Canine leishmaniosis and kidney disease: Q&A for an overall management in clinical practice

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MANUSCRIPT

1. Introduction

Canine leishmaniosis (CanL) is a systemic zoonotic disease caused by the protozoan *Leishmania infantum* (Paltrinieri *et al.* 2010) that is endemic in more than 70 countries (Solano-Gallego *et al.* 2011). There is evidence of spread to traditionally non-endemic areas such as North America (Gaskin *et al.* 2002, Duprey *et al.* 2006) and, especially, northern European countries such as the UK (Teske *et al.* 2002, Shaw *et al.* 2009, Geisweid *et al.* 2012, Maia & Cardoso 2015, Silvestrini *et al.* 2016, Medlock *et al.* 2018). Increases in the number of CanL cases in the UK might well be associated with increased importation of dogs into the UK, often involving dogs rescued from southern or eastern Europe (Norman *et al.* 2020, Traversa 2020).

CanL is a common cause of glomerulonephritis, which can cause proteinuria and may progress to renal failure (Zatelli *et al.* 2003, Aresu *et al.* 2013, dos Santos *et al.* 2013, Koutinas & Koutinas 2014). Azotaemia due to renal impairment has been described frequently in dogs with leishmaniosis, and some dogs may present with severe renal failure alone, which might ultimately be fatal (Slappendel 1988, Ferrer 1992, Ciaramella *et al.* 1997, Koutinas *et al.* 1999, De Freitas *et al.* 2012, Foglia Manzillo *et al.* 2013, Ribeiro *et al.* 2013). However, whilst proteinuria is commonly recognised at the time of diagnosis, renal azotaemia is reported to be a rare clinical finding (Zatelli *et al.* 2003, Planellas *et al.* 2009, Paltrinieri *et al.* 2016, Meléndez-Lazo *et al.* 2018).

The Canine Leishmaniosis Working Group (CLWG) was formed in November 2005 to develop a science-based consensus for management of CanL. Since then, several papers on diagnosis, clinical classification, treatment, prognosis and prevention of the disease have been developed (Maroli *et al.* 2010, Oliva *et al.* 2010, Paltrinieri *et al.* 2010, Roura *et al.* 2013). The aim of the current manuscript is to explore the relationship between *Leishmania* spp. infection and kidney disease in dogs, and

create guidelines for veterinarians to assist with management of those cases that develop renal disease. These guidelines are based on existing references and/or the experience of the CLWG members; however, veterinary clinicians should critically evaluate their potential applicability when approaching cases of CanL.

2. Do all dogs with leishmaniosis have renal disease?

This depends upon the diagnostic criteria used to establish the existence or absence of renal disease. If the diagnosis was based exclusively on the presence of azotaemia, the reported prevalence of kidney disease ranges between 5.9% (Meléndez-Lazo *et al*. 2018) and 38.1% (Koutinas *et al.* 1999) whilst, if pathological renal proteinuria was the criterion, then the prevalence would be approximately 50% (Font 1999, Cortadellas *et al.* 2006). If, however, diagnosed was based upon diagnostic imaging, kidney biopsy or direct visualisation of the kidneys, prevalence rises up (Polzin *et al*. 2005). In this respect, several studies (Poli *et al.* 1991, Nieto *et al*. 1992, Palacio *et al.* 1997, Costa *et al*. 2003, Plevraki *et al.* 2006, Aresu *et al*. 2013, Braga *et al*. 2015, Batista *et al.* 2020) have reported almost all dogs would be considered to renal disease. Therefore, veterinarians should be aware that, whilst only about half of dogs with leishmaniosis will have clinical evidence of renal disease based upon common diagnostic criteria, almost all are likely to be affected in some way.

3. Do we need to evaluate the presence of proteinuria in all dogs with leishmaniosis?

The answer to this question is yes, because, as discussed above, approximately 50% of dogs may have clinicopathologically detectable kidney disease at the time of diagnosis of CanL (Font 1999, Cortadellas *et al.* 2006). The renal disease is primarily of glomerular origin, usually involving different histopathological forms of glomerulonephritis, whilst renal amyloidosis occurs, only very rarely (Poli *et al.* 1991, Costa *et al.* 2003, Zatelli *et al.* 2003, Plevraki *et al.* 2006, Saridomichelakis 2009). Initially, asymptomatically-infected dogs with renal involvement present with moderate-to-severe proteinuria without azotaemia. As the disease progresses, tubulointerstitial lesions and azotaemia develop, ultimately leading to end-stage renal failure, which remains the most significant cause of death in CanL (Font 1999, Cortadellas *et al.* 2006, Koutinas & Koutinas 2014). Proteinuria is also an important marker of the progression of kidney disease in dogs with azotaemia, and is associated with greater risk of development of clinical signs and death (Jacob *at al.* 2005). Therefore, in order to establish an earlier diagnosis and improve the prognosis for existing kidney disease, quantification of proteinuria should be mandatory in all dogs with leishmaniosis.

4. **Is there a reason to measure blood pressure in dogs with leishmaniosis?**

Kidney diseases are the main cause of secondary systemic hypertension in dogs, associated or not to leishmaniosis, and different studies report a prevalence of between 9% and 93% (Acierno *et al.* 2018). In these dogs, a sustained increase in systolic blood pressure (SBP) may result in target organ damage affecting the eyes, heart, brain and kidneys (Jacob *et al.* 1999 and 2003, Cortadellas *et al.* 2006, Acierno *et al.* 2018). Further, in dogs with induced renal failure, the greatest SBP measurements were associated with increased proteinuria, a greater reduction in

glomerular filtration rate (GFR) and increased severity of renal injury (Finco 2004). Therefore, the concurrence of kidney changes and hypertension in dogs with leishmaniosis potentially exacerbate pre-existing chronic kidney disease (CKD), increasing the risk of mortality (Jacob *et al.* 2003). Given that the prevalence of systemic hypertension in dogs with leishmaniosis is reported to be between 29% (Braga *et al.* 2015) and 62% (Cortadellas *et al.* 2006), SBP should be measured in all CanL cases, in accordance with the recommendations of the International Renal Interest Society (IRIS 2019) and other published guidelines (Acierno *et al.* 2018).

5. What we know about the innate immune response in CanL?

The role of innate immunity in responding to canine infection with *Leishmania infantum* has been investigated in recent years, with a focus on the expression of the pattern-recognition receptors, Toll-like receptors (TLR) involved in initial recognition of microbial antigens by classical antigen presenting cells such as dendritic cells or macrophages. Overall, disease progression in leishmaniosis is associated with decreased expression of TLR, suggesting that the parasite subverts innate immunity by down-regulating expression of these molecules (Hosein *et al.* 2017). Gene expression studies of brain and splenic tissue from affected dogs revealed organ-specific patterns of up- or down-regulation of TLR-encoding genes (Grano et al. 2018). The association between a susceptibility phenotype for CanL and single nucleotide polymorphisms in genes encoding TLR has been investigated, but the associations identified did not achieve statistical significance (Soutter *et al.* 2019). The role of neutrophils as early effector cells in *Leishmania* infection has been explored by *in vitro* studies of neutrophil function including phagocytosis, chemotaxis and oxidative and non-oxidative intracellular pathways (Pereira *et al.*

2017). Neutrophil and macrophage recruitment to infected tissue is enhanced by the effect of IL-17 produced by T helper (Th) 1 cells (Toepp & Petersen, 2020).

In recent years, the involvement of acute phase proteins (APP) in a wide variety of canine infectious, neoplastic, inflammatory, and immune-mediated diseases have been investigated. Acute phase proteins provide another measure of an inflammatory response in addition to traditional assessment of leucocyte counts and profiles or the measurement of serum cytokine concentrations (Ceron *et al.* 2018). The concentration of APP (including C-reactive protein [CRP], haptoglobin ferritin, and others) is inevitably increased in CanL cases, and correlates with the severity of inflammatory disease, but do not contribute directly to the immunopathogenesis of renal lesions in CanL (Ceron *et al.* 2018). Nonetheless, they can provide a measurable index of the inflammatory response, charting progression and remission.

6. What we know about the adaptive immune response in CanL?

The complex immunopathology of CanL has been studied extensively and reviewed several times (Baneth *et al.* 2008, Day 2011, Koutinas & Koutinas 2014, Papadogiannakis & Koutinas 2015, Hosein *et al.* 2017, Toepp & Petersen 2020). The immunopathology that occurs in this disease is a consequence of interactions occurring between the *Leishmania* parasite, the sand fly vector (specifically the immunomodulatory properties of its saliva) and the local (cutaneous) and the systemic immune system of the host. Genetic background (breed) of affected dogs may also influence the immune system (Quilez *et al.* 2012, Hosein *et al.* 2017).

Fundamentally, there are two well-recognised, polarised adaptive immune responses that may be made to this infection (Day 2011, Toepp & Petersen 2020). It is these

differing immune responses that are thought to account for the variety in pathological changes and associated clinical signs recognised in dogs with overt clinical disease. In this respect, genetically-resistant dogs mount a robust Th1 immune response in which the signature cytokine (interferon [IFN]-γ) produced by Th1 cells; IFN-γ signals parasitized macrophages, enabling them to destroy intracytoplasmic *Leishmania* amastigotes and, thereby, limiting both the infection and associated inflammatory reactions which, ultimately, limit the reservoir capacity of the infected dog. In contrast, genetically-susceptible dogs mount a systemic immune response dominated by Th2 cells, regulatory T cells (Tregs) and regulatory B cells (Day 2011, Toepp & Petersen 2020). The signature cytokines released by Th2 cells include interleukins [IL]-4 and IL-13, which promote 'inappropriate' humoral immune responses by B lymphocytes, accounting for hypergammaglobulinaemia, autoantibody production, and formation of circulating immune complexes. The activity of Tregs and regulatory B cells (via the signature cytokine IL-10) downregulates the protective Th1 immune response and accounts for the persistence and chronicity of lesions, and of the infectious status of the parasitized dog (Day 2011, Toepp & Petersen 2020).

