DW. 2020. The Etiology of Cancer - Section C: Cancer-Causing Viruses. In: Withrow and MacEwen's Small Animal Clinical Oncology. 6th ed. Elsevier. p. 19–26.

Maekawa N, Konnai S, Asano Y, Otsuka T, Aoki E, Takeuchi H, Kato Y, Kaneko MK, Yamada S, Kagawa Y, et al. 2023. Molecular characterization of feline immune checkpoint molecules and establishment of PD-L1 immunohistochemistry for feline tumors. PLoS One. 18(1 January). doi:10.1371/journal.pone.0281143.

Maggs DJ, Collins BK, Thorne JG, Nasisse MP. 2000. Effects of L-lysine and L-arginine on in vitro replication of feline herpesvirus type-1. Am J Vet Res. 61(12):1474–1478. doi:10.2460/ajvr.2000.61.1474.

Maggs DJ, Lappin MR, Reif JS, Collins JK, Carman J, Dawson DA, Bruns C. 1999. Evaluation of serologic and viral detection methods for diagnosing feline herpesvirus-1 infection in cats with acute respiratory tract or chronic ocular disease. J Am Vet Med Assoc. 214(4):502–7.

Male D, Brostoff J, Roth DR, Roitt IM. 2012. Immunology. 8th ed. Elsevier.

Mari K de, Maynard L, Sanquer A, Lebreux B, Eun H-M. 2004. Therapeutic effects of recombinant feline interferon-omega on feline leukemia virus (FeLV)-infected and FeLV/feline immunodeficiency virus (FIV)-coinfected symptomatic cats. J Vet Intern Med. 18(4):477–82. doi:10.1892/0891-6640(2004)18<477:teorfi>2.0.co;2.

Marra G, Chang CL, Laghi LA, Chauhan DP, Young D, Boland CR. 1996. Expression of human MutS homolog 2 (hMSH2) protein in resting and proliferating cells. Oncogene. 13(10):2189–96.

Martin SA, Lord CJ, Ashworth A. 2010. Therapeutic targeting of the DNA mismatch repair pathway. Clin Cancer Res. 16(21):5107–13. doi:10.1158/1078-0432.CCR-10-0821.

Martini V, Bernardi S, Marelli P, Cozzi M, Comazzi S. 2018. Flow cytometry for feline lymphoma: a retrospective study regarding pre-analytical factors possibly affecting the quality of samples. J Feline Med Surg. 20(6):494–501. doi:10.1177/1098612X17717175.

Meinke A, Barahmand-Pour F, Wo¨hrl S, Wo¨hrl W, Stoiber D, Decker T. 1996. Activation of Different STAT5 Isoforms Contributes to Cell-Type - Restricted Signaling in Response to Interferons. Mol Cell Biol. 16(12):6937–6944.

Michiels L, Day MJ, Snaps F, Hansen P, Clercx C. 2003. A retrospective study of non-specific rhinitis in 22 cats and the value of nasal cytology and histopathology. J Feline Med Surg. 5(5):279–285. doi:10.1016/S1098-612X(03)00044-5.

Mooney SC, Hayes AA. 1986. Lymphoma in the cat: an approach to diagnosis and management. Semin Vet Med Surg Small Anim. 1(1):51–57.

Moore PF, Woo JC, Vernau W, Kosten S, Graham PS. 2005. Characterization of feline T cell receptor gamma (TCRG) variable region genes for the molecular diagnosis of feline intestinal T cell lymphoma. Vet Immunol Immunopathol. 106(3–4):167–78. doi:10.1016/j.vetimm.2005.02.014.

Morcinek JC, Weisser C, Geissinger E, Schartl M, Wellbrock C. 2002. Activation of STAT5 triggers proliferation and contributes to anti-apoptotic signalling mediated by the oncogenic Xmrk kinase. Oncogene. 21:1668–1678. doi:10.1038/sj/onc/1205148. www.nature.com/onc.

Mroz EA, Tward AD, Pickering CR, Myers JN, Ferris RL, Rocco JW. 2013. High intratumor genetic heterogeneity is related to worse outcome in patients with head and neck squamous cell carcinoma. Cancer. 119(16):3034–3042. doi:10.1002/cncr.28150.

