Interpretation of Automated Hematology

Andrew Loar, DVM, DACVIM
(Internal Medicine and Oncology
October 13, 2011

Interpretation of Automated Hematology - Omissions, errors and reviews

• Normal & Not-normal results
• Instruments: In-House vs Commercial Lab
• Criteria for slide review
  – Lab tech versus Pathologist
  – Compliance
• How does primary clinician troubleshoot?

Automated Hematology: Instrument limitations

• Platelets
• Neutrophils vs bands vs monocytes
• Unclassified cells
• Red cell and leukocyte morphology
• Nucleated red blood cells
• When, who and how to review slide
**Photos of platelets**

Normal field for estimate
Low numbers in field
Big and little clumps

**Platelets and Automated Hematology**

- **NOT IDENTIFIED**
  - Presence or absence of clumps
  - Small platelets (particularly feline)
  - Megaplatelets
- **Commercial laboratory platelet comments**
  - Examples
  - If count is low - **MUST** address presence of clumps
  - If count is low **WITH** clumps - the count isn’t low
  - Normal count **WITH** clumps - were clumps ‘counted’
  - What is a ‘manual’ platelet count

**Photos of leukocytes**

Normal field for estimate
Neutrophils/bands/myelocytes
Leukocytes and Automated Hematology

- NOT identified
  - Bands, myelocytes
  - Counted as segmented neutrophil or monocytes
- May overcount monocytes
  - Undercounting neutrophils
  - Physiologically significant?
- May undercount lymphocytes (as neutrophils)
- How/When to verify

Photos of Unclassified Cells

Reactive lymphs, lymphoblasts, immature hematopoetic cells & other unidentified blast forms

Leukocytes and Automated Hematology

- Unclassified cells NOT identified
  - Counted as monocytes or lymphocytes
  - Represents:
    - Reactive lymphocytes
    - Immature granulocytes
    - Neoplastic round cells: lymphoma, other leukemias
  - Significant in low proportion
  - Tech Review finding & criteria for Path Review
  - How/When to verify
Automated Hematology: Instrument Flags

Photos of Abnormal Red Cells
Hypochromasia, polychromasia, spherocytes, inclusions & reticulocytes
Cell morphology and Automated Hematology: Red Cells

- Not identified
  - Hypochromasia, anisocytosis, polychromasia, spherocytosis & inclusions
  - Indices suggest some of these
  - Review of anemic specimens confirms
  - Reflex reticulocyte counts
  - Significance of absence

Photos of abnormal leukocytes

- Toxic changes, left shift (see previous), parasites, Pelger-Huet

Cell morphology and Automated Hematology: Leukocytes

- Toxic changes
- Left shift
- Parasites/Inclusions
- Pelger-Huet syndrome
- Blast forms
Photos of NRBCs

Examples of NRBCs

Nucleated Red Cells (NRBCs) and Automated Hematology

- NRBCs generally identified as lymphocytes
- Requires slide review
- > 10 NRBCs per 100 WBCs requires correction of reported WBC
- Clinical significance
  - Appropriate response to abrupt anemia
  - Common response to acute hypoxemia

Criteria for Slide Review

- Commercial Lab*
  - PCV < 30%
  - WBC > 20,000 per ul
    - Lymphs > 5,000; Monocytes > 2,000
  - Platelet count < 150,000
  - Other parameters
- Criteria for Path Review
- In-House instrument recommendations
  - Manual review on all slides
Feline Herpesvirus Keratoconjunctivitis: Overview and Update

Nicole Roybal
Veterinary Specialty Hospital of San Diego
October 13, 2011

Introduction

• Virology and Epidemiology
• Clinical signs
• Differential Diagnoses
• Sequelae and associated conditions
• Diagnostic testing
• Treatment

Virology

• Feline Herpesvirus type 1 (FHV-1)
  – Highly species specific
• Double-stranded DNA virus
• Subfamily alphaherpesvirinae
  – Acute cytoplytic disease followed by latency
  – Largest subfamily and includes most clinically important diseases
  • Herpes simplex type 1 and 2, Varicella Zoster Virus
Virology