7. What we know about the pathophysiology and immunology of organ lesions associated to CanL?

The pathological basis of the multisystemic lesions of CanL therefore varies between target tissues. Granulomatous inflammation in a spectrum of organs (e.g. skin, lymph nodes, bone marrow, liver, intestinal tract) likely relates to the balance of activity between Th1 effector cells and T and B regulatory cells as discussed above. Depending upon the extent of the inflammation, organ function may be

impaired (Day 2011). A range of tissue autoantibodies may be induced in dogs with leishmaniosis, with possible mechanisms for their induction including polyclonal B-cell activation, inappropriate Th2-mediated activation of autoreactive B lymphocytes, release of autoantigens following tissue damage, or molecular mimicry between parasite or sand fly salivary antigens and host autoantigens (Day 2011). The ensuing plasma cell secretion of autoantibodies contributes to immune-mediated haemolysis (Ciaramella *et al.* 1997) and thrombocytopenia (Terrazzano *et al.* 2006) and the production of antinuclear (Lucena & Ginel 1998, Chaabouni *et al.* 2018), anti-histone (Ginel *et al.* 2008), anti-myofibre (Brandonisio *et al.* 1990; Vamvakidis *et al.* 2000), and peripheral antineutrophil cytoplasmic antibodies (Karagianni *et al.* 2012). In dogs with *Leishmania*-associated pemphigus foliaceus, the pathogenesis likely involves triggering of autoantibody, which binds to interepithelial desmosomal proteins within the epidermis. In human endemic pemphigus foliaceus (Fogo Selvagem) in Brazil, a sand fly salivary antigen triggers production of immunoglobulin (Ig) G4 antibodies that cross-react with desmoglein-1 (Qian *et al.* 2012), but there is no evidence for such a mechanism in CanL.

As discussed in more detail below, the presence of excess circulating antigen, antibodies specific for *Leishmania* bind to this antigen and create immune complexes that can lodge on vascular walls in regions of turbulent blood flow with endothelial damage; these complexes trigger local complement fixation, neutrophil recruitment, vascular damage and local leakage of fluid, proteins and inflammatory cells (Gizzarelli *et al.* 2020). In a recent proteomic study the composition of circulating immune complexes isolated from the blood of experimentally infected dogs was explored; the constituents that were most represented were molecules of the complement pathway and of the serpin family (Cacheiro-Llaguno *et al.* 2020),

the latter of which are serine protease inhibitors involved in modulation of numerous proteolytic cascades. The classical target sites for deposition of such complexes in CanL are the renal glomerulus, the anterior uvea, dermis, nasal mucosa and synovial membrane (Koutinas & Koutinas 2014). The chronic inflammatory state in CanL may also lead to tissue deposition of reactive amyloid in some cases.

Tissue infiltration by lymphoid cells, indicative of cell-mediated or cytotoxic immune reactions may also be part of the pathogenesis of some tissue pathology in CanL (Hosein *et al.* 2017). The 'interface dermatitis', seen histopathologically in depigmenting nasal lesions of dogs with leishmaniosis, is indistinguishable from the reaction characterizing discoid lupus erythematosus, suggesting similar immunopathology in the infectious and idiopathic immune-mediated diseases (De Lucia *et al.* 2017).

8. What we know about the pathophysiology and immunology of kidney lesions associated with CanL?

The kidney lesions associated with CanL are described in more detail below, but include chronic interstitial inflammation (associated with local infection) and glomerulonephritis (GN) (leading to proteinuria or nephrotic syndrome). A range of glomerular and secondary tubular lesions are recognised, including membranoproliferative GN, membranous GN and mesangioproliferative GN, progressing to interstitial fibrosis, glomerulosclerosis and end-stage renal disease (Costa *et al.* 2003, Aresu *et al*. 2013, dos Santos *et al.* 2013, Esch *et al.* 2015, Wilson *et al.* 2017). The major cause of glomerular pathology in CanL is thought to be deposition of preformed circulating immune complexes at different levels of the

glomerular unit; this is determined by the size and charge (cationic) of the complex. In membranoproliferative GN, immune complexes deposit within the mesangium and on either or both the epithelial or endothelial sides of the glomerular basement membrane (GBM). This results in GBM thickening with proliferation of mesangium and epithelial and/or endothelial cells. In membranous GN, immune complex deposition occurs on the epithelial side of the GBM, resulting in basement membrane thickening without cellular proliferation or inflammation. In mesangioproliferative glomerulonephritis, larger immune complexes deposit within the mesangium, leading to cellular proliferation and accumulation of mesangial matrix (Costa *et al.* 2003, Aresu *et al.* 2013, dos Santos *et al.* 2013, Wilson *et al.* 2017).

Classically, immune complex disease is associated with depletion of complement components and subnormal concentrations of circulating complement factors (e.g. C3 and C4). However, reduced concentrations of complement factors have not been consistently demonstrated in dogs with immune complex glomerulonephritis (Acierno *et al.* 2006). Circulating immune complexes could be detected in the blood of dogs with leishmaniosis (Brandonisio *et al.* 1990, Lopez *et al.* 1996, Cacheiro-Llaguno *et al.* 2020, Gizzarelli *et al.* 2020) and, in one clinical case, haemodialysis was used successfully as an adjunct to treatment of the disease (Baneth *et al.* 2018). Glomerular immunoreactants have also been demonstrated in affected kidneys by immunofluorescence labelling and by transmission electron microscopy (Aresu *et al.* 2013). Such glomerular immune complexes contain *Leishmania* antigens, antibodies (generally of the IgG or IgM classes) and complement molecules (specifically the third component of the complement pathway, C3) (Costa *et al.* 2003, Soares *et al.* 2009, Esch *et al.* 2015). Immunoreactants may also be found

within the glomeruli of clinically normal dogs without renal disease and this has been the case when control dogs were compared with dogs with leishmaniosis in some studies (Soares *et al*. 2009, Costa *et al.* 2010). In contrast to the data supporting the deposition of preformed antigen in the glomerulus, there is no evidence for the alternative mechanism of complexes forming locally between *Leishmania* antigens, which deposits first and subsequently binds ('captures') circulating antibody. That said, it has been suggested that histone proteins and anti-histone antibodies in dogs with leishmaniosis might act in such a fashion (Ginel *et al.* 2008). Similarly, there is no evidence that infected dogs with glomerular disease have circulating tissue autoantibodies specific for glomerular antigens that bind to their targets *in situ* and trigger localised inflammation. In such cases, immunofluorescence labelling would reveal linear deposition of Ig or complement along the GBM, which may, in time, become granular. Limited studies have explored the transcriptional expression of other immunologically relevant molecules within the kidneys of dogs with clinical leishmaniosis, including the induction of genes associated with autophagy and formation of inflammasomes (Esch *et al.* 2015).

The infiltration of $CD4^+$ and $CD8^+$ T lymphocytes into the kidneys of dogs with visceral leishmaniosis has been evaluated, together with the expression of adhesion molecules involved in T-cell recruitment into tissues. $CD4^+$ T cells generally predominate over $CD8^+$ cells, but the pattern of CD4/CD8 infiltration does not vary significantly with different forms of glomerulonephritis (Costa *et al.* 2010). There was upregulation of intercellular adhesion molecule (ICAM)-1 and P-selectin in the kidneys of infected versus control dogs (Costa *et al.* 2010). In contrast, there was

less apoptosis and expression of tumour necrosis factor (TNF)-α in the kidneys of infected versus control dogs (Costa *et al.* 2010).

9. What about circulating immune complexes and precipitin reactions?

As described above, the nature of the individual immune response determines whether the protozoan infestation will be controlled (e.g. in the presence of a cell-mediated response) or whether the dog will develop clinical signs due to the deposition of circulating immune complexes (e.g. in case of a humoral response involving the formation of antibodies) (Day 1999 and 2011). The presence of antibodies and antigens alone is not sufficient to produce circulating immune complexes since the latest have precise characteristics in order to be soluble and to circulate in the bloodstream (Lopez *et al.* 1996, Day 1999, Gizzarelli *et al.* 2020). For example, immune complexes that are too large are cleared by phagocytic cells, whilst complexes that are too small are unable to activate complement (Day 1999, Noris & Remuzzi 2013). Formation of immune complexes is best understood by considering the classical precipitin reaction:

1) An excess of antibody over antigen in a sensitised individual creates large immune complexes, which form at the site of antigens exposure leading to localised inflammatory disease, the Arthus reaction (Day 1999 and 2011).

2) An excess of antigen over antibody creates soluble immune complexes of small dimensions, which are more likely to circulate and deposit in the capillary beds of predilection sites such as the skin, synovia, uvea and renal glomerulus. This mechanism is the one that is thought to underlie the pathogenesis of immune-mediated glomerulonephritis (Poli *et al*. 1991, Nieto *et al*. 1992, Costa *et al*.

2000, Zatelli *et al.* 2003, Aresu *et al*. 2007, Aresu *et al*. 2013, dos Santos *et al*. 2013, Esch *et al.* 2015).

The deposition of circulating immune complexes also depends on several factors including: the size of the complex, the nature of the antigen (e.g. its chemical composition and charge), the nature of antibodies (e.g. complement fixing antibodies), vascular permeability and endothelial damage, and blood pressure and flow. With high pressure, turbulent flow and altered vascular permeability, the soluble circulating immune complexes deposit within the vessel wall, causing platelet aggregation and inflammation mediated by extracellular degranulation of neutrophils that cannot phagocytise the immune complexes (Day 1999, Warren 2006).

10. **What differences are there between the pathophysiology and immunology of lesions in the kidney and other organs?**

Although human patients with visceral leishmaniosis develop renal lesions (Clementi *et al.* 2011, Ortiz *et al*. 2015), and kidney pathology is known to occur in experimental infections of rodent species (Prianti *et al.* 2007), there have been relatively few detailed studies of renal immunopathology in these species. In contrast, the dog is regarded as a model for the likely changes occurring in infected people (Esch *et al.* 2015). Inflammation is reported in the kidneys of people with visceral leishmaniosis, and this is associated with *Leishmania* amastigotes, immune complex deposition and expression of adhesion molecules promoting T lymphocyte recruitment (Clementi *et al.* 2011, Ortiz *et al*. 2015). Circulating immune complexes of *Leishmania* antigens with immunoglobulin and complement have been identified (Clementi *et al.* 2011). Human patients with visceral leishmaniosis also develop a

spectrum of autoantibodies, including serum rheumatoid factor, antinuclear antibodies, anti-smooth muscle and anti-platelet antibodies; the antinuclear antibodies cross-react with *Leishmania* antigens, suggesting molecular mimicry as an underlying mechanism (Argov *et al.* 1989).

The murine model of experimental infection with *Leishmania major* in mice of different inbred strains was widely employed in the 1980s to dissect the Th1-Th2 paradigm in immunology. BALB/c mice are susceptible to infection and mount a classical Th2 immune response, whilst C57Bl/6 mice mount a Th1 response and are of resistant phenotype (Sadick *et al.* 1986, Loeuillet *et al.* 2016); however, these studies did not focus on renal immunopathology. Experimental infection of BALB/c mice with *Leishmania chagasi* led to lesions consistent with mesangioproliferative glomerulonephritis and IgG immune complex deposition; consistent with findings in the dog (Prianti *et al.* 2007).