Mukaratirwa S, Van Der Linde-Sipman JS, Gruys E. 2001. Feline nasal and paranasal sinus tumours: Clinicopathological study, histomorphological description and diagnostic immunohistochemistry of 123 cases. J Feline Med Surg. 3(4):235–245. doi:10.1053/jfms.2001.0141. [accessed 2022 May 14]. https://journals.sagepub.com/doi/10.1053/jfms.2001.0141?url_ver=Z39.88-2003&rfr_id=ori:rid:crossref.org&rfr_dat=cr_pub%20%200pubmed.

Murphy K, Weaver C. 2016. Janeway’s immunobiology. Garland science.

Nagata K, Lamb M, Goldschmidt MH, Duda L, Walton RM. 2014. The usefulness of immunohistochemistry to differentiate between nasal carcinoma and lymphoma in cats: 140 cases (1986-2000). Vet Comp Oncol. 12(1):52–57. doi:10.1111/j.1476-5829.2012.00330.x.

Nelson RW, Couto CG. 2019. Small Animal Internal Medicine. 6th ed. Elsevier.

Nemanic S, Hollars K, Nelson NC, Bobe G. 2015. Combination of Computed Tomographic Imaging Characteristics of Medial Retropharyngeal Lymph Nodes and Nasal Passages Aids Discrimination Between Rhinitis and Neoplasia in Cats. Vet Radiol Ultrasound. 56(6):617–27. doi:10.1111/vru.12279.

Page BDG, Khoury H, Laister RC, Fletcher S, Vellozo M, Manzoli A, Yue P, Turkson J, Minden MD, Gunning PT. 2012. Small molecule STAT5-SH2 domain inhibitors exhibit potent antileukemia activity. J Med Chem. 55(3):1047–55. doi:10.1021/jm200720n.

Pedretti E, Passeri B, Amadori M, Isola P, Di Pede P, Telera A, Vescovini R, Quintavalla F, Pistello M. 2006. Low-dose interferon-alpha treatment for feline immunodeficiency virus infection. Vet Immunol Immunopathol. 109(3–4):245–54. doi:10.1016/j.vetimm.2005.08.020.

Van Pelt DR, Lappin MR. 1994. Pathogenesis and treatment of feline rhinitis. Veterinary Clinics of North America - Small Animal Practice. 24(5):807–823. doi:10.1016/S0195-5616(94)50102-5.

Petitjean B, Jardin F, Joly B, Martin-Garcia N, Tilly H, Picquenot J-M, Brière J, Danel C, Mehaut S, Abd-Al-Samad I, et al. 2002. Pyothorax-associated lymphoma: a peculiar clinicopathologic entity derived from B cells at late stage of differentiation and with occasional aberrant dual B- and T-cell phenotype. Am J Surg Pathol. 26(6):724–32. doi:10.1097/00000478-200206000-00005.

Pham HTT, Maurer B, Prchal-Murphy M, Grausenburger R, Grundschober E, Javaheri T, Nivarthi H, Boersma A, Kolbe T, Elabd M, et al. 2018. STAT5BN642H is a driver mutation for T cell neoplasia. J Clin Invest. 128(1):387–401. doi:10.1172/JCI94509.

Polak KL, Chernosky NM, Smigiel JM, Tamagno I, Wjackson M. 2019. Balancing STAT activity as a therapeutic strategy. MDPI AG.

Raskin RE, Meyer DJ, Boes K m. 2021. Canine and Feline Cytopathology - A Color Atlas and Interpretation Guide. 4th ed. Elsevier, Inc.

Reece WO, Erickson HH, Goff JP, Uemura EE, editors. 2015. Dukes' Physiology of Domestic Animals. 13th ed. John Wiley & Sons.

Reed N. 2020. Chronic Rhinitis in the Cat: An Update. Veterinary Clinics of North America - Small Animal Practice. 50(2):311–329. doi:10.1016/j.cvsm.2019.10.005.

