

MISTERY SLIDES SESSION - Cytology -

Carlo Masserdotti DVM Dipl ECVCP, Spec Bioch Clin IAT Brescia



Case #1

- 11-year-old, DSH, neutered male cat.
- Sublingual lump, with mandibular involvement.
- FNCS of the lesion
- MGG stain





























Cytologic findings

- Malignant epithelial cells
 - Cells with cuboidal cytoplasm
 - Malignant squamous cells
- Pavement arrangement
- Osteoblasts
- Osteoclasts
- Inflammatory cells
 - Emperipolesis





Diagnosis

Cytologic diagnosis:

 Squamous cell carcinoma (SCC) with bone involvement

• Histological diagnosis:

Infiltrative SCC with bone invasion











- In a study of 52 cats with oral SCCs, bone invasion was present at diagnosis in 38 (73%) Postorino R et al. Oral squamous cell carcinoma in the cat. JAAHA, 1993(29):438-441
- In another study of 18 feline oral SCCs, bone invasion was present at diagnosis in 9 (50%) Gendler A et al. Computed tomographic features of oral squamous cell carcinoma in cats: 18 cases (2002-2008). JAVMA, 2010(236):319-325
- Among the prognostic indicator, bone involvement seems to be not evaluated Munday JS Tumors of the alimentary tract. In Meuten Tumors in Domestic Animals Vth ed. 2017:508





- Prognostic factos in feline oral SCC Munday
 - Maxillary location: longer survival time
 - Lymph node or distant metastasis: MST 24 days
 - No metastasis: 90 days
 - Few studies determine whether the histological grade is prognostic





- In this case, cytological features suggest that SCC invades the regional bone
- Is this feature helpful in management of the clinical case?
 - Need for radiographic evaluation?
 - Prognostic considerations?





Cell-in-cell events

- Cannibalism:
 - The outer cell engulfs the inner cell



Entosis:

 One cell of the same phenotype invades another



• Emperipolesis:

 Heterotypic cells transit other cell



Cell-in-cell events

• Enclysis:

 Cell-in-cell formation in a manner similar to pinocytosis, but so far is considered specific to hepatocytes engulfing CD4+ T lymphocytes



Phagoptosis:

 A form of cell death in which a macrophage engulf viable cells (not dead or dying cells)



- Emperipolesis. What's new??
- In <u>human being</u>, in cancers such as oral squamous cell carcinoma, cell-in-cell events have been linked to aggressiveness, metastasis, and therapeutic resistance (Siquara da Rocha et al, Front Oncol, 2022)
- Should emperipolesis be related with biological behaviour of a neoplasia?
- Starting point for future studies?





Case #2

- 14-year-old, neutered female, Beagle dog
- Clitoral mass
- FNCS of the lesion
- MGG stain



















Cytologic findings

- Epithelial cells with cuboidal to columnar indistinct cytoplasm
- Marked anisokaryosis and anisocytosis
- Arrangement in large irregular, bidimensional sheets
- Presence of microacinar structures
- Presence of brief palisade arrangement





Diagnosis

Cytologic diagnosis

 Malignant epithelial neoplasia of apocrine cell origin (possibly clitoral carcinoma)

Histologic diagnosis

• Apocrine, infiltrative carcinoma











Histology







Canine Clitoral Carcinoma: A Clinical, Cytologic, Histopathologic, Immunohistochemical, and Ultrastructural Study

Ranieri Verin¹, Francesco Cian², Jennifer Stewart³, Diana Binanti⁴, Amy L. MacNeill⁵, Martina Piviani⁶, Paola Monti⁷, Gianna Baroni⁸, Sophie Le Calvez⁹, Timothy J. Scase¹⁰, and Riccardo Finotello⁶



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Our case

- Very marked anisocytosis and anisokaryosis
- Acinar arrangement
- No naked nuclei

Published cases

- Anisokaryosis mild to moderate
- Rosette-like arrangement
- Naked nuclei

Tubular structures

 Tubular, rosette-type and solid pattern




- Apocrine carcinoma vs clitoral carcinoma
- Need for IHC, not here performed
- Possible that from apocrine gland of clitoris a carcinoma similar to others cutaneous sites may arise





