Clinical Updates in Small Animal Practice

Session: Alabama Rot / Cutaneous Renal glomerular vasculopathy (CRGV)

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Cutaneous and Renal Glomerular Vasculopathy Study Notes

What is CRGV?
- It is cutaneous and renal glomerular vasculopathy
- The disease manifests as ulcerative skin lesions affecting the distal extremities, ventrum, muzzle and / or oral cavity. It is variably associated with clinically significant acute kidney injury (AKI).
- It is a thrombotic microangiopathy (TMA)

What is a thrombotic microangiopathy?
- Damage to the endothelial lining of blood vessels results in platelet activation
- Leading to widespread formation of microthrombi
- These occlude the small blood vessels in affected organs (particularly the renal glomeruli and skin), compromising blood supply and causing organ damage, then organ failure and death
- Red cells trying to squeeze past the clots in affected vessels undergo shear damage
- These damaged red cells (schistocytes) are targeted for destruction in the spleen; but some are so fragmented that they break down in the blood vessels
- The resultant haemolysis is known as ‘microangiopathic’ and leads to anaemia and jaundice

TMA’s in Humans:
- Haemolytic uraemic syndrome (HUS)
  - Causes AKI and microangiopathic haemolysis
  - Generally seen in babies and infants
  - Triggered by shiga-toxin – generally as a result of *E.coli* diarrhoea
  - Small geographical clusters
  - Temporal clusters (summer)
- Atypical haemolytic uraemic syndrome (aHUS)
  - Genetic abnormalities in the complement system lead to endothelial deposition of complement and platelet aggregation, with loss of normal regulation
  - A trigger is still suspected as not all patients with the genetic abnormalities will develop disease
  - Causes AKI, haemolysis and, in some patients, ulcerative skin lesions
- Thrombotic thrombocytopenic purpura (TTP)
  - Deficiency of a protein which cleaves von Willebrands’ factor, reducing thrombosis (ADAM TS 13).
  - The small vessels in the brain appear most affected
  - Patients generally suffer neurological signs rather than AKI
Previous experiences with CRGV / HUS in animals

- 1973: six calves reported with ‘HUS’
ARE HUS and CRGV THE SAME?

This is not known for sure, HOWEVER:

- None of the dogs reported to have HUS had skin lesions
- All of the CRGV cases had skin lesions
- Diarrhoea was present in 4/5 of the dogs reported to have HUS
- Diarrhoea was only identified in 14 of the first 65 CRGV cases
- This suggests that HUS and CRGV in dogs may be 2 distinct illnesses

UK Cases

- Number of confirmed cases: between Nov’12 and Dec’16, there have been 82 confirmed cases
- Number of suspected azotaemic cases: 90 (82 non survivors plus 8 survivors)
- Number of non-azotaemic cases?: unknown since diagnosis can only be confirmed via renal histopathology, generally obtained PM
- Survivors: all non-azotaemic cases; possibly 8 azotaemic cases

Case Number by Year

- November 2012-October 2013: 10 confirmed cases
- Between November 2013-October 2014: 30 confirmed cases
- Between November 2014-October 2015: 19 confirmed cases
- Between November 2015-December 2016: 23 confirmed cases

Number of CRGV Cases By Month Nov 2012-Dec 2016
**Location:**

- 26 counties affected from across the UK
- Apparent small ‘clusters’ but no actual location overlap
- BUT – difficult to pinpoint since dog walking routes generally very varied; it is unknown whether there is an environmental trigger and if so, what is the temporal relationship between exposure and illness