11. **What pathological patterns in the kidney are associated with CanL?**

The renal lesions observed in CanL are consistent with immune complex-mediated glomerulonephritis (ICGN), suggesting an immune-mediated pathogenesis (Marcussen *et al*. 1989, Poli *et al*. 1991, Nieto *et al*. 1992, Costa *et al*. 2000, Zatelli *et al.* 2003, Aresu *et al*. 2007, Aresu *et al*. 2013, dos Santos *et al*. 2013, Esch *et al.* 2015). However, there is inconsistency in the published literature in both the identification of a glomerular pattern of injury and in the terminology used to classify ICGN (Poli *et al*. 1991, Nieto *et al*. 1992, Costa *et al*. 2000, Zatelli *et al.* 2003). This as a consequence of a poorly-defined classification scheme for canine glomerular disease and the traditional adoption of a human classification system that does not entirely apply to canines (see questions 8, 9 and 10). The terminology and

pattern definitions used in this review are consistent with those of the most recent literature on CanL (Cianciolo *et al.* 2016, Cianciolo *et al*. 2018).

Membranoproliferative glomerulonephritis (MPGN) is the most commonly-reported glomerular pattern of injury in CanL (Marcussen *et al*. 1989, Poli *et al*. 1991, Nieto *et al*. 1992, Costa *et al*. 2000, Aresu *et al*. 2007, Aresu *et al*. 2013, dos Santos *et al*. 2013, Esch *et al.* 2015), with the key features of MPGN being mesangial-cell proliferation, mesangial matrix expansion, endocapillary hypercellularity and glomerular basement membrane (GBM) thickening. There is both ultrastructural (e.g. transmission electron microscopy) and immunofluorescence (e.g. labelling of renal biopsy tissues with antibodies specific for canine IgG, IgM or complement C3, plus conjugated to a fluorochrome) evidence of mesangial and membranous immune deposits. According to the ultrastructural location of the deposits in the GMB, lesions can be subclassified as MPGN (only subendothelial deposits) or MPGN with mixed pattern (multiple membranous locations, including subendothelial, intramembranous or subepithelial). Immune deposits are mostly composed of IgG and IgM, while C3 and IgA are reported less frequently (Poli *et al*. 1991, Nieto *et al*. 1992, Esch *et al.* 2015).

Other types of ICGN, such as mesangioproliferative GN and membranous GN, are less frequently reported in CanL (Poli *et al*. 1991, Costa *et al*. 2000, Zatelli *et al.* 2003, Aresu *et al*. 2013). Mesangioproliferative GN is characterised by mesangial matrix expansion and hypercellularity with no GBM thickening or endocapillary hypercellularity. Immune deposits are located exclusively in the mesangium. In contrast, membranous GN is typically defined by GMB thickening with no significant hypercellularity and membranous deposits (subepithelial location).

According to the stage of the disease, there is variable degree of tubulo-interstitial damage with fibrosis and lymphoplasmacytic infiltration. However, tubulointerstitial lesions are thought to be secondary and are not observed without glomerular lesions, which are considered to be the primary lesion (Zatelli *et al.* 2003). The lymphocytic population is dominated by $CD4^+$ lymphocytes (T-helper cells) (Costa *et al*. 2000, Costa *et al*. 2010).

However, Poli *et al.* (1991) also reported the presence of immune deposits on the tubular basement membrane, identified by immunofluorescence. This observation suggests that at least part of the tubular damage might also have an immune-mediated nature. Glomerular amyloidosis is reported sporadically, but its association with *Leishmania* infection is not proven (George *et al*. 1976, Poli *et al*. 1991). Finally, intraparenchymal macrophages with intracytoplasmic amastigotes and vasculitis are uncommon findings in kidneys of dogs with leishmaniosis (Swenson *et al.* 1988, Pumarola *et al*. 1991).

12. What differences are there in renal histopathology between dogs and both humans and rats with leishmaniosis?

In both humans and dogs, leishmaniosis has a wide range of presentations (mainly visceral, cutaneous and mucosal) depending both on the species of *Leishmania* involved and the host immune response to the parasite. Visceral leishmaniosis in humans frequently affects immune-deficient patients, for example, those infected with the human immunodeficiency virus or organ transplant recipients (Clementi *et al*. 2011, Vassallo *et al*. 2014, Enriquez *et al*. 2015, El Jeri *et al.* 2017). Visceral leishmaniosis is also reported in association with other renal diseases such as those occurring as part of systemic lupus erythematous, *sicca* syndrome and diabetes (El

Jeri *et al*. 2017). Type III MPGN, amyloidosis and acute interstitial nephritis with intralesional parasites are the most common pathological findings in affected people (Enriquez *et al*. 2015, Vassallo *et al*. 2014). However, tubular necrosis is also described and thought to be secondary to the inflammation and/or ischemia due to small vessel obliteration by *Leishmania* parasites (Vassallo *et al*. 2014).

13. **What differences in clinical presentation are there between dogs with leishmaniosis with different histopathological patterns?**

Renal lesions in CanL are mainly attributed to the deposition of soluble circulating immune complexes within capillary beds of the glomerular tuft, leading to ICGN (see question 11) (Marcussen *et al*. 1989, Poli *et al*. 1991, Nieto *et al*. 1992, Costa *et al*. 2000, Zatelli *et al.* 2003, Aresu *et al*. 2007, Aresu *et al*. 2013, dos Santos *et al*. 2013, Esch *et al.* 2015). However, the clinical presentation of dogs with glomerulonephritis can be extremely variable, mainly depending on the stage of disease at diagnosis. Advanced glomerulonephritis, involving both the glomerulus and the tubulointerstitial compartment, can be characterised by severe proteinuria and renal failure, while the same histological alterations at an earlier stage can lead to mild or moderate proteinuria in the absence of renal failure. Regardless of these general premises, the severity of proteinuria varies with the different forms of glomerulonephritis, but it represents a marker identifiable in all types of glomerulonephritis (Poli *et al*. 1991, Costa *et al*. 2000, Aresu *et al*. 2013).

Membranoproliferative glomerulonephritis (MPGN) is the most commonly-reported glomerular pattern in dogs with leishmaniosis (Marcussen *et al*. 1989, Poli *et al*. 1991, Nieto *et al*. 1992, Costa *et al*. 2000, Aresu *et al*. 2007, Aresu *et al*. 2013, dos Santos *et al*. 2013, Esch *et al.* 2015). MPGN is also characterised by moderate

proteinuria and is typically progressive, and the overall renal prognosis is guarded-to-poor. No specific therapy has yet been shown definitively to modify the natural course of MPGN, and data confirming efficacy of glucocorticoids, cytotoxic agents or immunosuppressive drugs are absent.

Other types of ICGN are less frequently described in CanL. These include mesangioproliferative GN and membranous GN (Poli *et al*. 1991, Costa *et al*. 2000, Zatelli *et al.* 2003, Aresu *et al*. 2013). Mesangioproliferative GN is characterised by mild proteinuria, with many dogs being asymptomatic for years, never developing renal insufficiency. Anecdotal evidence suggests that dogs with mesangioproliferative GN have low risk of renal insufficiency, particularly those responding to antiproteinuric therapy.

In membranous GN, proteinuria is usually severe, and the most common clinical presentation is the nephrotic syndrome. The clinical course of membranous GN is affected by several factors, but the stage of the disease at the time of diagnosis is fundamental. Unfortunately, with severe persistent proteinuria, the renal damage is typically progressive and overall renal prognosis is poor, with a large number of dogs developing end-stage renal disease (Benderitter *et al.* 1988, Poli *et al*. 1991, Costa *et al*. 2000, Aresu *et al*. 2013).

14. **How do we diagnose leishmaniosis in dogs?**

There is no perfect single test for CanL and, therefore, diagnosis will depend on the clinical decision of the veterinarian made after evaluating a range of clinical and laboratory factors. The likelihood of diagnosing leishmaniosis increases when a dog that shows compatible clinical signs and laboratory alterations also has a markedly

positive antibody titre with serological testing (e.g. IFA antibody titter ≥1/320 if the cut-off of the test is 1/40), and when the parasite can be identified within tissue samples. Therefore, veterinarians should use information from multiple sources in order to make a diagnosis, including: clinical history, physical examination findings, laboratory changes (e.g. haematological, biochemical and on urinalysis), tests to detect the parasite (e.g. cytology, histopathology and polymerase chain reaction [PCR]), tests that evaluate the immune response of the host (e.g. serology), and response to treatment to support or refute the diagnosis of leishmaniosis (Paltrinieri *et al.* 2010, Rodríguez-Cortes *et al*. 2010, Solano-Gallego *et al.* 2011).

The main aim of diagnosis in dogs with compatible clinical signs is to demonstrate a cause-effect relationship with both pathological alterations and the presence of *Leishmania* parasites. Without such confirmation, there is a risk of falsely concluding that leishmaniosis is present, which can be problematic for dogs living in areas endemic for the disease. In this situation, first-line investigations should include both direct and indirect diagnostic tests (Saridomichelakis *et al.* 2005, Maia & Campino 2008, Paltrinieri *et al.* 2010, Solano-Gallego *et al.* 2011, De Tommasi *et al*. 2014). Direct tests involve identifying intralesional amastigotes using cytology, tissue biopsy, immunohistochemistry or PCR of injured tissues (if tissue damage is present), or cytology and/or PCR of bone marrow or lymph node aspirates (if there are no accessible lesions); indirect include qualitative and quantitative serological assays.

In dogs without clinical signs, based on a complete physical examination and a minimum database (e.g. haematological and serum biochemical profiles and urinalysis), the first-line diagnostic approaches should include specific indirect diagnostic tests as quantitative serological assays. This is because of the poor

sensitivity of qualitative serological assays in ruling out potential infection with *Leishmania* (Solano-Gallego *et al.* 2014). For screening in areas endemic for CanL, this quantitative serology is best performed between February and April; this is sufficient time after the end of the last seasonal period, but before the beginning of the *Phlebotomus* sand fly season. If indicated from the results of serology, more accurate and specific direct tests should then be performed including quantitative PCR assay on bone marrow or lymph node aspirates or conjunctival swabs or blood; PCR is preferred because of it's ability to detect the DNA of *Leishmania* and thereby confirming the infection (Paltrinieri *et al.* 2010, Solano-Gallego *et al.* 2011).

In both clinical situations, the use of vaccines for leishmaniosis in southern Europe and in some Latin American countries, known to elicit long-standing low-to-medium titre of antibodies against *Leishmania*, may further complicate the interpretation of serology in vaccinated dogs (Solano-Gallego *et al.* 2017).