Rojko JL, Hardy Jr RG. 1994. Feline leukemia virus and other retroviruses. In: The cat: diseases and clinical management. 2nd ed. New York. p. 263–432.

Russo M, Lamb CR, Jakovljevic S. 2000. Distinguishing rhinitis and nasal neoplasia by radiography. Vet Radiol Ultrasound. 41(2):118–24. doi:10.1111/j.1740-8261.2000.tb01464.x.

Sadrzadeh H, Abtahi SM, Fathi AT. 2012. Infectious pathogens and hematologic malignancy. Discov Med. 14(79):421–433.

Santagostino SF, Mortellaro CM, Boracchi P, Avallone G, Caniatti M, Forlani A, Roccabianca P. 2015. Feline Upper Respiratory Tract Lymphoma: Site, Cyto-histology, Phenotype, FeLV Expression, and Prognosis. Vet Pathol. 52(2):250–259. doi:10.1177/0300985814537529.

Savary KC, Price GS, Vaden SL. 2000. Hypercalcemia in cats: a retrospective study of 71 cases (1991-1997). J Vet Intern Med. 14(2):184–9. doi:10.1892/0891-6640(2000)014<0184:hicars>2.3.co;2.

Schmidt JW, Wehde BL, Sakamoto K, Triplett AA, Anderson SM, Tsichlis PN, Leone G, Wagner K-U. 2014. Stat5 Regulates the Phosphatidylinositol 3-Kinase/Akt1 Pathway during

Mammary Gland Development and Tumorigenesis. Mol Cell Biol. 34(7):1363–1377. doi:10.1128/mcb.01220-13.

Schoenborn WC, Wisner ER, Kass PP, Dale M. 2003. Retrospective assessment of computed tomographic imaging of feline sinonasal disease in 62 cats. Vet Radiol Ultrasound. 44(2):185–95. doi:10.1111/j.1740-8261.2003.tb01269.x.

Sfiligoi G, Théon AP, Kent MS. 2007. Response of nineteen cats with nasal lymphoma to radiation therapy and chemotherapy. Vet Radiol Ultrasound. 48(4):388–93. doi:10.1111/j.1740-8261.2007.00262.x.

Shelton GH, Grant CK, Cotter SM, Gardner MB, Hardy WD, DiGiacomo RF. 1990. Feline immunodeficiency virus and feline leukemia virus infections and their relationships to lymphoid malignancies in cats: a retrospective study (1968-1988). J Acquir Immune Defic Syndr (1988). 3(6):623–630.

Singh B. 2018. Dyce, Sack and Wensing's Textbook of Veterinary Anatomy. 5th ed. Elsevier Inc. http://evolve.elsevier.com/Singh/.

Spada E, Perego R, Sgamma EA, Proverbio D. 2018. Survival time and effect of selected predictor variables on survival in owned pet cats seropositive for feline immunodeficiency and leukemia virus attending a referral clinic in northern Italy. Prev Vet Med. 150:38–46. doi:10.1016/j.prevetmed.2017.12.001.

Stark GR, Darnell JE. 2012. The JAK-STAT Pathway at Twenty. Immunity. 36(4):503–514. doi:10.1016/j.immuni.2012.03.013.

Stützer B, Simon K, Lutz H, Majzoub M, Hermanns W, Hirschberger J, Sauter-Louis C, Hartmann K. 2011. Incidence of persistent viraemia and latent feline leukaemia virus infection in cats with lymphoma. J Feline Med Surg. 13(2):81–7. doi:10.1016/j.jfms.2010.09.015.

Swerdlow SH, Campo E, Pileri SA, Harris NL, Stein H, Siebert R, Advani R, Ghielmini M, Salles GA, Zelenetz AD, et al. 2016. The 2016 revision of the World Health Organization classification of lymphoid neoplasms. Blood. 127(20):2375–90. doi:10.1182/blood-2016. www.bloodjournal.org.

Sykes JE. 2022. Greene's infectious diseases of the dog and cat. Elsevier Health Sciences.