• Targets epithelial cells of upper respiratory tract and conjunctiva
  – Less tropism for corneal epithelium
• Migrates up sensory nerves
  – FHV-1 establishes latency in trigeminal ganglion
• Recrudescence
  – Viral reactivation and migration back to epithelial cells resulting in clinical signs

Virology

• Spread via direct, aerosol, fomite transmission
  – Persists for 1 hour in fluorescein and proparacaine solutions but 5 days in saline eye wash.
• Relatively unstable in environment
  – 18 h in moist conditions, less when dry
  – Susceptible to routine disinfection

Epidemiology

• 97% of cats are seropositive
• 80% of infected cats develop latency
  – 45% shed virus spontaneously
  • Variably associated with clinical signs
  – 70% shed virus after steroid administration
Primary Infection

- Naive cats, usually kittens
  - 8-12 weeks old as maternal antibodies wane
- 2-6 day incubation period
- Associated with transient viremia
- Clinical signs more severe vs. recrudescence
  - Fever, malaise, sneezing, nasal and ocular discharge, conjunctivitis
- Vaccination reduces severity of primary infection
  - Does not prevent latency, reactivation or shedding

Recrudescent Disease

- Clinical signs vary in severity
  - Mild serous discharge to severe keratoconjunctivitis
  - Unilateral or bilateral
    - Unilateral cases tend to recur in the same eye
    - Concurrent mild upper respiratory signs uncommon

Clinical Signs

- Most common manifestation is conjunctivitis, keratitis/corneal ulceration is second
- Targets epithelium = superficial ulceration
  - Cell lysis allows viral spread to adjacent cells
    - Pathognomonic for feline herpesvirus
- Prominent neutrophil response
  - Purulent discharge even without secondary bacterial infection
Differential Diagnoses

- Chlamyphila felis
  - Upper respiratory component mild to absent
  - Severe chemosis common, no corneal involvement
  - Rule out via cytology
- Feline Calicivirus
  - Minor conjunctiva pathogen, no corneal involvement

Sequelae and Related Conditions

Confirmed
- Symblepharon
- Stromal keratitis
- Tear film deficiencies
- Spastic entropion

Unconfirmed
- Corneal sequestrum
- Eosinophilic keratitis

Symblepharon

- Conjunctival and corneoconjunctival adhesions
  - Result of severe ulceration and subsequent fibrosis
- More likely with primary infection
- Treatment
  - Early lesions can be manually disrupted with topical anesthesia and cotton-tipped applicator
  - Mature lesions require surgical intervention if significantly obstructing vision
  - Technically complex and prone to recurrence
Stromal Keratitis

• Uncommon, immune-mediated
• Lymphocytic infiltration of corneal stroma with fibrosis and vascularisation
• Viral antigen enters stroma during periods of epithelial ulceration and is ineffectually cleared.
• Can progress to blindness 2, 6
• Treatment requires both antiviral and judicious anti-inflammatory therapy. 2

Tear Film Deficiencies

• Qualitative tear film deficiency
  — Inadequate mucin production
  • Mucin promotes smooth, cohesive tear film
  • Normally produced by conjunctival goblet cells
  • Conjunctivitis destroys goblet cells
  — Reduced tear film break up time (TFBUT)
    • Persisted for > 1 month post inoculation 11
    — Clinical application: mucinomimetic tear replacements can be beneficial for corneal health and comfort
• Quantitative tear film deficiency
  — Reduced Schirmer Tear Test values
  — Viral destruction of lacrimal gland? Stricture of lacrimal ductules?
  — Reported, uncommon 4

Spastic Entropion

• Secondary to chronic blepharospasm
  — Most common type of entropion in cats
  — Not specific to herpesvirus
• Self-perpetuating
  — Physical irritation of entropion exacerbates blepharospasm
• Surgical correction often indicated
  — Conservative approach with analgesia and therapeutic soft contact lens may help
Corneal Sequestrum

- Plaque of necrotic corneal stroma
  - Starts amber, progresses to brown-black
  - Composition of pigment uncertain
  - Can progress to full thickness and perforation
- Not specific to herpes
  - Secondary to chronic superficial ulceration and exposure of anterior stroma
  - Breed predisposition in brachycephalic cats
- Spontaneous resolution can occur with sufficient vascular response
- Keratectomy usually required to speed healing and prevent progression