Case #3

- 6-year-old, DSH, male cat
- Cutaneous nodule in axillary region, cystic in appearance (liquid content)
- FNCS of the lesion
- MGG stain















Cytologic findings

- Poor quality of sample (weak staining)
- Round cells of epithelial origin
 - Pigmented cytoplasm, large achromatic globules
 - Irregular borders
 - Mild anisokaryosis and anisocytosis
- Arrangement in dischoesive sheets, sometimes with psuedo-papillary arrangement
- Presence of pigmented keratin





Diagnosis

Cytologic diagnosis

- Pigmented neoplasia of adnexal origin
- More likely apocrine origin

Histologic diagnosis

Trichoblastoma with cystic spaces















- Unusual presentation of a trichoblastoma
 - Due to the fact that the cystic area has been sampled
- The morphological features of the cells depend from the transformation that occurs in a cystic space
 - Similar to cells when exfoliate in an effusion
 - Loss of contact
 - Irregular outline of the cytoplasm
- Transformation make the cytological diagnosis almost impossible





- Trichoblastoma (according with TL Gross, 2005)
 - Ribbon type trichoblastoma
 - Trichoblastoma with outer root sheath differentiation
 - Trabecular type trichoblastoma
 - Spindle cell type trichoblastoma
 - Granular cell type trichoblastoma







Ribbon type





Spindle cell type





Granular cell type



 Trichoblastoma with outer root sheath differentiation (according with TL Gross):

- "lesions are well circumscribed with multilobular and trabecular architecture and large areas of cystic degeneration"
- "the cystic zones appear to be a result of apoptosis, acantholysis and keratinocyte drop-out"
- "the trichoblast are frequently melanized"
- "the primary DD is apocrine ductular adenoma"





Case #4

- 9-month-old, male, French bulldog dog
- Icterus and liver failure
- FNCS of the liver
- MGG stain





CBC (automated count) and biochemistry

RBC	6,91x10 ⁶	5,70	7,90	WBC	,	12,5x10 ³	3	6	11	PLT	457x10 ³	120	350
HGB	15,9 g/dl	12,0	18,0	Neut	rofili	68 %		2,5	7,0	MPV	7,5 fL	7,9	12,3
НСТ	47 %	37,0	55,0	Linfo	citi	30 %		1,3	5,5	PDW	%	10	27
MCV	68,1 fL	60,0	76,0	Mono	ociti	2 %		0,06	0,25	STIMA	Ad		
МСН	23 pg/L	20,0	25,4	Eosi	nofili			0,07	0,8				
МСНС	33,8 g/dl	32,0	39,0	Baso	Basofili			0	0,1				
RDW	13,9%	12,0	16,0										
СРК	286 U/L		!	90	320		сно		29 mg/d	I	95	210	
AST	107 U/L			15	35		TRIG	i	41 mg/dl		19	81	
ALT	257 U/L		;	32	87		BUN		18 mg/d	l i i i i i i i i i i i i i i i i i i i	32	64	
ALP	1396 U/L			19	70		CRE		0,36 mg/	/dl	0,95	1,85	
GGT	5.9 U/L		(0,1	0,6		GLU		128 mg/	dl	86	116	
TBILI	2,86 mg/dl		(0,14	0,26		CA		9,2 mg/c	11	9,3	11,2	
ТР	4,5 g/dl		(6,3	7,8		Ρ		7,1 mg/c	1	3,5	6,6	
ALB	2,8 g/dl		;	3	4		CI		103 mg/	dl	112	119	
GLO	1,7 g/dl		;	3	4,5		Na⁺		133 meg	ı/L	145	152	
A/G	1,65		(0,72	1.25		K+		3,3 meq	/L	3,5	4,7	



















Cytological findings

- Reversible aspecific damage of the hepatocytes
 - Microvesicular steatosis
- Presence of a «high» number of cuboidal cells in small aggregate
 - Biliary origin likely





Diagnosis

- Cytologic diagnosis
 - Microvesicular steatosis
 - Proliferative disease of bilary epithelium of unknow origin
 - Reactive causes more likely
 - Congenital hepatic fibrosis was suggested
 - No signs of neoplasia