15. **How should CanL be classified from a clinical perspective?**

In clinical practice, clinical staging is recommended in order to establish proper management, treatment and prognosis in dogs with leishmaniosis. The main aim is to decide whether the dog is: (1) infected but healthy, (2) infected and sick for other reasons, or (3) infected and sick due to leishmaniosis. These distinctions have become more complicated because vaccines are likely to induce seropositivity. A correct clinical staging must be relevant for the time of diagnosis, during the follow-up period, and also to help the clinician decide: (1) whether the dog should or should not be treated for leishmaniosis; (2) whether the dog needs additional treatments depending on the clinical signs displayed; and (3) what is the clinical prognosis.

The two best-known clinical staging systems for leishmaniosis in dogs are published, those produced by LeishVet (Solano-Gallego *et al*. 2011) and CLWG (Paltrinieri *et al.* 2010, Roura *et al.* 2013). However, another classification of CanL based on the severity of APP changes has been recently published (Ceron *et al.* 2018). A complete clinical and laboratory-based assessment of each dog at the time of diagnosis, together with serological responses and parasite detection, are necessary to characterise the severity of disease and to assign the case to a clinical stage (Paltrinieri *et al.* 2016, Ceron *et al.* 2018, Meléndez-Lazo *et al*. 2018, IRIS 2019). Following diagnosis, the dog should periodically be re-evaluated and re-classified in line with disease progression or regression (Oliva *et al.* 2010, Paltrinieri *et al.* 2010, Solano-Gallego *et al.* 2011, Roura *et al.* 2013, Paltrinieri *et al.* 2016, Meléndez-Lazo *et al*. 2018). The CLWG system (Table 1) classifies dogs as exposed, infected and sick, with the latter being further classified by severity (Oliva *et al.* 2010, Paltrinieri *et al.* 2010, Roura *et al.* 2013). *Exposed dogs* are clinically unremarkable, have a low-titre positive serology and are negative either by PCR, by cytology or with both. These dogs do not need treatment for leishmaniosis and have a favourable prognosis. *Infected dogs* are healthy or have clinical signs or clinicopathological alterations associated with other causes, but are positive either by PCR or cytology or both, based on samples from bone marrow, lymph node, spleen, skin or peripheral blood. These dogs only need treatment for clinical signs associated with other diseases, and the prognosis for leishmaniosis is favourable. *Sick infected dogs* have clinical signs or clinicopathological alterations associated with leishmaniosis. These dogs need treatment for leishmaniosis, and the prognosis is favourable-to-guarded. *Severely sick dogs* show severe clinical conditions, for example severe proteinuria associated with nephropathy, chronic kidney disease

(e.g. IRIS stages III and IV), ocular disease causing blindness or severe joint disease impairing motility. These dogs could require immunosuppressive drugs in addition to treatment for leishmaniosis, and their prognosis is guarded-to-poor.

16. **What differences are there amongst dogs with different clinical and serological statuses?**

Dogs with different clinical and serological statuses differ in the way they are treated and followed up. Seropositive dogs without clinical signs or laboratory alterations associated with leishmaniosis do not need treatment, even if they have a high positive antibody titre. However, there are promising results with the use of immunotherapeutic drugs, such as domperidone or nucleotides, that modulate and activate the immune response so as to prevent the development of clinical signs in this population of dogs (Sabaté *et al.* 2014, Hosein *et al.* 2017, Segarra *et al.* 2018). Seropositive dogs require close follow-up to enable early detection of clinical signs or clinicopathological alterations compatible with leishmaniosis (Oliva *et al.* 2010, Solano-Gallego *et al*., 2011, Solano-Gallego *et al*., 2017). The intervals for follow-up should be shorter when the antibody titre is higher especially when the dog is proteinuric, because there is an association between high positive antibody titre and the presence of clinical signs (Paltrinieri *et al.* 2010, Pierantozzi *et al.* 2013, Paltrinieri *et al.* 2016).

In contrast, although the other two groups of dogs with clinical signs or laboratory alterations secondary to *Leishmania* infection are seronegative, they should receive anti-*Leishmania* treatment as soon as possible so as to obtain a better long-term clinical response (Miró *et al*. 2008, Oliva *et al.* 2010, Solano-Gallego *et al*. 2011).

575

576 **17. How can we diagnose renal disease in dogs with leishmaniosis?**

577 The initial diagnostic investigation of renal disease in dogs with leishmaniosis
578 should include measurement of serum or plasma creatinine (ideally assessed twice
579 in a fasted, well-hydrated and normotensive dog) and urinalysis (to include urine
580 specific gravity [USG], dipstick, urine sediment examination and urinary protein-
581 creatinine ratio [UPC]). Depending upon the results obtained, other diagnostic
582 procedures may be indicated including serum symmetric dimethylarginine (SDMA)
583 concentration or systolic blood pressure measurement. Once the evaluation is
584 completed, the dog should be classified according to the IRIS staging scheme (Table
585 2; IRIS 2019).

586 Evaluation of proteinuria initially involves a dipstick test, allowing semi-
587 quantitative measurement of its magnitude. Although, traditionally, cystocentesis
588 samples were recommended, so that protein contamination from the lower urinary
589 tract was avoided, voided samples are appropriate provided that sediment is inactive
590 (Beatrice *et al.* 2010). In dogs, a negative dipstick result indicates that the dog is
591 probably non-proteinuric, therefore eliminating the need for further determination of
592 the UPC. When the dipstick result is 30 mg/dl or above, pathological proteinuria is
593 suspected and quantification with UPC is indicated (Zatelli *et al.* 2010, Roura *et al*.
594 2017). Ideally, pre-renal and post-renal causes of proteinuria should be excluded
595 before measuring the UPC (Lees *et al.* 2005, IRIS 2019). However, this is not
596 always possible in dogs with leishmaniosis, because both severe hyperproteinaemia
597 (causing overload proteinuria) and renal proteinuria can frequently coexist. To this
598 end, proteinuria should be re-evaluated after leishmanicide therapy. There may be
599 differences in UPC between samples obtained at home and those obtained at the

hospital (Duffy *et al*. 2015), so samples should always be obtained under the same conditions. The current recommendation is to consider that a dog is non-proteinuric when UPC < 0.2, while those dogs with 0.2 ≤ UPC ≥ 0.5 are borderline proteinuric and need to be re-evaluated within 2 months. Finally, dogs with a UPC > 0.5 are considered to be proteinuric (Roura *et al*. 2017, IRIS 2019). If proteinuria is persistent (e.g. in three separate samples over a 2-3 week period or documented on a pooled sample of three voidings) (Lees *at al.* 2005, LeVine *et al.* 2010, Paltrinieri *et al*. 2016), pre-renal and post-renal causes of proteinuria have been excluded, and the urine sediment is inactive, then a UPC ≥ 2.0 usually is due to a glomerular disease, whilst a UPC between 0.5 and 2.0 could indicate either a glomerular or a tubular renal disease (Lees *et al.* 2005). That said, primary tubulointerstitial proteinuria has occasionally been described in dogs with a UPC > 2.0, so this does not necessarily preclude primary tubulointerstitial disease (Schneider *et al*. 2013).

In addition to UPC measurement, urine protein electrophoresis using sodium dodecyl sulphate polyacrylamide gel (SDS-PAGE), which separates proteins according to their molecular mass, may help to determine whether proteinuria is of tubular (e.g. low molecular weight proteins) or glomerular (e.g. intermediate and high molecular weight proteins) origin (Zaragoza *et al.* 2003, Zini *et al.* 2004, Roura *et al.* 2017). Results of SDS-PAGE correlate well with histopathology of renal biopsy samples, especially for the differentiation between glomerular and severe tubulointerstitial damage (Zini *et al.* 2004, Brown *et al*. 2010). A majority of dogs with leishmaniosis have a mixed glomerular and tubular pattern, although pure glomerular proteinuria can be seen in early stages (Zatelli *et al*. 2003).

Different authors have investigated the usefulness of measuring the activity of some urinary markers of glomerular (IgG, c-reactive protein, and ferritin) or tubular (N-

acetyl-β-D-glucosaminidase, γ-glutamyl transpeptidase, retinol-binding protein, and β-glucuronidase) damage in dogs with leishmaniosis (Palacio *et al.* 1997, Ibba *et al.* 2016, Pardo-Marín *et al.* 2017, Paltrinieri *et al.* 2018). Although the results of some of these studies are promising, more investigations are warranted before such tests can be widely recommended.

Recently, serum SDMA has been investigated for early detection of decreased GFR in dogs with leishmaniosis; although measuring SDMA could help, it did not increase diagnostic sensitivity compared with UPC measurement (Torrent *et al.* 2018, Giapitzoglou *et al.* 2020). This is to be expected because proteinuria is the first clinicopathological finding that indicates nephropathy associated with leishmaniosis (Koutinas & Koutinas 2014, Paltrinieri *et al.* 2016), and GFR is not usually decreased in the initial stages of glomerular disease, instead being normal or increased (Cortadellas *et al.* 2008). Although this limits the usefulness of SDMA as an early indicator of renal disease in Can. It might still be useful for detecting kidney dysfunction caused by other pathogenic mechanisms. If the results of the laboratory evaluation are all normal, the existence of renal disease might be further investigated by performing kidney biopsy, although this not always easily justified from a clinical perspective.

18. **When should renal biopsy be performed?**

In general, renal biopsy is indicated a definitive diagnosis can contribute to improve clinical management of the dog, thereby improving outcome (Lees & Bahr 2011). The main indication for a kidney biopsy in dogs with leishmaniosis would be to investigate the existence of an active immune-mediated component that could indicate therapeutic intervention with immunosuppressive drugs (IRIS *et al.* 2013b).

Additionally, in the presence of proteinuria, renal biopsy can establish if a renal lesion not related to leishmaniosis is present (Costa *et al.* 2003, Zatelli *et al.* 2003, IRIS *et al.* 2013b) a kidney biopsy may help to establish if a renal lesion not related to leishmaniosis is present. Nevertheless, this does not mean that renal biopsy should be performed in all seropositive and proteinuric dogs. As discussed previously, dogs with leishmaniosis and associated renal disease should receive leishmanicide therapy and, when indicated, renal treatment according to the severity of the disease. By itself, the leishmanicide treatment can ameliorate the severity of renal disease (Plevraki *et al.* 2006, Pierantozzi *et al.* 2013). Moreover, standard therapy for renal disease (IRIS 2019) can also help preserve renal function and extend survival (Grauer *et al.* 2000, Jacob *et al.* 2002, Cortadellas *et al.* 2014, Zatelli *et al.* 2016). Therefore, when an adequate response to these therapies is observed, performing renal biopsies will probably not alter outcome. However, if there has not been an adequate response, renal biopsy could be indicated especially if SDS-PAGE did not clarify the origin of urinary proteins. It has been recommended that biopsy should be considered when proteinuria is substantial (UPC $\geq$ 3.5) in dogs already receiving standard therapy for glomerular disease, if renal disease is progressive, if administration of immunosuppressive drug therapy is being considered or when the kidney disease is not end-stage (IRIS *et al.* 2013c).