Eosinophilic Keratitis

- Immune mediated, proliferative white-pink plaques on corneal and conjunctival surfaces
- Characteristic cytology
  - Eosinophils, mast cells
- Possibly herpes-related
  - 45/59 (76%) cats PCR (+) vs. 6% of normal cats
  - Unilateral or bilateral
- Often require long-term anti-inflammatory (steroids or cyclosporine) with antiviral

Diagnostic Testing

- Results can be difficult to interpret for the individual clinical patient
  - False Positives:
    - Stress/illness induced viral shedding
    - Subclinical virus harbored in conjunctiva and cornea
    - 50% of normal cats harbor herpesvirus DNA in their corneas
    - Cross-reaction with vaccine virus
  - False Negatives:
    - Intermittent viral shedding
    - Inadequate sample collection or handling
    - Reduced test sensitivity
Diagnostic Testing

• More informative in research and epidemiology settings
  • Tracking trends in larger populations
• Presumptive diagnosis often based on signalment, history, clinical signs, response to treatment

• Fluorescent Antibody Testing
  – Low sensitivity and specificity, affected by fluorescein stain, limited clinical value
• Serum Antibody Titers
  – Does not differentiate between vaccine and wild-type virus
  – No difference in seroprevalence between affected and clinically normal cats

• Virus Isolation
  – Considered gold standard
  – Meticulous sample handling requirements
    • Impractical for clinical settings
    • Lower sensitivity
• Cytology
  – Non-specific: neutrophils and epithelial cells
    • Intranuclear viral inclusion bodies difficult to see
  – Primarily to rule out *C. felis*
Diagnostic Testing

- Polymerase Chain Reaction
  - Highly sensitive, specific for FHV
    - Possibly too sensitive?
      - Coincidence? Consequence? Cause? 1,2
  - Sample handling less strict
  - More clinically applicable (and sensitive) than VI14
  - Different transit times and temperatures made no difference in ability to identify DNA 15

Treatment

- Variables to consider
  - Age, immune status, primary vs. recrudescent, level of discomfort 1,2,6,16
  - Stress of treatment can exacerbate signs
- Treatment needs vary between patients and within individuals
  - Benign neglect vs. topical antiviral vs. topical and systemic antivirals and antibiotics

Antiviral Therapy

- No veterinary antiviral drugs available
- Nucleoside Analogues
  - Interfer with viral DNA replication
  - In vitro FHV-1 efficacy studies:
    - Trifluridine >> idoxuridine = ganciclovir >> cidofovir = penciclovir > vidarabine >> acyclovir 17,18
  - Complicated metabolism
    - Phosphorylation by virus, host or both to reach active form
  - Systemic treatment often limited by toxicity
  - Virustatic
    - Require frequent application (q1-4h) for optimum efficacy, one week beyond resolution of clinical signs 5
Topical Antiviral Medications

• Trifluridine 1% solution (Viroptic)
  — Only commercially available product evaluated in cats
  — Effective but cost and common topical irritation limit use
• Idoxuridine 0.1% solution, 2.5% ointment
  — Available through compounding pharmacies
  — Well-tolerated, reasonably effective, most common
• Vidarabine 3% ointment
  — Available through compounding pharmacies
  — Similar clinical efficacy to idoxuridine 1,2,16

Topical Antiviral Medications

• Ganciclovir 0.15% gel (Virgan)
  — Relatively new for humans, not yet evaluated in cats
• Cidofovir 0.5% solution
  — Compounded
  — Long half-life results in BID dosing
  — Recent study showed improved clinical signs and reduced viral shedding 19
• Interferons
  — Cytokines that induce antiviral effects and stimulate immunologic defenses, may be synergistic with antiviral drugs
  — In vitro studies promising 20 but in vivo studies less convincing 21

Systemic Antiviral Medications

• Acyclovir (Zovirax)
  — Previously the only systemic antiviral evaluated in cats
  — Limited bioavailability and efficacy against FHV-1
  — Risk of bone marrow suppression
  — Pro-drug valacyclovir (Valtrex) – better bioavailability but more toxic
    • Fatal liver and kidney necrosis 1,6,16
Systemic Antiviral Medications