Histologic diagnosis

Hepatic ductular reaction









- Ductular reaction (DR) is a stereotypical periportal response to injury, characterized by the proliferation of reactive bile ducts
- The origin of active cells during DR can be cholangiocytes, hepatocytes, or hepatic progenitor cells
- DR is pathologically recognized as bile duct hyperplasia and is commonly observed in biliary disorders
- It can also be identified in various liver disorders
 - Cholestatic liver diseases
 - Inflammatory liver diseases
 - Viral infections
 - Liver fibrosis
 - Senescence







Self-proliferation of cholangiocytes

- Ductular reaction type 1 (according with Desmet, 2011)
- Generally observed in acute cholestasis
- Interaction between inflammatory cells, stroma and cholangiocytes
- Elongation, branching and luminal widening of biliary tubes



Bile duct regeneration driven by hepatocytes

- Ductular reaction type 2A (according with Desmet, 2011)
- Also called "ductular metaplasia of hepatocytes"
- Generally observed in chronic cholestasis and apparently due to the intracellular accumulation of retained bile acids
- The newly formed ductules retain the labyrinthic, anastomosing pattern of hepatocellular plates
- Ductular reaction type 2B
- Generally centrolobular
- Induced by hypoxia

Bile duct regeneration driven by LPCs (liver progenitor cells – oval cells

- Ductular reaction type 3 (according with Desmet, 2011)
- Generally observed in case of massive loss of parenchymal cells
- Not able to recognize primary cause
- Not able to differentiate among different DR types
 - Need of IHC
- Morphological features mostly suggestive (by me) of
 - Toxic disease
 - Acute viral disease





Case #5

- 8-year-old, male, Labrador dog
- Nodule on the gingiva
- FNCS of the lesion
- MGG stain















Cytologic findings

High number of epithelial cells

- Bluish, cuboidal cytoplasm
- Small cytoplasmic globules
- Round to ovoid nuclei
- Anisokaryosis and aniscytosis are mild
- Trabecular arrangement
 - Peripheral palisading very frequent





Diagnosis

Cytologic diagnosis

- Benign epithelial neoplasia, morphologically suggestive of tumor of odontogenic origin (ameloblastoma most likely)
- Histological diagnosis
 - Ameloblastoma











- The cardinal features of odontogenic epithelium include: (according with Murphy BG, Bell CM, Soukup JW. Veterinary Oral and Maxillofacial Pathology, 2020, Wiley Blackwell)
 - Palisading of the basilar epithelium
 - The palisading epithelial cells have antibasilar nuclei (nuclei located at the apical pole)
 - Palisading cells can have a basilar clear zone within the cytoplasm
 - Centrally located cells of the differentiated enamel organ: the odontogenic islands are (should be, may be ...) comprised of polygonal cells with long intercellular desmosomal bridges reminiscent of the stellate reticulum











Case #6

- 6-year-old, female, Labrador dog
- Nodule on the nailbed
- FNCS of the nodule
- MGG stain























Cytologic findings

- Round cells
 - Plasmacytic appearance
 - Arcoplasma
 - Flame figures
 - Bluish, needle-shaped cytoplasmic structures
 - Cytoplasmic Auer rods
- Eccentric nuclei
 - Anisokaryosis
 - Frequent double nuclei





Diagnosis

Cytologic diagnosis

• Round cell neoplasia, more likely extramedullary plasmacytoma

Histologic diagnosis

- Round cell neoplasia, more likely extramedullary plasmacytoma
- Focal involvement of phalanx bone
- Lymph node: hyperplasia, no evidence of metastasis
 - IHC: MUM1 +++











- Auer rods are needle-shaped basophilic, cytoplasmic bodies
- In human being they are described in many lympho and plasmaproliferative disorders
 - Multiple myeloma
 - Chronic lymphocytic leukemia
 - Acute myeloid leukemia
- The composition of these inclusions is uncertain, some authors suggest a lysosomal origin (Zhanxi Gao, 2019), but others(Hristov, 2010; O'Peters 1984) hypothesize they are abnormal immunoglobulins which precipitate in the cytoplasm