**Map of UK cases to date:**

**Previous Investigations / Testing**

- No bacterial genetic material identified in tissues from affected dogs
- No viral genetic material identified in tissues from affected dogs
- No known ‘toxins’ (including heavy metals) identified in the urine, or kidneys of affected dogs; or in the environment
- No common bacterial organisms cultured from blood, urine, faeces, skin lesions or tissues
- Extensive testing for *E.coli* and Shiga toxin all negative
- No convincing evidence for Leptospirosis (via antibody testing, PCRs on blood and urine, PCRs on tissue, or FISH)
- A site visit was not suggestive for any plant (tree, shrub, grass, fungus) being causative – including giant hogweed
- No evidence for any link with military ordnance / radioactive substances
- No evidence for *Borrelia, Leishmania, Bartonella, Dog Circovirus*
- No common diet, treat, geographical location or behaviour
Clinical Signs:
- Skin lesions in 100%
- Anorexia
- Vomiting
- Lethargy
- Pyrexia (early in course of disease)
- Hypothermia (if become azotaemic)
- Icterus
- Lameness
- PU/PD
- Diarrhoea
- Petechiae
- Neurological signs (typically terminally)
- Signs of bleeding (epistaxis, haematochezia, haematemesis)

Physical examination:

Skin Lesions:
- Lesions variable in appearance, but typically affect the paws, distal limbs, ventrum, muzzle and / or tongue
- Generally full thickness ulcers, with variable surrounding bruising, oedema and erythema
- Some lesions are more subtle and can appear like a cut or graze
- Size can vary from a few millimetres, to extensive, coalescing lesions affecting large areas of the body

Remaining Physical Examination:
- Variable – some animals have no findings other than skin lesion(s)
- Icterus
- Petechiae
- Pyrexia / hypothermia
- Lameness
- Abdominal pain

Diagnosis:
- Thorough history (including possible access to toxins / medications)
- Haematology and biochemistry, including blood smear examination
- Urinalysis including sediment examination, culture and UP:Cr
- SDMA
- Systolic BP measurement
- GFR (iohexol clearance)
- Histopathology of skin biopsies? Often only non-specific findings
- If azotaemic, testing to exclude other causes of AKI, such as Leptospira serology / PCR, basal cortisol
- Abdominal ultrasonography
  - Haematological abnormalities:
    - VARIABLY:
      - Thrombocytopenia
      - Burr cells, schistocytes
      - Anaemia
      - Neutrophilia
    - Biochemical abnormalities:
      - VARIABLY:
        - Elevated serum liver enzyme activity
        - Hyperbilirubinaemia
        - Azotaemia
        - Azotaemia is believed to develop in ~25-30% of cases
        - Median time from appearance of skin lesions to development of azotaemia was 4 days (range: concurrent to 9 days) in the first 30 confirmed cases. REFERENCE: Holm et al, Vet Rec 2015 Apr 11; 176(15): 384
      - Elevated serum muscle enzyme activity
      - Hyperphosphataemia
      - Abnormal spec PI / snap
    - Urinalysis findings:
      - Proteinuria
      - Casts
      - Haematuria / haemoglobinuria / myoglobinuria
      - Isosthenuria
      - Glycosuria
      - Polyuria
      - oliguria / anuria
    - Imaging:
      - Abdominal ultrasonography: often unremarkable
Some cases have hyperechoic renal cortices

Abdominal effusion is present in some cases

Can try to exclude other conditions, such as CKD, pyelonephritis, renal lymphoma, or other neoplastic disease

**Approach to non-azotaemic cases:**

- **In-patient monitoring:** Admit for IVFT and monitoring: unknown if IVFT in the euvolaemic pre-azotaemic CRGV patient reduces the risk of AKI developing, however, in human infants, the severity of the AKI seen with HUS is reduced by early IVFT

- Ideally, monitoring should include daily haematology, biochemistry (including SDMA), urinalysis and (at least subjective) monitoring of urine output.

- HR, RR and effort, body weight and systolic BP should ideally be monitored BID-QID

- Assess for presence of moist nasal discharge, ascites, oedema, chemosis

- A GFR (iohexol clearance) test can be performed prior to initiating IVFT

- Duration of IVFT and daily monitoring: 7-9 days?

- **Outpatient monitoring:** the frequency and level of monitoring is likely to be (at least in part) determined by owner wishes / concerns and financial considerations.

  Monitoring at least the SDMA or BUN/creatinine q 24-48hrs will give some information, however it is worth noting that the urine output may fall and thrombocytopenia may develop in CRGV patients with AKI, up to 12-24hrs before they become azotaemic

**AKI:**

**AKI Definition:**

- AKI is characterised by a rapid reduction in renal function, leading to an inability to regulate fluid balance, electrolyte concentrations and acid-base status.