• Famcyclovir (Famvir)
  – Complicated non-linear pharmacokinetics
  – Optimum dosing schedule not yet determined
    • Wide published range: 62.5 mg/cat q24h to 90 mg/kg PO TID
    • 62.5 mg PO TID didn’t achieve adequate plasma levels for viral inhibition but 90 mg/kg was sufficient
  – Minimal systemic side effects reported

L-Lysine

• Amino acid, available OTC
• Reduces viral replication by competitive inhibition with arginine
  – 500 mg PO BID: reduced clinical signs post-inoculation
  – 400 mg PO q24h: reduced viral shedding with latently infected
• However...
  – No effect noted in large population of shelter cats
  – Shelter cats fed diet supplemented with lysine had more severe clinical signs and more frequent viral shedding
• Bottom line...
  – No severe reported side effects or controlled study in client-owned cats, may be effective when given in bolus form, safe for long-term use in chronically affected cats

Conclusions

• Feline herpesvirus is ubiquitous and a common cause of ocular disease in cats
• Recognizing typical history, signalment and clinical signs may be more helpful than diagnostic testing
• Treatment needs vary with each patient
• Owner education important
  – Recognizing potential for disease recurrence
  – Minimizing stress whenever possible in affected pets
Questions?

San Marcos: Wednesday-Friday
Sorrento Valley: Saturday
nicole.roybal@vshsd.com

Sources


Sources

Sources


Image Sources

- Eosinophilic Keratitis: http://davidlwilliams.org.uk/?p=136
- Sequestrum: http://www.petvetsdoctor.com/620640.html
- Entropion: http://vetspecialistsrochester.com/top_feline_conditions.php
- Stromal Keratitis, Chlamydia Histo: http://davidlwilliams.org.uk/?p=136
- Dendritic ulcer: http://vetspecialistsrochester.com/top_feline_conditions.php
- Stromal Keratitis, Chlamydia Histo: http://davidlwilliams.org.uk/?p=136
What’s New: A review of recently approved drugs
Onsior, Incurin, Trifexis, Propoflo 28 and more!
Margo Karriker, PharmD, FSVHP

Objectives

- Review new FDA approvals for small animal products
- Discuss these products’ place in therapy
- Review where to find information on these products

Robenacoxib

*Brand name:* Onsior
*Sponsor:* Novartis
*Approval date:* March 8, 2011
*Release date:* 2012
*Therapeutic class:* Non-steroidal anti-inflammatory drug (NSAID)
*Presentation:* 6mg, non-scored tablets

*Label indications:*
Control of postoperative pain and inflammation associated with orthopedic surgery, ovariohysterectomy and castration in cats > 5.5 lbs (2.5 kg) and > 6 months of age; for up to a maximum of 3 days

*Dosing:* 1 mg/kg orally once daily, for a maximum of three days. Preoperatively: Administer dose approximately 30 minutes prior to surgery. May be given with or without food. Tablets are not scored and should not be broken.

*Place in Therapy:*

- Highly Cox-2 selective NSAID approved for cats
- Shown to be non-inferior to ketoprofen in cats with signs and symptoms of acute pain and inflammation in musculoskeletal disorders
- Extra-label use in dogs has been studied
- Shown to be non-inferior to carprofen in dogs with OA in a 12 week study
- Healthy cats: 10month old, 2x and 5x for 3 days did not produce toxicity
- Healthy dogs: 10mg/kg/day for 6 months, no toxicity

Estriol

*Brand name:* Incurin
*Sponsor:* Merck Animal Health (Intervet, Inc)
*Approval Date:* July 15, 2011
*Release date:* 2012 (per Merck Animal Health Technical Services)
*Therapeutic class:* Hormone
*Presentation:* Single-scored, 1mg tablets
*Label indications:*
For the control of estrogen-responsive urinary incontinence in ovariohysterectomized female dogs

*Dosing:*
Initial dose of 2mg (2 tabs) orally once daily for a minimum of 14 days. After incontinence is controlled, the lowest effective dose should be determined in a step-wise fashion. Dose is not dependant on body weight. Minimum of 7 days between adjustments. Max dose of 2mg per day.