- Extramedullary plasma cell tumor is generally benign, despite the appearance of the cells
- Subclassification, based on the variable morphologic features: (according with Cangul et al. 2002)
 - Hyaline type
 - Mature type
 - Cleaved type
 - Asyncronous type
 - Polymorphous-blastic type
- No significant correlations were observed between the cell type and the location of the tumour, presence of amyloid or prognosis





- In our dog staging of the neoplasia was negative
- The only malignant feature was a focal infiltration of phalangeal bone.
- After excision (5 months) no signs of recurrency or metastasis are been detected
- Should Auer bodies be indicative of aggressive behaviour in dog too?





Case #7

- 3-year-old, female, mixed-breed dog
- Abdominal effusion
 - Hemorrhagic appearance
 - TCC: 6,1x10⁶
 - TP: 5,9 g/dl
- Flushing
- MGG stain





CBC (automated count) and biochemistry

RBC	4,8x10 ⁶	5,70	7,90	١	WBC	27,7x10 ³		6	11	PLT	90x10 ³	120	350
HGB	11,3g/dl	12,0	18,0	I	Neutrofili	91		2,5	7,0	MPV	8fL	7,9	12,3
НСТ	31,3%	37,0	55,0	I	Linfociti	1		1,3	5,5	PDW	11.3%	10	27
MCV	65fL	60,0	76,0	I	Monociti	5		0,06	0,25	STIMA	Not ad		
МСН	23,5pg/L	20,0	25,4	I	Eosinofili	1		0,07	0,8				
МСНС	36,2g/dl	32,0	39,0	I	Basofili	2		0	0,1				
RDW	12,5%	12,0	16,0										
СРК	110 U/L			90	320		сно		140 mg/o	IL	95	210	
AST	70 U/L			15	35		TRIG		30 mg/dl		19	81	
ALT	130 U/L			32	87		BUN		50 mg/dl		32	64	
ALP	90 U/L			19	70		CRE		1,7 mg/d	I	0,95	1,85	
GGT	4 /L			0,1	0,6		GLU		90 mg/dl		86	116	
TBILI	0,3 mg/dl			0,14	0,26		CA		9.1 mg/d	I	9,3	11,2	
ТР	5.1 g/dl			6,3	7,8		Ρ		4,1 mg/d	I	3,5	6,6	
ALB	2,9 g/dl			3	4		CI		110 mg/a	IL	112	119	
GLO	2.2 g/dl			3	4,5		Na⁺		150 meq	/L	145	152	
A/G	1,3			0,72	1.25		K+		4,5 meq/	Ĺ	3,5	4,7	


















Cytologic findings

- Bloody background
 - Macrophagic erythrophagocytosis
- Reactive mesothelial cells
- Many leukocytes (may we call this "inflammatory cells"?)
- Microfilariae





Diagnosis

- Hemorrhagic effusion with heartworm infection
 - Suggested subacute (or chronic) peritoneal hemorrhage
 - No cytological evidence of platelet
 - Presence of macrophagic erythrophagocytosis
 - No evidence of phagocytosis of heme-breakdown pigments
- SNAP Heartworm RT test: **positive**
 - The SNAP® Heartworm RT Test is an in vitro test for the detection of **Dirofilaria immitis** antigen in canine serum, plasma, or anticoagulated whole blood.
 - Sensitivity: 84%
 - Sensibility: 97%
 - Accuracy: 86%





J Parasit Dis (July-Sept 2017) 41(3):805–808 DOI 10.1007/s12639-017-0892-8

ORIGINAL ARTICLE



Microfilaruria by *Dirofilaria immitis* in a dog: a rare clinical pathological finding

Marina Mitie Monobe¹ · Rodrigo Costa da Silva¹ · João Pessoa Araujo Junior² · Regina Kiomi Takahira³

- "The obstruction of blood flow by the parasite leads to congestive right heart failure, which cause abdominal effusion".
- "Moreover, microfilariae may occlude and rupture small vessels. In this way, the microfilariae are released in atypical places, i.e., peritoneal and abdominal effusion and, even, lower urinary tract"