Sykes JE, Sturges BK, Cannon MS, Gericota B, Higgins RJ, Trivedi SR, Dickinson PJ, Vernau KM, Meyer W, Wisner ER. 2010. Clinical signs, imaging features, neuropathology, and outcome in cats and dogs with central nervous system cryptococcosis from California. J Vet Intern Med. 24(6):1427–38. doi:10.1111/j.1939-1676.2010.0633.x.

Taylor SS, Goodfellow MR, Browne WJ, Walding B, Murphy S, Tzannes S, Gerou-Ferriani M, Schwartz A, Dobson JM. 2009. Feline extranodal lymphoma: response to chemotherapy and survival in 110 cats. J Small Anim Pract. 50(11):584–592. doi:10.1111/j.1748-5827.2009.00813.x.

Thamm DH. 2019. Novel Treatments for Lymphoma. Veterinary Clinics of North America - Small Animal Practice. 49(5):903–915. doi:10.1016/j.cvsm.2019.04.004.

Thomasy SM, Shull O, Outerbridge CA, Lim CC, Freeman KS, Strom AR, Kass PH, Maggs DJ. 2016. Oral administration of famciclovir for treatment of spontaneous ocular, respiratory, or dermatologic disease attributed to feline herpesvirus type 1: 59 cases (2006-2013). J Am Vet Med Assoc. 249(5):526–38. doi:10.2460/javma.249.5.526.

Tizard IR. 2017. Veterinary Immunology. 10th ed. Elsevier.

Torlakovic EE, Nielsen S, Francis G, Garratt J, Gilks B, Goldsmith JD, Hornick JL, Hyjek E, Ibrahim M, Miller K, et al. 2015. Standardization of positive controls in diagnostic immunohistochemistry: recommendations from the International Ad Hoc Expert Committee. Appl Immunohistochem Mol Morphol. 23(1):1–18. doi:10.1097/PAI.0000000000000163.

Tromblee TC, Jones JC, Etue AE, Forrester SD. 2006. Association between clinical characteristics, computed tomography characteristics, and histologic diagnosis for cats with sinonasal disease. Vet Radiol Ultrasound. 47(3):241–8. doi:10.1111/j.1740-8261.2006.00134.x.

Vail DM, Moore AS, Ogilvie GK, Volk LM. 1998. Feline lymphoma (145 cases): proliferation indices, cluster of differentiation 3 immunoreactivity, and their association with prognosis in 90 cats. J Vet Intern Med. 12(5):349–554. doi:10.1111/j.1939-1676.1998.tb02134.x.

Vail DM, Pinkerton M. 2020. Hematopoietic Tumors - Section B: Feline Lymphoma and Leukemia. In: Withrow and MacEwen's Small Animal Clinical Oncology. 6th ed. Elsevier. p. 715–730.

Vail DM, Thamm DH, Liptak JM. 2020. Withrow & MacEwen's Small Animal Clinical Oncology. 6th ed. Elsevier Inc.

Weaver RF. 2011. Molecular Biology. 5th ed.

Wei Q, Guan Y, Cheng L, Radinsky R, Bar-Eli M, Tsan R, Li L, Legerski RJ. 1997. Expression of five selected human mismatch repair genes simultaneously detected in normal and cancer cell lines by a nonradioactive multiplex reverse transcription-polymerase chain reaction. Pathobiology. 65(6):293–300. doi:10.1159/000164141.

Weiss ATA, Klopfleisch R, Gruber AD. 2010. Prevalence of feline leukaemia provirus DNA in feline lymphomas. J Feline Med Surg. 12(12):929–35. doi:10.1016/j.jfms.2010.07.006.

Weiss ATA, Klopfleisch R, Gruber AD. 2011. T-cell receptor γ chain variable and joining region genes of subgroup 1 are clonally rearranged in feline B- and T-cell lymphoma. J Comp Pathol. 144(2–3):123–34. doi:10.1016/j.jcpa.2010.08.001.

Werner JA, Woo JC, Vernau W, Graham PS, Grahn RA, Lyons LA, Moore PF. 2005. Characterization of feline immunoglobulin heavy chain variable region genes for the molecular diagnosis of B-cell neoplasia. Vet Pathol. 42(5):596–607. doi:10.1354/vp.42-5-596.