**User Safety:**
Women who are of child-bearing age or those who are breastfeeding should use caution when administering INCURIN Tablets. Wash your hands with soap and water after administration to avoid exposure to the drug.

**Place in Therapy:**
- An approved product with a similar efficacy profile to DES
- Canine approved, commercially available
- Estrogenic effects seen in 5-9% of dogs at 2mg every 24 hours

**Spinosad and Milbemycin oxime**

*Brand name:* Trifexis  
*Sponsor:* Elanco  
*Approval Date:* January 4, 2011  
*Release Date:* 2011

**Therapeutic class:** Antiparasitic  
**Presentation:** chewable tablets – range of sizes, 6pk

**Label indications:** Prevention of heartworm disease (Dirofilaria immitis); kill fleas; the prevention and treatment of flea infestations (Ctenocephalides felis), and the treatment and control of adult hookworm (Ancylostoma caninum), adult roundworm (Toxocara canis and Toxascaris leonina) and adult whipworm (Trichuris vulpis) infections in dogs and puppies 8 weeks of age or older and 5 pounds of body weight or greater.

**Place in Therapy:**
- Combination parasiticide; appropriate for year-round heartworm prevention
- Oral option
- Combination did not cause neurotoxicity in collie dogs when administered above the labeled dose

**Propofol multi-dose (with benzyl alcohol)**

*Brand name:* Propoflo 28  
*Sponsor:* Abbott Animal Health  
*Approval date:* February 4, 2011 (supplemental approval)  
*Release date:* 2011

**Therapeutic class:** intravenous anesthetic  
**Presentation:** 10mg/mL, 20ml multi-dose vials, 5 vial pack

**Label indications:** Induction of anesthesia; maintenance of general anesthesia by intermittent bolus injections for short procedures; induction of general anesthesia where maintenance is provided by inhalant anesthetics

**Place in therapy**
- Multi-dose product
- Safety and efficacy previously established
- Extra-label use in cats
- Benzyl alcohol content 20mg/mL
- Toxicity not seen at these levels

**Orbifloxacin oral suspension**

*Brand name:* Orbax  
*Sponsor:* Intervet (Merck)  
*Approval date:* March 25, 2010 (supplemental approval)  
*Therapeutic class:* Antimicrobial - quinolone  
*Presentation:* 30mg/mL oral suspension, 20mL bottle, 6 pack  
*Label indications:*  
- Cats: treatment of skin infections (wounds and abscesses) caused by susceptible strains of Staphylococcus aureus, Escherichia coli, and Pasteurella multocida  
- Dogs: treatment of UTIs in dogs caused by susceptible strains of Staph. pseudintermedius, Proteus mirabilis, E. coli and Enterococcus faecalis skin and soft tissue infections caused by susceptible strains of Staph. pseudintermedius, Staph. aureus, coagulase positive staph., Pasteurella multocida, Proteus mirabilis, Pseudomonas spp., Klebsiella pneumoniae, E. coli, Enterobacter spp., Citrobacter spp., Enterococcus faecalis, Beta hemolytic strep.(Group G) and Strep. equisimilis.

*Place in therapy:*  
- Safety and efficacy previously established for oral tablets  
- Only approved oral suspension  
- Improved palatability  
- Discard 30 days after opening, does not require refrigeration

**Propofol microemulsion**

*Brand name:* PropoClear  
*Sponsor:* Pfizer  
*Approval date:* May 21, 2010  
*Therapeutic class:* intravenous anesthetic  
*Presentation:* 10mg/mL, multi-dose, 20mL; 50mL; 100mL vial  
*Label indications:* Induction and maintenance of anesthesia and for induction followed by maintenance with an inhalant anesthetic, in cats and dogs.

**References:**

Freedom of Information Summaries:  
[www.fda.gov](http://www.fda.gov) > Animal & Veterinary > Products > FOIA Drug Summaries
**Onsior:**


**Incurin**


**Trifexis**


**PropoClear**