Clinicians should also consider situations where renal biopsy is contraindicated; examples include: dogs with IRIS advanced stage 3 or stage 4 CKD where biopsy will probably not provide any valuable information, concurrent coagulopathies, renal cystic disease, moderate-to-severe hydronephrosis, pyelonephritis, perirenal fluid, uncontrolled hypertension, or severe anaemia. Finally, the clinician should have access to a specialist renal diagnostic pathology centre that can perform

electron microscopy and immunofluorescence microscopy as well as light microscopy. Several studies performed in dogs with glomerular disease have shown that, although light microscopy leads to correct diagnosis in 73-77% of cases, immunofluorescence and electron microscopy are required in the remainder, not least to confirm of an immune-mediated component to the disease (Schneider *et al.* 2013, Cianciolo *et al.* 2016, Aresu *et al.* 2017). Therefore, approximately 1 in 4 dogs could be erroneously classified if only light microscopy is available, although this ratio could differ in leishmaniotic dogs where minimal change glomerulonephritis is less frequent.

19. **How should renal biopsy be performed?**

Once it has been decided that renal biopsy could be of benefit in a particular dog, the clinician needs to decide how to obtain the sample and where to submit it for the histopathological examination. Different methods of obtaining kidney biopsies have been described including percutaneous techniques (e.g. laparoscopy, keyhole technique, ultrasound-guided, or blind) and surgical biopsy (Osborne *et al.* 1996, Rawlings *et al*. 2003, Vaden 2004, Vaden & Brown 2017). A detailed description of these procedures is outside the scope of these guidelines. Although, obtaining kidney tissue is considered a safe and relatively low-risk procedure, it is not exempt from complications that can be severe (Vaden 2004, Vaden *et al.* 2005). Therefore, it is not advisable for inexperienced operators to perform this procedure. Ideally, the least invasive procedure capable of rendering satisfactory results should be chosen, with ultrasound-guided needle biopsy producing satisfactory results in a majority of cases (Zatelli *et al.* 2005, Lees *et al.* 2011, Crivellenti *et al.* 2018). However, other

699 methods could be selected if preferred and if the benefits outweigh the risks (Vaden
700 *et al.* 2005).

701

702 20. **What is the treatment protocol for kidney disease associated with CanL?**

703 Any dog diagnosed with kidney disease should be classified and treated following
704 the IRIS recommendations (IRIS 2019), as detailed in questions 21-25. This is also
705 the case for dogs with leishmaniosis, although clinicians have the advantage of
706 knowing the cause of the renal disease (IRIS *et al.* 2013b). Treatment should be
707 tailored to each individual case, and the risk:benefit ratio of each treatment should
708 be carefully considered (IRIS *et al.* 2013b, Baneth *et al.* 2018). However, when
709 leishmaniosis-induced kidney disease is identified, independent of creatinine
710 concentration, specific treatment for leishmaniosis should be started immediately,
711 either alone or together with the standard treatment recommended for the IRIS
712 (Table 2; Torres *et al*. 2011, IRIS *et al.* 2013b, Pierantozzi *et al.* 2013, Baneth *et al.*
713 2018, Daza González *et al.* 2019, IRIS 2019). Such dogs should then be closely
714 followed up to determine the efficacy of treatment (Figure 1; Segev *et al.* 2008,
715 Oliva *et al*. 2010, Solano-Gallego *et al*. 2011, IRIS *et al.* 2013b).

716

717 21. **How is proteinuria associated with CanL treated?**

718 Proteinuria associated with CanL requires different therapeutic approaches,
719 depending on the dog's clinical condition and the stage of disease (Figure 1;
720 Plevraki *et al.* 2006, Oliva *et al.* 2010, IRIS *et al.* 2013b, Pierantozzi *et al.* 2013,
721 Cortadellas *et al.* 2014, Proverbio *et al.* 2016, Zatelli *et al*. 2016, Daza González *et*
722 *al.* 2019). In dogs with UPC ≤ 3.0 and active leishmaniosis and hose that are sick or

723 severely sick (Table 1; Paltrinieri *et al.* 2010, Roura *et al.* 2013), should be given
724 leishmanicide therapy and renal parameters should be re-evaluated afterwards (IRIS
725 *et al.* 2013b, Pierantozzi *et al.* 2013). If, at follow-up and regardless of serum
726 creatinine concentration, the dog is still proteinuric (UPC > 0.5), therapy for
727 proteinuria should be administered (Plevraki *et al.* 2006, IRIS *et al.* 2013b,
728 Pierantozzi *et al.* 2013, Cortadellas *et al.* 2014, Proverbio *et al.* 2016, Zatelli *et al*.
729 2016, IRIS 2019).

730 A sequential treatment protocol thas been recommended (Figure 1) as follows: a)
731 identify and treat with a leishmanicide drug plus allopurinol; b) revaluate and stage
732 renal disease after finishing the leishmanicide treatment; c) if proteinuria is not
733 controlled, continue with allopurinol and start a low-phosphorus therapeutic diet
734 alone or together with an angiotensin converting enzyme inhibitor (ACEi) such as
735 enalapril or benazepril at 0.5 mg/kg q12h PO; d) after 4 weeks of antiproteinuric
736 treatment, revaluate and restage renal disease; e) if proteinuria is still not controlled,
737 increase the ACEi dose (maximum 2 mg/kg q24h PO) if it was not prescribed yet,
738 add an angiotensin-receptor blockers (ARB) such as telmisartan (1 mg/kg q24h PO)
739 or losartan (0.125-0.25 mg/kg q12h PO) or administer approximately 80 mg/kg
740 q24h PO of polyunsaturated fatty acids (particularly an omega-3 fatty acid such as
741 eicosapentaenoic); f) administer low-dose acetylsalicylic acid (1-5 mg/kg q24h PO)
742 or clopidogrel (1-3 mg/kg q24h PO) in all dogs when serum albumin persistently
743 remains below 2.0 g/dl (< 20 g/l).

744 Close monitoring of kidney function, blood pressure and plasma potassium
745 concentration are essential when administering high doses of an ACEi especially
746 when combined with an ARB requires.

747 Hypercoagulability is a potential risk in dogs with severe persistent
748 hypoalbuminemia (IRIS 2019), althought it cannot always be predicted from the
749 serum albumin concentration in dogs with protein-losing nephropathy (White *et al.*
750 2016).

751 These dogs with leishmaniosis and proteinuria could be treated or not with this *step-*
752 *by-step* protocol, depending on the clinical evolution and severity of proteinuria
753 (Zatelli *et al*. 2016). The approach to proteinuria described above can also be used
754 in dogs either exposed to or infected with *Leishmania* according to the CLWG
755 classification but who do not require leishmanicide therapy (Table 1; Paltrinieri *et*
756 *al.* 2010, Roura *et al.* 2013). However, in sick or severely sick dogs in active
757 leishmaniosis (Table 1; Paltrinieri *et al.* 2010, Roura *et al.* 2013) and UPC >3.0
758 concurrent leishmanicide (Pierantozzi *et al.* 2013, Proverbio *et al.* 2016, Daza
759 González *et al.* 2019) and antiproteinuric therapy could be considered, possibly even
760 using all antiproteinuric treatments at once (IRIS *et al.* 2013b, IRIS 2019).

761

762 22. **Do all dogs with leishmaniosis and kidney disease need ACEi?**

763 Not all dogs with leishmaniosis and kidney disease need ACEi (Plevraki *et al.* 2006,
764 IRIS *et al.* 2013b, Pierantozzi *et al.* 2013, Cortadellas *et al.* 2014, Proverbio *et al.*
765 2016, Zatelli *et al*. 2016). For example, some may have non-proteinuric renal
766 disease or be dehydrated (IRIS *et al.* 2013a and 2013b, IRIS 2019), whereas
767 treatment with leishmanicides might lead to a reduction in UPC < 0.5. In such cases,
768 ACEi can still be introduced if there is a subsequent increase in UPC or if systemic
769 hypertension is detected at follow-up examinations (see question 21).

In some countries, enalapril is the only ACEI registered for the treatment of proteinuria in dogs, although others drugs from the same class (e.g. benazepril) are likely to have the same effect. For both drugs, 0.5 mg/kg q12h PO is most effective at reducing the magnitude of proteinuria (Cortadellas *et al.* 2014, Zatelli *et al*. 2016, IRIS 2019, Keene *et al.* 2019).

23. **Do all dogs with leishmaniosis and kidney disease need antihypertensive therapy?**

Not all dogs with leishmaniosis require antihypertensive therapy, and decisions on intervention should be made in accordance with internationally-accepted recommendations (Table 2; IRIS 2019). Anti-hypertensive therapy is usually administered when there is evidence of persistent SBP above 160 mmHg, given the risk of further damage to the kidney, leading to the progression of renal disease (Acierno *et al.* 2018, IRIS 2019). Any dog with leishmaniosis and SBP > 160 mmHg needs therapy with antihypertensive drugs in order to reduce blood pressure below this reference value (Acierno *et al.* 2018, IRIS 2019). Dogs with evidence of target organ damage (e.g. eye, central nervous system or heart) should be treated, even if persistent hypertension cannot be demonstrated (Acierno *et al.* 2018, IRIS 2019).

Angiotensin converting enzyme inhibitors are the first choice for the treatment of systemic hypertension in dogs but, if hypertension cannot be controlled, the clinician should consider increasing the ACEi dose, adding a calcium channel blocker such as amlodipine (0.1-0.5 mg/kg q24h PO) or adding an ARB such as telmisartan (Acierno *et al.* 2018, IRIS 2019). If combinations of ACEi, amlodipine and ARB are used, close monitoring of kidney function and blood pressure are

required given the risks of hypokalaemia and systemic hypotension (see questions 20-22).

24. **Do all dogs with leishmaniosis and kidney disease need a renal diet?**

Nephropathic dogs with leishmaniosis can have proteinuria, azotaemia or both. In all of the above conditions, administration of a low phosphorus therapeutic diet is recommended. Exceptions are made for sick and severely sick dogs according to the CLWG classification (Table 1; Paltrinieri *et al.* 2010, Roura *et al.* 2013); such dogs may not need dietary and, as described above (IRIS *et al.* 2013b, Pierantozzi *et al.* 2013), are treated initially with leishmanicide therapy and subsequently re-evaluated and staged according to the IRIS staging system (IRIS 2019) (see question 21).

25. Is there any benefit of using glucocorticoids or other immunosuppressive drugs in the management of dogs with leishmaniosis and kidney disease?