Yao Z, Cui Y, Watford WT, Bream JH, Yamaoka K, Hissong BD, Li D, Durum SK, Jiang Q, Bhandoola A, et al. 2005. Stat5a/b are essential for normal lymphoid development and differentiation. Proceedings of the National Academy of Sciences. 103(4):1000–1005. www.pnas.orgcgidoi10.1073pnas.0507350103.

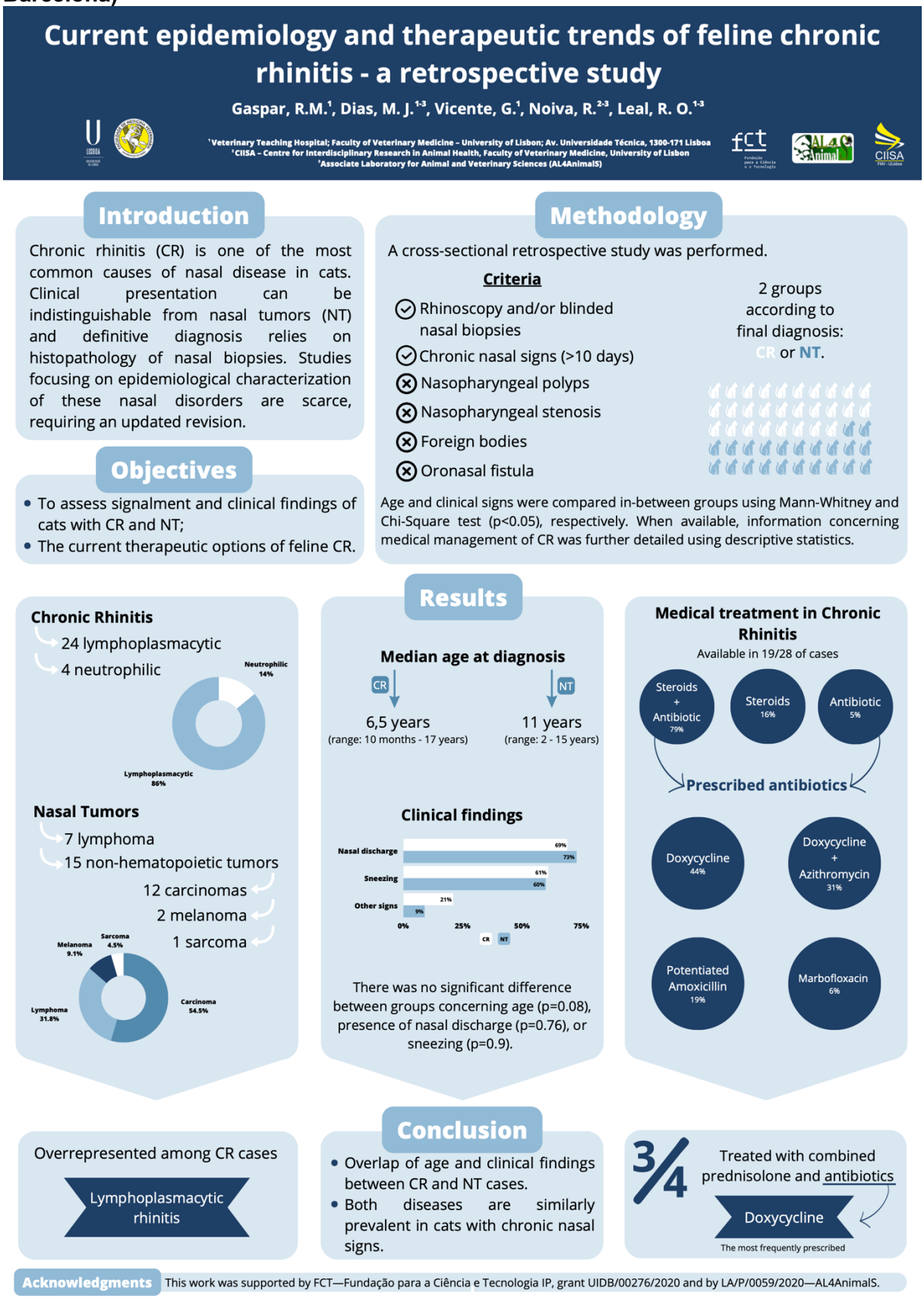
Zachary JF. 2022. Pathologic Basis of Veterinary Disease. 7th ed. Elsevier, Inc.