- No others published data about microfilariae in effusions of dog with D.immitis infection (better: not able to retrieve it...)
- Description of microfilariae in ascitic fluid in **D. Repens** infection
 - Katarzyna Pasdzior-Czapulaet al. Dirofilaria repens-An etiological factor or an incidental finding in cytologic and histopathologic biopsies from dogs. Vet Clin Pathol, 2018 Jun;47(2):307-311
- Hemorrhagic effusion described in cat:
 - Biasato et al. Pulmonary artery dissection causing hemothorax in a cat: potential role of Dirofilaria immitis infection and literature. J Vet Cardiol, 2017(19):82-87
 - Investigation of the effusion not performed
- «Hemorrhagic effusion may be seen with... Heartworm infection»
 - Valenciano AC, Arndt TP and Rizzi TE, in Cowell and Tyler's Diagnostic Cytology and Hematology of the Dog and Cat, IVth ed, 2014(260):





- This dog was not protected with heartworm prophylaxis and lived in heartworm endemic areas
- Which pathological process leads to the presence of microfilariae in the effusion of our dog?
 - Cavitary hemorrhage due to others causes?
 - Active penetration of larvae through the pleura?
 - Mild thrombocytopenia may concur?





Case #8

- 7-year-old, male, Newfoundland dog
- Subcutaneous nodule on the thorax
- FNCS of the lesion
- MGG stain



















Cytologic findings

- Large birifrangent bodies with small spherules inside
 - Vegetal components?
- Suppurative and macrophagic septic inflammation
 - Occasional neutrophilic phagocytosis of coccoid bacteria





Diagnosis

- Cytologic diagnosis
 - Mixed septic inflammation; vegetal foreign body
- Histologic diagnosis
 - The nodule was surgically removed and submitted for histopathologic examination:
 - Mixed inflammation with large vegetale foreign body













- Are the large birifrangent bodies observed in cytologic sample recognizable as selected part of the vegetal foreign body?
- Which clinical way to investigate the presence of a foreign body are know and used?
- What is not clear to me:
 - Very slow number of bacteria
 - Mixed inflammation instead that pyogranulomatous inflammation (strange to me!!!)





Case #9

- 2-year-old, DSH, neutered male cat
- FeLV +
- Cutaneous nodules on the thorax and abdomen
- FNCS of the lesions
- MGG stain

















Cytologic findings

- Round cell with lymphoid appearance
 - Medium size
 - Erythrophagocytosis
 - Sometimes cytophagocytosis





Diagnosis

Cytologic diagnosis

- Lymphoma with erythrophagocytosis
- Suspicion of cutaneous involvement in γ - δ T cell lymphoma





- The day after the cytological diagnosis, before any others investigations, the cat died
 - Necropsy was not performed
 - No final diagnosis was obtained
 - So sorry...
- Hypothesis???
 - Cutaneous (subcutaneous) localization of hepatosplenic lymphoma???
 - A specific form of cutaneous lymphoma??





Case #10

- 6 years old, neutered female cat
- 2 weeks history of anemia/leukopenia
- Cutaneous nodules and papules
- FeLV FIV negative (PCR)
 - Biochemistry unremarkable
 - No treatment
- Blood and bone marrow

Catherine Trumel, DVM, PhD, ECVCP Dipl., ENV Toulouse France Delphine Rivière, DVM, DU Cyto-Hematology, InovieVet Montpellier France








Additional tests

Skin lesion biopsies

- Histiocytic infiltration suggestive of a progressive feline histiocytosis
 - ✓ Weak to moderate atypia

✓ Immunohistochemistry: Iba1+++, CD204+, CD117 (cKit) –
 ✓ In agreement with histiocytic proliferation





CBC data

Variables	Value s	Reference intervals
Ht %	21,5	30,3 - 52,3
Hb g/dL	6,2	9,8 - 16,2
MCV fL	79,3	35,9-53,1
MCMH g/dL	26,8	28,2-35,8
Retic 10 ⁹ /L	129,8	3,0-50,0
 Leucocytes 10⁹/L Neutrophils Lymphocytes Eosinophils Basophils 	2,51 0,75 0,91 0,05 0,02	2,87 - 17,02 2,30 - 10,29 0,92 - 6,88 0,17 - 1,57 0,01 - 0,26
Platelets 10 ⁹ /L	10	151 – 600