Clinicians have long debated the use of glucocorticoids in CanL, but robust evidence one way or another is lacking. Based upon personal experience, some argue that they can ameliorate clinical signs and improve outcomes (Bonavia *et al.* 1995, Cortese *et al.* 2008). In contrast, others discourage their use given possible because negative effects (Center *et al.* 1987, Waters *et al.* 1997, IRIS *et al.* 2013e). Several studies have reported beneficial effects associated with the use of glucocorticoids (mainly prednisone and prednisolone at variable doses) in dogs with leishmaniosis and clinical presentations potentially caused by immune complex deposition (Bergeaud 1988, Blavier *et al.* 2001, Cortese *et al.* 2008, Sbrana *et al.* 2014). However, only two studies (Bergeaud 1988, Bonavia *et al.* 1995) evaluated

dogs with renal disease and neither included a control group, making it difficult to interpret the results. Other studies have been small, including dogs with arthritis (Sbrana *et al.* 2014) and designed to evaluate the haemostatic function in CanL (Cortese *et al.* 2008), whilst one final study did not support the recommendation for using glucocorticoids in dogs with leishmaniosis (Blavier *et al.* 2001).

Nevertheless, evidence against the use of glucocorticoids is also not strong. Adamama-Moraitou *et al* (2005) discouraged the use of prednisolone, at an immunosuppressive dose, in dogs with leishmaniosis given the risk of promoting parasite replication by increasing serum iron concentration and decreasing copper concentration. However, this did not actually occur in any dog included in their study. In contrast, reactivation of the disease after prolonged treatment with glucocorticoids has been described in human patients and murine models (Rousseau *et al.* 1998, Ortiz *et al.* 2015).

The IRIS group currently recommends using immunosuppressive drugs in dogs with an active ICGN, which could be the case in many dogs with leishmaniosis and renal disease (IRIS *et al.* 2013b). This recommendation is based on the prediction that suppression of humoral or cell-mediated immunity and the associated glomerular inflammatory response will favourably influence the progression, severity and clinical outcome of the disease (Day 1999, Noris & Remuzzi 2013) (see question 9). Ideally, this decision should be based on results of a kidney biopsy (see question 18). However, if dogs are already receiving standard therapy for glomerular disease, but they have progressive increases in serum creatinine or evidence of rapidly progressive glomerular disease, and no concurrent infectious disease has been detected, then immunosuppressive therapy could be considered, even in absence of a histopathological diagnosis (IRIS *et al.* 2013d, IRIS *et al.* 2013e).

844 Considering that severe complications are rare, current international
845 recommendations are to use mycophenolate mofetil (10 mg/kg q12 h PO) as the first
846 choice for dogs with rapidly progressive glomerular disease, with
847 cyclophosphamide (50 mg/m^2 q48h PO) considered to be an alternative when
848 mycophenolate appears is ineffective (IRIS *et al.* 2013e). Although glucocorticoids
849 are not recommended as sole treatment, due to slow onset of action, an
850 immunosuppressive dose of prednisolone (2 mg/kg q24h initially, then tapered) can
851 be used in combination with these drugs (IRIS *et al.* 2013e). Dogs with stable or
852 slowly-progressive disease (defined as minimally-progressive proteinuria and
853 azotaemia, with normo-albuminaemia or minimal hypoalbuminaemia), and without
854 evident oedema or clinical signs of uraemia, can be treated with the above
855 mentioned drugs or with drugs with more delayed onset including chlorambucil
856 (0.1-0.2 mg/kg q24h PO) alone, or in combination with azathioprine (1-2 mg/kg
857 q24h PO) on alternating days, or ciclosporin (5-10 mg/kg q12-24h PO)(IRIS *et al.*
858 2013e). In absence of adverse effects, at least 8–12 weeks of therapy should be
859 administered (IRIS *et al.* 2013e).

860 It should be noted that these recommendations are based upon evidence of efficacy
861 in human patients (Emancipator 1998) and uncontrolled clinical experience in dogs
862 (IRIS *et al.* 2013e). In fact, there is only a single case report describing the use of
863 mycophenolate in a dog with glomerulonephritis of uncharacterised pathology
864 (Banyard & Hassett 2001). In contrast, it cannot be assumed that all proteinuric dogs
865 with leishmaniosis have ICGN. Although this may be true for a majority of cases,
866 the veterinary literature has described situations in proteinuric dogs with
867 leishmaniosis in which immunosuppressive drug administration would be
868 contraindicated. These include dogs with uncommon renal amyloidosis (George *et*

869 *al.* 1976, Poli *et al.* 1991) and those with chronic glomerulosclerosis without
870 immune complex deposits (Aresu *et al.* 2013).

871 In summary, robust evidence supporting the use and dosage of immunosuppressive
872 drugs in the management of dogs with renal disease secondary to leishmaniosis is
873 lacking. The use of prednisone or prednisolone at anti-inflammatory dosage (0.7
874 mg/kg q24h over a 3-10 days period) to reduce the renal inflammation secondary
875 due to deposition of immune complexes, and not to decrease their formation and
876 circulation, is based only on expert opinions (Figure 1). Therefore, once the decision
877 to treat a dog with leishmaniosis with immunosuppressive drugs is taken, the
878 clinician should discuss with the owners the arguments for and against the use of
879 those drugs. Considering their potential side effects, these agents should be
880 administered cautiously, adjusting doses and with close and careful monitoring
881 (IRIS *et al.* 2013b).

882

883 26. **How should dogs with leishmaniosis and kidney disease be treated?**

884 In dogs, the objectives of anti-*Leishmania* treatment are typically: (1) to induce
885 reduction in the parasite load in order to produce clinical and clinicopathological
886 improvement, (2) to restore normal immune function, (3) to avoid clinical relapses,
887 and, (4) to reduce the chance of further infection of sand flies (Gradoni *et al.* 1987,
888 Vouldoukis *et al.* 1996, Bourdoiseau *et al.* 1997, Noli & Auxilia 2005, Mateo *et al.*
889 2009, Oliva *et al.* 2010, Miró *et al*. 2011, Solano-Gallego *et al.* 2011).

890 Given that the treatment of CanL is always a clinical decision, the clinician must
891 decide the best treatment in each case, based on clinical presentation, published
892 scientific evidence, and owner factors. However, for sick dogs with leishmaniosis

(Table 1), the combination of meglumine antimoniate at 50-100 mg/kg q12-24h SQ for 1 month and allopurinol (10 mg/kg q12 PO, or q24h with presence of xanthinuria, for at least 12 months) is the most widely described and effective treatment. If this treatment regimen is not possible, an alternative is a combination of miltefosine (2 mg/kg q24h PO for 28 days) and allopurinol (Oliva *et al*. 2010, Solano-Gallego *et al*. 2011).

Several studies have demonstrated that all of these drugs can improve and prevent progression of kidney disease in dogs with leishmaniosis (Plevraki *et al.* 2006, Torres *et al*. 2011, Pierantozzi *et al.* 2013, Proverbio *et al.* 2016, Paltrinieri *et al.* 2018, Daza González *et al.* 2019) (see questions 20-21). However, to date, there has been just one study of pathological renal damage, but without clinical or clinicopathological alterations, induced by meglumine antimoniate in dogs (Bianciardi *et al.* 2009). In contrast, two recent studies have indicated no impact of meglumine antimoniate treatment for CanL on kidney function on kidney function (Daza González *et al.* 2019, Kasabalis *et al.* 2019). The increase of azotaemia or proteinuria in some dogs treated with meglumine antimoniate is most likely due to the kidney-specific patho-mechanism of formation and deposition of immune complexes than to nephrotoxicity of the drug (Koutinas & Koutinas 2014, Kasabalis *et al.* 2019) (see question 9). The routine use of other protocols or drugs to treat leishmaniosis in dogs with kidney disease is no longer recommended (Pineda *et al.* 2017).

27. **When and how do we need to control the evolution of leishmaniosis in dogs?**

The ideal frequency, the tests used or the best protocol for managing leishmaniosis in dogs with or without treatment has not been defined (Paltrinieri *et al.* 2016, Meléndez-Lazo *et al.* 2018). In general, dogs are followed according to their individual needs, which are primarily driven by health status and clinicopathological situation at the time of assessment.

In dogs that are exposed or infected (Table 1), but not receiving treatment because there are no clinical signs or pathological alterations, it makes sense to undertake a physical examination, minimum database (haematological and serum biochemical examinations and urinalysis), and serological testing every 6-12 months in order to confirm that retain the same clinical classification. To interpret better the results of serology, testing should be undertaken close to the beginning of the sand fly season (Oliva *et al.* 2010, Roura *et al.* 2013, Paltrinieri *et al.* 2016).

In sick dogs without any renal changes or with kidney disease IRIS stage 1 at the beginning of the treatment (Table 1-2), it makes sense to undertake a physical examination, haematological and serum biochemical examination (that could include protein electrophoresis and APP measurement, depending on the clinician's criteria), and urinalysis (especially USG and UPC) (Pardo-Marín *et al.* 2017, Ceron *et al.* 2018) at the end of leishmanicide treatment [1-7 days for meglumine antimoniate (Pierantozzi *et al.* 2013, Solano-Gallego *et al.* 2016); 3-4 weeks for miltefosine (Proverbio *et al.* 2016)] used. Later on, these assessments, including quantitative serology for leishmaniosis, could be repeated every 3-4 months during the first year after leishmanicide treatment. Subsequently, assessments (including real-time qPCR) should be undertaken every 6-12 months for life, to inform future decisions about clinical staging, treatment and prognosis for each dog with leishmaniosis (Oliva *et al.* 2010, Paltrinieri *et al.* 2010, Martínez *et al.* 2011, Roura

942 *et al.* 2013, Paltrinieri *et al.* 2016). However, the schedule and the tests evaluated in
943 these controls could vary, depending on the health status of the dogs and the clinical
944 decisions of the clinicians.

945 In sick dogs with severe clinical signs (e.g. uveitis or kidney disease of IRIS stages
946 2-4) (Tables 1-2), as well as the previously-described assessments, tests for
947 secondary conditions should be considered as per published recommendations
948 (Oliva *et al.* 2010, Paltrinieri *et al.* 2010, Roura *et al.* 2013, Paltrinieri *et al.* 2016).
949 When IRIS stages 2-4 are present, a further check should be performed 3-5 days
950 after starting meglumine antimoniate, to evaluate for a possible worsening clinical
951 status due to immune complexes deposition, and to determine the need for whether
952 adjustments to therapy are needed (see question 25 and Figure 1).