Zhou L-Z, Xiao H-Q, Chen J. 2024. Mismatch repair gene MSH6 correlates with the prognosis, immune status and immune checkpoint inhibitors response of endometrial cancer. Front Immunol. 15. doi:10.3389/fimmu.2024.1302797.

Zwahlen CH, Lucroy MD, Kraegel SA, Madewell BR. 1998. Results of chemotherapy for cats with alimentary malignant lymphoma: 21 cases (1993-1997). J Am Vet Med Assoc. 213(8):1144–9.

# 5. Annexes

## Annex 1 – Poster presented at the 33rd Annual Congress of the European College of Veterinary Internal Medicine - Companion Animals (ECVIM-CA) (21-23 September 2023, Barcelona)



## Annex 2 – Abstact submitted for poster presentation at the 33rd Annual Congress of the European College of Veterinary Internal Medicine - Companion Animals (ECVIM-CA) (21-23 September 2023, Barcelona)

**Current epidemiology and therapeutic trends of feline chronic rhinitis - a retrospective study**

Gaspar, R.M. [1], Dias, M. J. [1-3], Vicente, G[1], Noiva, R.[2,3], Leal, R. O.[1-3]

[1] Veterinary Teaching Hospital; Faculty of Veterinary Medicine – University of Lisbon; Av. Universidade Técnica, 1300-171 Lisboa

2 CIISA – Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon

3 Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS)

Chronic rhinitis (CR) is one of the most common causes of nasal disease in cats. Clinical presentation can be indistinguishable from nasal tumors (NT) and definitive diagnosis relies on histopathology of nasal biopsies. Studies focusing on epidemiological characterization of these nasal disorders are scarce, requiring an updated revision.

This study aims to assess signalment and clinical findings of cats with CR and NT and evaluate the current therapeutic trends of feline CR.

A cross-sectional retrospective study of feline cases submitted for rhinoscopy and/or blinded nasal biopsies due to chronic nasal signs (>10 days) in a veterinary teaching hospital between January 2018 and December 2021 was conducted. A total of 50 cats were included and divided into two groups according to final diagnosis: CR or NT. Cats with nasopharyngeal polyps, oronasal fistula, foreign bodies, and stenosis were excluded. Age and clinical signs were compared in-between groups using Mann-Whitney and Chi-Square test, respectively ($p < 0.05$ considered significant). When available, information concerning the medical management of CR was further detailed using descriptive statistics.

A total of 50 cases were recruited; CR cases totaled 28/50 (56%) and 22/50 (44%) were NT cases. Median age at diagnosis was 6.5 years (range: 10 months-17 years) for CR and 11 years (range: 2-15 years) for NT. Nasal discharge and sneezing were the most reported clinical findings, being present in 35/50 (70%; 19 CR versus 16 NT) and 30/50 (60%; 17 CR versus 13 NT), respectively. There was no significant difference between groups concerning age ($p=0.08$), presence of nasal discharge ($p=0.76$), or sneezing ($p=0.9$). Among CR cases, 24/28 (86%) were classified as lymphoplasmacytic while 4/28 (14%) were neutrophilic. Concerning NT, 7/22 (32%) were lymphoma while 15/22 (68%) were non-hematopoietic tumors (12 carcinomas, 1 sarcoma, and 2 melanomas). Information regarding medical treatment was available in 19/28 (68%) of CR cases, of which 15/19 (79%) received steroids combined with antibiotics, 3/19 (16%) only received steroids (oral ± inhaled), and 1/19 (5%) only received antibiotic treatment. Among those receiving antibiotics (16/19; 84%), doxycycline was prescribed in 7/16, combined doxycycline and azithromycin in 5/16, potentiated amoxicillin in 3/16, and marbofloxacin in 1/16.