- Glomerular filtration rate (GFR) decreases, eventually leading to increased serum creatinine concentration and possibly to reduced urine production.

**Phases of AKI**

- **Initiation** – nephrotoxic or ischaemic insult (for CRGV dogs it is an ischaemic insult due to occlusion of blood supply by microthrombi)

- **Extension** – ischemia, hypoxia, inflammation, and cellular injury continue, leading to cellular apoptosis, necrosis, or both.

- **Maintenance** - characterized by azotaemia; may last for days to weeks. Oliguria (<0.5 mL urine per kg body weight per hour) or anuria (no urine production) may occur, although urine production is highly variable

- **Recovery** - renal tubules undergo repair. Marked polyuria may occur as a result of partial restoration of renal tubular function and of osmotic diuresis of accumulated solutes. Renal function may return to normal, or the animal may be left with residual renal dysfunction

- Clinical and laboratory abnormalities may not be evident during the first 2 stages.
**International Renal Interest Society (IRIS)**

- IRIS was created to advance the scientific understanding of kidney disease in small animals. It is led by experts in veterinary nephrology.
- The mission of IRIS is to help veterinary practitioners to better diagnose, understand and treat renal disease in dogs and cats.
- One of the organization's primary objectives is to establish an internationally recognized set of guidelines on the diagnosis and treatment of kidney disease in small animals.
- Acute kidney injury (AKI) represents a continuous spectrum of renal injury from mild, clinically inapparent, nephron loss to severe acute kidney failure.
- To emphasize this concept, IRIS recommends that AKI is graded to accurately characterize the severity of the disorder.
- The IRIS AKI Grading scale (I-5) for dogs and cats is based on fasting blood creatinine determination and clinical parameters, such as urine output.

### AKI Grade

<table>
<thead>
<tr>
<th>AKI grade</th>
<th>Blood creatinine concentration (µmol/l)</th>
<th>Subgrade</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade I</td>
<td>&lt;140</td>
<td>Each AKI grade is further subgraded as:</td>
</tr>
<tr>
<td>Grade II</td>
<td>141-220</td>
<td>- Non-oliguric (NO) or oligoanuric (O)</td>
</tr>
<tr>
<td>Grade III</td>
<td>221-439</td>
<td>- Requiring renal replacement therapy</td>
</tr>
<tr>
<td>Grade IV</td>
<td>440-880</td>
<td>- Oliguria: urine production &lt;1 ml/kg/hr</td>
</tr>
<tr>
<td>Grade V</td>
<td>&gt;880</td>
<td>- Anuria: no urine produced over 6 hrs</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Non-oliguric: urine production &gt;1 ml/kg/hr</td>
</tr>
</tbody>
</table>
Goals of management:

- Improve glomerular filtration rate (GFR)
- Manage fluid balance
- Maintain normal electrolyte concentrations
- Manage haematological abnormalities and their consequences
- Keep the patient comfortable (manage pain and nausea)
- Skin lesion management
- Nutritional support
- Address possible complement abnormalities

Management:

- ‘Standard' management for AKI (matching fluid input, to urine output, plus insensible and other losses)
  - Placement of an indwelling urinary catheter
  - Every 1-2 hours, the urine output is measured (plus any vomit / diarrhoea)
  - The insensible losses are calculated as 0.5-1ml/kg/hour
  - The total estimated fluid output of the dog is worked out by adding all of these values together.
  - This figure as total output per hour, is then used to determine the fluid therapy rate for the next hour.

Example calculation

![Example calculation table]
Ongoing Monitoring:

- HR
- RR and effort
- Body weight (ideally QID)
- Systolic BP (ideally TID)
- Development of new skin lesions
- Presence of chemosis, moist nasal discharge oedema, ascites (q 2-4 hours)
- At least SID urea, creatinine and electrolytes
- Daily urine dipstick and sediment

Indwelling urinary catheters:

- Foley catheters are suitable for use as indwelling urinary catheters
- They should be placed in a sterile way. Sterile lube and a guidewire are usually required.
- Much easier in ♂s; ♀s usually need sedation
- The bulb is then inflated as per the manufacturers instructions to keep the catheter in place
- A collection bag is then attached. It should be kept clean and handled with gloves

Oliguria / Anuria

- Olig- or anuria is a medical emergency.
- Oliguria is usually defined as <0.5ml/kg/hour of urine production, however when managing dogs with AKI, we ideally want urine output at >2ml/kg/hr.
- Clinicians may therefore instruct intervention even if urine output is still >1ml/kg/hr.