953

954 **28. When and how do we need to control the evolution of kidney disease in**
955 **dogs with leishmaniosis?**

956 Most dogs that have been thoroughly evaluated and are considered to be free of
957 renal disease at the time that leishmaniosis is diagnosed do not to develop it
958 (Planellas *et al*. 2009). Therefore, no specific renal management is required beyond
959 routine monitoring for dogs with leishmaniosis. In contrast, renal function should be
960 revaluated periodically in dogs with renal disease require, so as to optimise the
961 drugs and dosages that are requiered (IRIS *et al.* 2013a, IRIS *et al.* 2013b, Roura *et*
962 *al.* 2013). Although other alterations can be present, the pillars of monitoring renal
963 disease in dogs with leishmaniosis are to control changes in proteinuria, azotaemia,
964 hypoalbuminaemia, blood pressure and hyperphosphataemia. The following
965 recommendations only apply to dogs that are clinically stable and are receiving

966 ambulatory treatment; animals requiring hospitalisation because of their poor
967 clinical condition require a different monitoring protocol.

968 Although ACEi and ARB are considered safe when used in stable dogs, they are not
969 free of side effects, the most relevant of which are hyperkalaemia, hypotension and
970 reduction of GFR, resulting in increases in serum creatinine concentration. The
971 authors advise that dogs treated with ACEi, ARB or their combination have
972 creatinine, potassium and SBP reassessed within 5-7 days (even earlier for dogs at
973 IRIS advanced stage 3 or stage 4) of starting the treatment or when the dose is
974 increased. Increases of serum creatinine concentration of 0.5 mg/dl (< 45 µmol/l)
975 and/or SDMA of < 2 µg/dl could be attributed to effect of the ACEi and/or ARB on
976 glomerular haemodynamics for dogs in IRIS stages 1, 2 and 3, while greater
977 changes suggest disease progression and require treatment adjustment. However,
978 any increase in serum creatinine is considered unacceptable for dogs in IRIS stage 4
979 (IRIS *et al.* 2013a).

980 As a general rule, we recommend reassessing changes in proteinuria, albumin,
981 creatinine and phosphate concentrations every 4-6 weeks until values are stable or
982 until the target value for UPC and plasma phosphate have been reached (IRIS 2019).
983 Increases in serum albumin concentration and UPC reductions suggest a positive
984 response to therapy. However, since these can also occur in dogs with progressive
985 excretory failure, such changes must be interpreted in conjunction with variations in
986 creatinine concentration (IRIS *et al.* 2013e). Subsequently, dogs at IRIS stages 1, 2
987 or early stage 3 are revaluated every three months for the first year and thereafter
988 every 6-12 months at IRIS stage 1, twice a year at IRIS stage 2, and three times a
989 year at IRIS stage 3. Dogs in IRIS advanced stage 3 or stage 4 should be evaluated
990 every 4-6 weeks. When assessing proteinuria, day-to-day variations in the UPC

should not be ignored, especially in dogs with UPC > 4 (Nabity *et al.* 2007). In these cases, consideration should be given to either averaging 2–3 serial UPC measurements or measuring UPC in urine sample that has been pooled from 2 to 3 collections (LeVine *et al.* 2010). Moreover, the required percentage of change to consider that variation in the UPC is significant decreases with the severity of proteinuria. For dogs with massive proteinuria (UPC > 12) > 35% change is required, while for dogs with UPC close to 0.5, > 80% of change is necessary (Nabity *et al.* 2007).

Regarding serum phosphate concentration, the authors' advice is to follow the IRIS recommendations (IRIS 2019). Initial SBP monitoring is conducted 1-14 days after starting therapy depending on the severity of hypertension, the IRIS stage of CKD and dog stability. Dogs at IRIS stages 1-2 with mild increase in SBP could be revaluated in 2 weeks, while unstable hospitalised dogs at IRIS stages 3-4 need to be reassessed daily (IRIS *et al.* 2013a, Acierno *et al.* 2018). Once the target SBP has been reached, monitoring should occur at least every 3-6 months (Acierno *et al.* 2018, IRIS 2019).

In dogs that are put on immunosuppressive treatment, initial assessments should be performed no later than 1–2 weeks after initiation of the treatment and every 2 weeks thereafter for the first 4–6 weeks of treatment. Subsequently, assessments are recommended at least every 4 weeks for the next 3 months and then at quarterly intervals until resolution of the disease (IRIS *et al.* 2013e).

29. **What is the relationship between allopurinol treatment, xanthinuria and kidney disease?**

Allopurinol is a leishmaniostatic drug used in treating CanL for long periods, usually at least 12 months, in order to maintain reduced parasite load, avoid relapses and reduce the transmission to people and other dogs (Plevraki *et al.* 2006, Oliva *et al.* 2010, Miró *et al.* 2011, Solano-Gallego *et al.* 2011, Torres *et al.* 2011, Manna *et al.* 2015). Traditionally, allopurinol was thought to be safe for use in dogs, with long-term and even lifelong therapy often required (Ginel *et al.* 1998, Denerolle & Bourdoiseau 1999, Noli & Auxilia 2005, Freeman 2010). However, both allopurinol resistance (Maia *et al.* 2013, Yasur-Landau *et al.* 2016) and xanthine urolithiasis (Koutinas *et al.* 2001, Pennisi *et al.* 2005, Torres *et al.* 2011, Torres *et al.* 2016) can occur with prolonged therapy. Urinary adverse effects of allopurinol can be detected as soon as 3 weeks post-treatment up to after 9 years of treatment, suggesting issues with both short- and long-term use (Torres *et al.* 2011, Torres *et al.* 2016). Given that kidney mineralisation and xanthine uroliths are usually irreversible, can seriously impact kidney function and this type of urolith cannot be dissolved medically, closer follow-up is required, including urinalysis (at the beginning of treatment and at the time of each follow-up assessment) and abdominal ultrasound if xanthinuria is detected (Torres *et al.* 2016). In the presence of such urinary problems, the dose of allopurinol should be reduced to 10 mg/kg q24h or less (Vercammen & De Deken 1995, Manna *et al.* 2015), water consumption should be increased and low-purine diet fed in order to maintain urinary xanthine concentration below its saturation point (Osborne *et al.* 2010), or to exchange allopurinol for nucleotides (Segarra *et al.* 2017).

30. **Do all dogs with leishmaniosis need to receive lifelong leishmanicide or leishmaniostatic drugs therapy?**

Although CanL is considered to be a chronic disease, the majority of dogs respond well to the recommended treatments and do not require lifelong leishmanicide and/or leishmanostatic drugs (Oliva *et al.* 2010, Solano-Gallego *et al.* 2011, Roura *et al* 2013).

Moreover, the occurrence of side effects or resistance associated with the use of these drugs suggests that the treatment should be suspended when the dog obtains the status of infected, but clinically healthy dog (see questions 15, 16, 29 and 34).

However, some dogs could relapse although they had received adequate treatment when clinicians: (1) withdraw allopurinol, or even (2) during the treatment with allopurinol. In the first situation, a complete treatment needs to be reinitiated again; in the second situation, the dog will again require treatment with a leishmanicide drug such as meglumine antimoniate or miltefosine. Furthermore, there are some dogs that, even when maintained on allopurinol treatment, need leishmanicide treatment every 4-12 months in order to remain clinically stable. These dogs have a guarded prognosis because their immunological and clinical responses are inadequate (Oliva *et al.* 2010, Solano-Gallego *et al.* 2011, Roura *et al* 2013).

31. Do all dogs with leishmaniosis and kidney disease need lifelong renal treatment?

The answer to this question is clearly no since remission of renal disease can occur in both proteinuric non-azotaemic and azotaemic dogs (Plevarki *et al.* 2006, Pierantozzi *et al.* 2013, Proverbio *et al.* 2016). For many practitioners, the general assumption is that all dogs with leishmaniosis with renal disease have CKD. However, in almost all cases, the initial diagnosis is based exclusively on the results

obtained after a single evaluation of the routine laboratory markers of renal function. Therefore, true severity and irreversibility of renal disease cannot be estimated when the disease is diagnosed. Treating the aetiological agent and providing therapy to support the kidneys can reduce the severity of the injuries or even completely cure of the disease, especially where an early diagnosis has been made. Unfortunately, this is not the case for all dogs, and there will be some that progress to CKD, requiring lifelong renal therapy and adjustment in treatment (IRIS 2019). This fact highlights the importance of periodically monitoring renal function, as treatment requirements may change with time.

32. **Will all dogs with leishmaniosis and kidney disease die?**

Many dogs with leishmaniosis and concurrent kidney disease have both a normal quality of life and lifespan. Factors that most influence both survival time and progression of the disease are early diagnosis and an adequate therapeutic approach and monitoring scheme.

33. **What is the prognosis for dogs with leishmaniosis?**

The prognosis of the disease varies depending on the clinicopathological situation and is, therefore, not the same for all dogs with leishmaniosis. Nowadays, the prognosis for the majority of cases is favourable, thanks to early diagnosis due to improvement in diagnostic techniques, and the use of more adequate protocols to treat leishmaniosis (Solano-Gallego *et al*. 2011, Roura *et al*. 2013). Discussion with owners about the prognosis for their dog with leishmaniosis can be difficult. The prognosis depends on: the clinical staging at the time of diagnosis and at each

follow-up assessment; the severity of the clinicopathological alterations that are present; the response to the treatment; and the number of relapses (Solano-Gallego *et al*. 2011, Roura *et al*. 2013). While the prognosis for both exposed and infected dogs is considered to be favourable, it is not the same if infection progresses to overt disease. For clinically affected dogs undergoing treatment, the prognosis varies from good-to-poor depending on many different factors, such as the severity of the clinical signs. For example, the presence of uveitis carries a poor prognosis for vision and the severity of kidney disease is clearly associated with reduced survival (Finco *et al*. 1999, Elliot & Watson 2008, Oliva *et al*. 2010, Solano-Gallego *et al*. 2011, Roura *et al*. 2013, IRIS 2019). Prognosis also depends on the anti-*Leishmania* treatment used, because clinical and laboratory alterations are more stable and the prognosis is better on the long term when meglumine antimoniate plus allopurinol treatment are used (Torres *et al*. 2011, Manna *et al.* 2015). In contrast, the prognosis is very poor in dogs with severe clinical signs of leishmaniosis with no treatment (Dos-Santos *et al*. 2008).

34. **What is the prognosis for dogs with leishmaniosis and kidney disease?**

Classically, the prognosis of dogs with leishmaniosis and kidney disease was very poor because advanced renal disease was the major cause of death or euthanasia (Mancianti *et al.* 1988, Slappendel 1988, Ferrer *et al.* 1995, Koutinas *et al.* 1999). However, in light of recent improvements in knowledge, diagnosis is often made earlier and treatment options are better such that prognosis is more favourable (Plevraki *et al.* 2006, Torres *et al*. 2011, Pierantozzi *et al.* 2013, Cortadellas *et al.* 2014, Proverbio *et al.* 2016, Zatelli *et al.* 2016, Paltrinieri *et al.* 2018).