Variables	Procyt e	Manual count
Neutro %	21,5	47
Lympho %	6,2	24
Mono %	31,1	17
Eosino %	28,8	0
Baso %	0	2
Blasts %	0	10
nRBC /100WBC		21















Blasts



Macrophage with black granules highly suggestive of hemosiderin

CBC and blood film examination

Quantitative and qualitative abnormalities

✓ Macrocytic regenerative anemia with dyserythropoiesis

✓ Leukopenia with neutropenia and blasts cells

✓ Presence of macrophages with a probable siderophagic activity

✓ Underestimation of platelet concentration (clumps)





Bone marrow evaluation

- Quality 4/4
- High cellular BM spicules
- Megakaryocytes
 2/4



Macrophages infiltration
 Increased number of blasts
 Erythroid lineage présent









- Increased number of blasts
- Erythroid lineage présent with dyserythropoiesis
- Regular abnormal mitoses



- Macrophages infiltration
- Siderophagic activity hemophagocytosis
- Plasma cells

Bone marrow differential (total : 300)

	Absolut		
MYELOID LINEAGE	count	Percentage	
Myeloblasts	1	0,6%	
Pro-myelocytes	1	0,6%	
Myelocytes	0	0%	
Neutrophil meta-metamyelocytes	2	1,1%	
Band neutrophils	7	4,0%	
Segmented neutrophils	3	1,7%	
Myeloid granulocytic lineage	14	8,0%	
OTHERS			
Eosinophilic cells	0	0%	
Lymphocytes	48	16%	
Plasma cells	15	5%	
Macrophages	43	14,3%	
Abnormal cells	86	28,7%	

е	ERYTHROID LINEAGE		
%	Rubriblasts	4	0,9%
%	Pro-rubricytes	14	3,1%
%	Rubricytes	37	8,3%
6	Meta-rubricytes	39	8,7%
6	Erythroid lineage	94	21%

M/E ratio	0,1
EMI	0,24
MMI	0,17





Blood and bone marrow abnormalities

- Marked macrocytic and regenerative anemia with dyserythropoiesis secondary to:
 - Erythro and erythroblastophagocytosis
 - Decreased/abnormal erythroid lineage?
 - Bone marrow infiltration by macrophages and blasts of probable monocytic/histiocytic origin

Neutropenia secondary to:

- Leukophagocytosis?
- Decreased myeloid lineage
 - Bone marrow infiltration by macrophages and blasts of probable monocytic/histiocytic origin

Circulating blasts and macrophages with bone marrow infiltration





Treatment and follow-up

- Corticotherapy (2,5mg/day) is prescribed waiting for the biological and histological results
- 8 days later, the cat is presented in emergency for dyspnea
- Dies within an hour





Discussion

Histiocytic/monocytic disorders reported in cats

- Hemophagocytic syndrom
- Histiocytic sarcoma
- Feline progressive histiocytosis
- Hemophagocytic histiocytic sarcoma
- Feline pulmonary langherans cell histiocytosis
- AML 4/5

Is there a relationship between the bone marrow hemophagocytic activity and the cutaneous lesion qualified as feline progressive histiocytosis?





Diagnosis and differential



Hemophagocytic syndrom



Feline progressive histiocytosis

- Cutaneous lesions
- Spicytopenia, erythrophagocytosis, BM cells infiltration

Hemophagocytic histiocytic sarcoma

- bicytopenia, siderophages (blood and BM), hemophagocytosis (BM)
- Inormal biochemistry, cutaneous lesion

Histiocytic sarcoma

- Multiple lesions, fatal and rapid evolution
- Low degree of atypia in cutaneous lesion, BM involvement, erythrophagocytosis, bicytopenia





Conclusions







Vittoria Castiglioni



Jaco Van der Lugt



Eleonora Piseddu



Fabio Aloisio



Vanessa Turinelli



Raffaella Bergottini

Manuele Manzocchi







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