Drugs aimed at promoting diuresis are:

- Furosemide – loop diuretic
- Mannitol – osmotic diuretic. Contraindicated in anuric renal failure
- Possibly dopamine – catecholamine. Dilates renal vascular beds
- Possibly diltiazem – calcium channel blocker. Decreases vascular resistance

- Furosemide: 2mg/kg bolus, which can be followed either by a CRI if effective (0.25-1mg/kg/hr) or by one or two incremental dose increases if ineffective (4mg/kg, then 6mg/kg)
- If furosemide ineffective, mannitol is given at 0.25-0.5g/kg over 10 minutes, and again, if effective, can be followed with a CRI.
- Dopamine: at <10ug/kg/min increases renal blood flow and urine output. Dose: 2-10 ucg/ kg/min
- Diltiazem: anecdotal evidence with AKI due to Leptospirosis. Dose: 0.3-0.5mg/kg over 10mins, followed by 3-5ucg/kg/min CRI. Monitor BP and HR and reduce dose if either below normal. Furosemide can be used in conjunction with diltiazem (ref: Veterinary Emergency and Critical Care Manual; 2nd edition, K A Mathews)
Supportive therapy:

- **Management for uraemic gastritis:**
  - omeprazole
  - ranitidine
  - sucralfate

- **anti-nausea medication:**
  - metoclopramide
  - maropitant
  - ondansetron
  - prochlorperazine

- **Antibiotics?**

- **Analgesia - opioids**

- **Management of vasculitis:**
  - Pentoxifylline – increases erythrocyte flexibility, increases fibrinolysis, reduces blood viscosity

- **Steroids?** Only used in 9 non-surviving cases - both anti-inflammatory and immunosuppressive doses. No survivors received steroids

- **Blood products:**
  - Anaemia can be severe enough to require blood transfusion
  - PRBCs
  - Whole blood - platelets also replaced?
  - Fresh frozen plasma? Unknown whether this could be beneficial if plasma exchange therapy is not possible

Options if Standard management fails:

- **Peritoneal dialysis:**
  - Indicated if olig- or anuria persists after attempted medical management
  - The dialysis catheters have to be placed surgically – thrombocytopaenia may be an issue
  - Require sterile handling / dressing changes
  - Home-made or commercial dialysate
  - Perform dialysis q every half hour to hour initially
  - If patient improves extend interval to 1-4 hours
  - Labour intensive and expensive, but may be cheaper than CRRT

- **Haemodialysis (continuous renal replacement therapy):**
  - Continuous renal replacement therapy (CRRT) is a relatively new, extracorporeal blood purification modality, used for the treatment of acute kidney injury in dogs
  - Only offered at the RVC in the UK

- **Plasma exchange therapy (PEX)?**
  - A Continuous Renal Replacement platform and a therapeutic plasma exchange filter are used. Vascular access is achieved via a dedicated jugular catheter. Anticoagulation is performed with citrate regional anticoagulation. 1.5 x plasma volumes are exchanged, replaced with 50% isotonic crystalloids and 50% fresh frozen plasma (FFP) over a period of 3.5 hours.
  - Several cycles would be performed if the patient appears to be improving
**Prognosis:**

- Out of 90 confirmed or strongly suspected azotaemic cases, only 8 have survived
- The prognosis therefore appears guarded if AKI develops
- Cases which do not develop AKI appear to recover fully, but skin lesions may take weeks to months to fully heal