For dogs with IRIS stages 1, 2 or early 3 CKD as well as those with severe or progressive proteinuria, the prognosis after treatment is generally favourable-to-guarded (Paradies *et al*. 2010, Torres *et al*. 2011, Rougier *et al*. 2012, Pierantozzi *et al.* 2013, Proverbio *et al.* 2016, Pineda *et al.* 2017). However, dogs with advanced IRIS stage 3 or stage 4 CKD, or those with severe or progressive proteinuria, the prognosis after treatment is guarded-to-poor (Koutinas *et al*. 1999, Plevraki *et al*. 2006, Planellas *et al*. 2009). When evaluating the prognosis of dogs with leishmaniosis and associated renal disease, the prognostic value of proteinuria needs to be considered after implementing the leishmanicide treatment (Koutinas *et al*. 1999, Plevraki *et al*. 2006, IRIS *et al.* 2013b, Pierantozzi *et al.* 2013, Proverbio *et al.* 2016).

As a result, most experts recommend that dogs with leishmaniosis and any renal alteration should be treated for leishmaniosis with the same protocol as dogs without renal involvement (see questions 20, 21 and 26), together with symptomatic and specific renal treatment necessary for each IRIS stage (Elliot & Watson 2008, IRIS *et al.* 2013b, IRIS 2019).

35. **Conclusions**

There is a growing international interest in CanL, because of the geographical spread of the disease and because modern diagnostic tests have allowed the identification of an increasingly large number of dogs showing clinical signs, that in the past were not identified, or were identified late in the course of the disease. Similarly, increased understanding and availability of diagnostics has led to identification of more dogs with nephropathy associated with leishmaniosis. Clinicians must be aware of this important aspect of CanL and consider specific

assessments of renal function at the time of initial diagnosis and staging, and at the time of periodic follow-up assessments over the life of those dogs. These guidelines summarise the current state-of-the-art of knowledge of the pathogenesis, clinical presentation, diagnosis, treatment, long-term management and prognosis for CanL-associated renal disease.

LEGENDS

Figure 1. Flow-chart of clinical management of dogs with leishmaniosis and proteinuria (UPC > 0.5)

Leish *Leishmania* spp., UPC urine protein creatinine ratio, LeishTx leishmanicide treatment, ACEi angiotensin-converting enzyme inhibitors, ARB angiotensin-receptor blockers

*with UPC > 3, the antiproteinuric therapy could also be instituted at the same time of leishmanicide treatment

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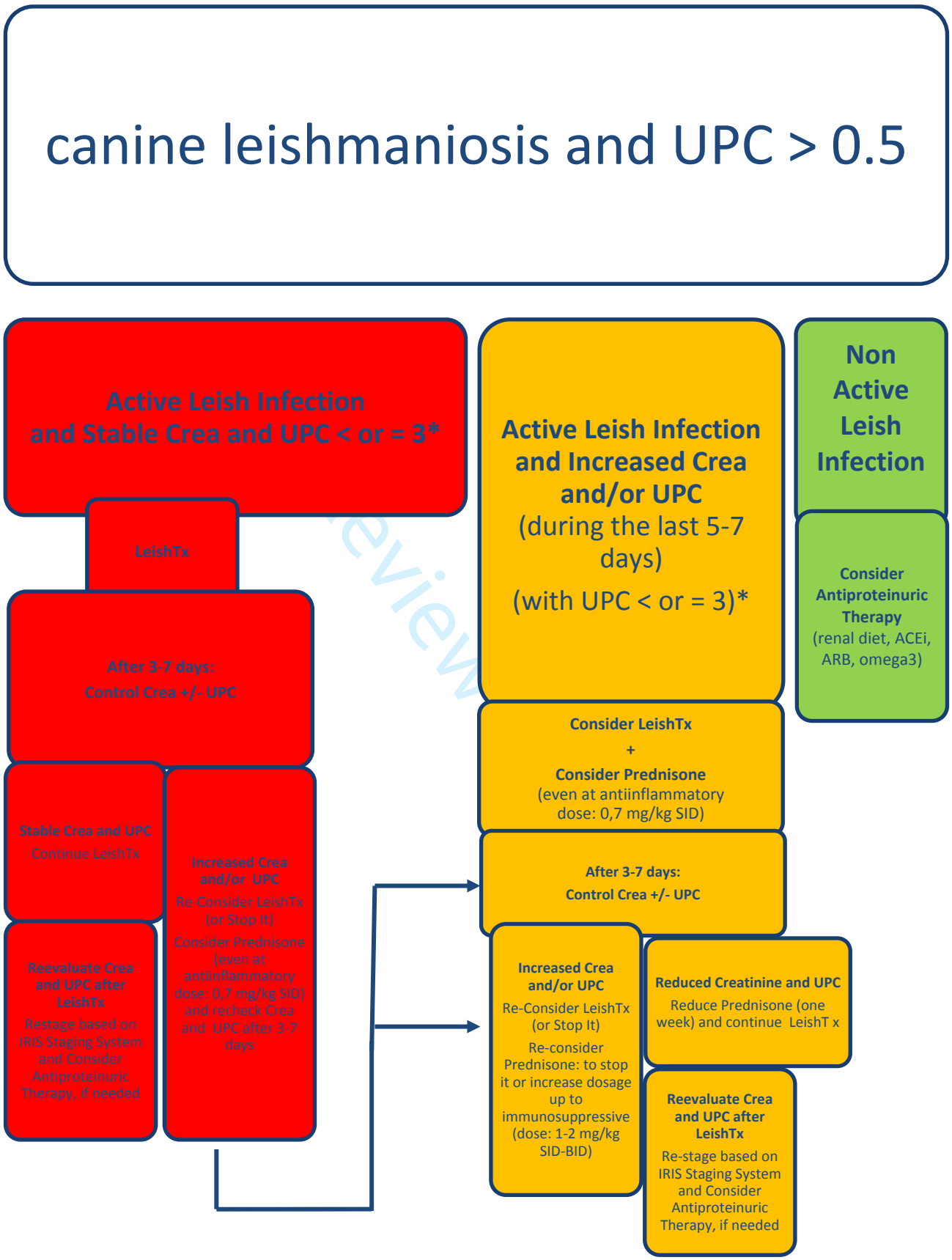
canine leishmaniosis and UPC > 0.5
Active Leish Infection and Stable Crea and UPC < or = 3*
LeishTx
After 3-7 days: Control Crea +/- UPC
Stable Crea and UPC
Continue LeishTx
Reevaluate Crea and UPC after LeishTx
Restage based on IRIS Staging System and Consider Antiproteinuric Therapy, if needed
Increased Crea and/or UPC
Re-Consider LeishTx (or Stop It)
Consider Prednisone (even at antiinflammatory dose: 0,7 mg/kg SID) and recheck Crea and UPC after 3-7 days
Active Leish Infection and Increased Crea and/or UPC (during the last 5-7 days) (with UPC < or = 3)*
Consider LeishTx + Consider Prednisone (even at antiinflammatory dose: 0,7 mg/kg SID)
After 3-7 days: Control Crea +/- UPC
Increased Crea and/or UPC
Re-Consider LeishTx (or Stop It)
Re-consider Prednisone: to stop it or increase dosage up to immunosuppressive (dose: 1-2 mg/kg SID-BID)
Reduced Creatinine and UPC
Reduce Prednisone (one week) and continue LeishT x
Reevaluate Crea and UPC after LeishTx
Re-stage based on IRIS Staging System and Consider Antiproteinuric Therapy, if needed
Non Active Leish Infection
Consider Antiproteinuric Therapy (renal diet, ACEi, ARB, omega3)

Table 1. CLWG clinical stage of canine leishmaniosis (adapted from Roura *et al.* 2013). [CLWG = Canine leishmaniosis working group; PCR = Polymerase chain reaction; IFA = Immunofluorescence assays; ELISA = Enzyme-linked immunosorbent assays; UPC = Urinary protein creatinine ratio; IRIS = International renal interest society; * IRIS stage of CKD in dogs (www.iris-kidney.com)]

Stage	Definition	Description
A	Exposed	Clinically normal or have clinical signs and/or clinicopathological abnormalities associated with other disease(s). Infection cannot be demonstrated by microscopy, culture or PCR, and a specific antibody titter is positive. Such dogs live or have lived during more than one 'transmission season' in a geographical region where sand flies are endemic.
B	Infected	Clinically normal or have clinical signs and/or clinicopathological abnormalities associated with other disease(s). Parasites have been demonstrated by microscopy, culture or PCR and a specific antibody titter is negative or positive to any extent.
C	Clinically sick	Dogs exhibit clinical signs and/or clinicopathological abnormalities associated with leishmaniasis. Infection is demonstrated by microscopy, culture or PCR and by positive specific antibody titter, to any extent. Given the variable clinical and clinicopathological expression of leishmaniosis, observed signs can differ from those commonly described. Dogs with clinical signs and/or clinicopathological abnormalities associated with leishmaniosis and an antibody titter ≥3 dilutions (IFA) or >40% (ELISA) of the laboratory cut-off value can also be considered clinically sick even if the parasite cannot be directly demonstrated.
D	Severely sick	Dogs with: (1) severe proteinuria (UPC>3); (2) severe kidney disease (IRIS* stage 3–4); (3) severe ophthalmic disease that can lead to functional loss and/or require immunosuppressive therapy; (4) severe joint disease leading to loss of motor function and/or require immunosuppressive therapy; and (5) severe concomitant disease(s).
Ea	Unresponsive to treatment	Dogs clinically unresponsive to recommended treatments of leishmaniosis.
Eb	Early relapse	Dogs with clinical relapse soon following cessation of recommended treatments of leishmaniosis.

Table 2. IRIS stage of CKD in dogs (adapted from IRIS 2019). (IRIS = International renal interest society, CKD = Chronic kidney disease, SDMA = Symmetric dimethylarginine, UPC = Urinary protein creatinine ratio).

Stage	Creatinine (mg/dL)	SDMA (μg/dL)	Description
1	<1.4	<18	No azotaemia
2	1.4-2.8	18-35	Mild azotaemia
3	2.9-5.0	36-54	Moderate azotaemia
4	>5.0	>54	Severe azotaemia

Sub-stage Proteinuria (UPC)	Nonproteinuric <0.2	Borderline proteinuric 0.2–0.5	Proteinuric >0.5

Sub-stage blood pressure (mmHg)	Normotensive <140	Prehypertensive 140-159 mmHg
	Hypertensive 160-179	Severely hypertensive ≥180