This study reinforces the overlap of age and clinical findings between CR and NT, being both similarly prevalent in cats with chronic nasal signs. Lymphoplasmacytic rhinitis were overrepresented among CR cases. More than three-quarters of CR cases were treated with combined prednisolone and antibiotics, being doxycycline the most frequently prescribed.

**Annex 3 – Poster's presentation for the ECVIM-CA Online Congress 2023**

**Annex 4 – Abstract submitted for oral presentation for the II Congress BioMedLab (1-3 March 2024, Coimbra)**

II CONGRESSO BioMedLab
ASSOCIAÇÃO PORTUGUESA DE CIÊNCIAS BIOMÉDICAS LABORATORIAIS

☐Bioquímica e Imunologia
☐Biotecnologia
☐Ciências Forenses e Tanatologia
☐Citopatologia
☐Genética
☐Gestão e Controlo de Qualidade
☐Hematologia e Ciências da Transfusão
☐Histopatologia
☒Histoquímica e Imunohistoquímica
☐Microbiologia
☒Oncobiologia
☐Patologia Molecular e Celular
☐Saúde Pública

**Selecione o tipo de apresentação:**

☒Comunicação oral ☐Poster

# Inflammation Or Proliferation? Exploring New Diagnostic Markers In Feline Nasal Lymphoma

Sandra Vieira De Carvalho1,2, Renata Gaspar1, Rodolfo Leal1,2, Rute Noiva1,2

1. Ciisa – Centro De Investigação Interdisciplinar Em Sanidade Animal, Faculdade De Medicina Veterinária, Universidade De Lisboa, Lisboa, Portugal; 2. Associate Laboratory For Animal And Veterinary Sciences (Al4animals), Lisboa, Portugal;

In a population of 100,000 cats, 200 will develop lymphoid neoplasia. Lymphoma is the most common nasal cavity tumor in cats. While aggressive nasal large B-cell lymphomas are the most common, epitheliotropic types often have longer survival. In comparison, feline lymphoplasmacytic rhinitis (FLPCR) is a progressive nasal disease with cycles of lymphoplasmacytic inflammation, tissue damage and repair. Not only are clinical signs similar for feline nasal lymphoma (FNL) and FLPCR, imaging findings in inflammatory mass-like lesions overlap with findings for nasal neoplasms. Importantly, lymphoid inflammatory infiltrates are also commonly seen in and around nasal lymphomas. Therefore, new tools with diagnostic or prognostic value are needed in clinical practice.

## Annex 5 – Antibody characteristics

| Antibody | Brand | Dilution | Antigen Retrieval | Time[1] | Positive Control |
|---|---|---|---|---|---|
| CD3 | DAKO, anti-CD3<br>Refª A0452 | 1/400 | EDTA buffer (pH 9.0)<br>Water bath at 96ºC for 20 minutes | 20' | Cat tonsil |
| CD20 | Thermo Scietific, anti-CD20<br>Refª RB-9013-P1 | 1/400 | EDTA buffer (pH 9.0)<br>Water bath at 96ºC for 20 minutes | 20' | Cat tonsil |
| Pax5 | DAKO, anti-Human B-Cell Specific Activator Protein Clone DAK-Pax5<br>Refª M7307 | 1/100 | Citrate buffer (pH 6.0)<br>Water bath at 96ºC for 20 minutes | 20' | Cat tonsil |
| STAT5 | Biorbyt, anti-Stat5 (phosphor-Y694/699)<br>Refª orb159639 | 1/50 | EDTA buffer (pH 9.0)<br>Water bath at 96ºC for 20 minutes | 120' | Tissue diagnosed as lymphoma |
| MLH1 | BD Pharmingen™, Purified Mouse Anti-MLH-1 with Control<br>Refª 551092 | 1/200 | Citrate buffer (pH 6.0)<br>Water bath at 96ºC for 20 minutes | Overnight | Tissue diagnosed as lymphoma |
| MSH6 | BD Transduction Laboratories™, Purified Mouse anti-MSH6<br>Refª 610919 | 1/200 | Citrate buffer (pH 6.0)<br>Water bath at 96ºC for 20 minutes | Overnight | Tissue diagnosed as lymphoma |

**[1] – Incubation time in minutes**