**Post Mortem:**

- **Gross Findings:**
  - Subcutis: oedema and icterus
  - Viscera: petechial haemorrhages
  - Pleural / peritoneal cavity: can have haemorrhagic effusion
  - Skin: erosion, ulceration, bruising, oedema, erythema
  - Kidneys: can be discoloured with pinpoint haemorrhage and / or infarcts visible
  - Lungs / liver: generally oedema / congestion

- **Microscopic findings**
  - **KIDNEY:**
    - Fibrinoid necrosis of glomerular arterioles
    - Frequently vessels are occluded by thrombi.
    - Glomerular tufts are frequently congested and partially occluded by haemorrhage.
    - Fibrinoid necrosis of intralobular and arcuate arteries is occasionally observed.
    - Tubular necrosis, ranging from mild to marked, often with concurrent evidence of tubular restitution is sometimes seen.
    - Micro-organisms, viral cytopathic effects and metazoan parasites are not identified.
  - **SKIN:**
    - The epidermis is generally focally to diffusely ulcerated.
    - The subjacent dermis is often undergoing coagulative necrosis.
    - The hair follicles have reduced to absent sebaceous glands, reduced cellularity and are separated by increased fibrous tissue and an attenuated follicular epithelium.
    - Affected follicles are often bordered by variable numbers of neutrophils, foamy macrophages and karyorrhectic debris.
    - Fibrinoid necrosis is sometimes observed in the small dermal arterioles.

**Prevention:**

- As the cause is unknown, it is very difficult to advise on prevention
- Bathing with warm water +/- suitable dog shampoo, after a walk to remove mud and any other substances is unlikely to do harm and may be beneficial
- There are no specific shampoos or chemicals recommended to prevent CRGV
- It is unknown whether certain walking locations should be avoided

**Risk of Spread?**

- Some dogs which have walked together, but not necessarily lived together, have all been affected
- Not all dogs in contact with a confirmed case develop disease
- Currently no evidence of direct transmission from one dog to another (or to humans)
- Currently no evidence of transmission in veterinary hospitals / surgeries, or other environments such as dog groomers
Therapy of Human TMAs

- **aHUS**
  - Plasma Exchange: removes overactive complement proteins, and replaces functionally defective ones – and is recommended for management of human aHUS
  - Eculizumab binds to C5, which acts at a late stage in the complement cascade. When activated, C5 is involved in attracting pro-inflammatory immune cells, while also destroying target cells by triggering pore formation (in aHUS it is the endothelial cells that are targeted). By inhibiting the complement cascade at this point, the normal, disease-preventing functions of proximal complement system are largely preserved, while the properties of C5 that promote inflammation and cell destruction are impeded. It is also used in human aHUS – cost ~£400K/person/year!

- **TTP**
  - caused by deficiency of ADAM TS 13, which cleaves von Willebrands’ factor - reducing blood clot formation.
  - The deficiency of ADAM TS 13 is often caused by anti-ADAM TS 13 antibody formation (i.e. the disease is often autoimmune)
  - but it can also be due to mutations in the gene encoding the ADAM TS 13 protein.
  - Plasma exchange represents the treatment of choice.
  - For refractory cases, a chimeric anti CD20 antibody called rituximab has also been shown to be efficacious.
  - Rituximab targets the B cells producing the anti-ADAM TS 13 antibodies.
  - Some patients remain in remission following treatment, but some patients experience recurrent disease relapses.

Research:

- Complement factors – a study is underway to compare the concentrations of various complement factors in dogs affected by CRGV with unaffected dogs
- *Aeromonas hydrophila* – another study is also in progress to investigate incidence of positive *Aeromonas* culture and serology in dogs with CRGV and those without
- ADAM TS 13 / ANTI ADAM TS 13 antibodies – a study is in the pipeline to identify whether there are any differences in ADAMTS 13 / anti-ADAMTS13 concentrations in dogs with CRGV versus those without

Goals of research:

- Identify if there is an environmental trigger: It may be something to which exposure could either be avoided, or for which preventative therapy (e.g. a vaccine) could be developed
- Potential for genetic tests to identify carriers if genetic abnormalities of the complement system are identified
- Develop better early diagnostic test(s): e.g measurement of complement factors, as in humans with aHUS
- Identify optimal treatment strategies
- Identify prognostic factors