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Efficacy of leflunomide for treatment of immune-mediated polyarthritis in dogs: 14 cases (2006–2008)

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Abstract

Objective—To evaluate efficacy and adverse effects of leflunomide for the treatment of naturally occurring immune-mediated polyarthritis (IMPA) in dogs.

Design—Retrospective case series.

Animals—14 dogs with cytologically confirmed IMPA.

Procedures—Medical records were used to identify dogs with a diagnosis of IMPA that were treated with leflunomide. Signalment, radiographic findings, laboratory data, dosage of leflunomide, treatment duration, treatment response, and occurrence of adverse effects were determined from medical records.

Results—Mean \pm SD initial dosage of leflunomide was 3.0 ± 0.5 mg/kg (1.4 ± 0.2 mg/lb) PO once daily. Treatment duration for the initial starting dosage ranged from 1 to 6 weeks. Of the 14 dogs treated with leflunomide, 8 had complete resolution of clinical signs of IMPA initially, 5 had partial response to treatment, and 1 had minimal response to treatment. Adverse effects from treatment with leflunomide were not observed during the treatment period.

Conclusions and Clinical Relevance—Oral administration of leflunomide was a safe and effective alternative to oral administration of corticosteroids for treatment of IMPA in dogs. On the basis of findings in this study, a starting dosage for leflunomide of 3 to 4 mg/kg (1.4 to 1.8 mg/lb) PO once daily for at least 6 weeks before making dose adjustments is recommended. Dose adjustments should be based on cytologic evaluation of synovial fluid and clinical signs of IMPA. Hematologic variables, serum biochemical analysis results, and clinical signs of IMPA should be monitored for evidence of adverse effects to treatment with leflunomide.

Immune-mediated polyarthritis is a common arthritic condition in dogs. First reported in the 1970s, IMPA is characterized as an inflammatory arthropathy, responsive to immunosuppressive therapy, for which no infectious etiology has been determined.^{1,2} Immune-mediated poly-arthritis is considered a type III hypersensitivity reaction in which an immunologic stimulus triggers creation and deposition of immune complexes within the basement membrane of the synovium. Through activation of the complement cascade, inflammatory cells, including neutrophils and macrophages, are recruited to the site of inflammation. The end result, after phagocytosis of the immune complexes, is release of nitric oxide, free radicals, and proteases that cause tissue destruction.^{3,4} Although findings in some retrospective studies^{4–6} suggest susceptibility of certain dog breeds or sex to IMPA, agreement among these reports does not exist.

Clinical features of IMPA include stiffness and lameness most commonly, as well as pyrexia, lymphadenopathy, inappetence, signs of pain in the lumbar area, signs of depression, exercise intolerance, and lethargy.^{2,4,5} Decreased range of motion, effusion, heat, and pain upon manipulation of affected joints may be appreciated. Bilaterally symmetric joint involvement is common with IMPA. Joints most often affected (in descending frequency) are the carpal, tarsal, stifle, and elbow joints.⁵

Treatment of IMPA requires both treatment of the underlying immunologic trigger, if identified, and treatment of joint inflammation. Failure to achieve this goal may result in persistence or recurrence of clinical signs of IMPA. Numerous regimens have been proposed and involve treatment with a single drug or combination treatment with corticosteroids, cytotoxic drugs, or newer immunomodulating drugs. Efficacy of individual drugs or dosages is difficult to assess as combination treatment is common and controlled prospective trials are unavailable. Regardless of treatment regimen chosen, efficacy is best assessed by both clinical signs and cytologic evaluation of synovial fluid samples.

Corticosteroids are the most widely used treatment for IMPA in dogs. Although initial response rate has been reported as high as 81%,⁵ adverse effects are common. Adverse effects range from polyuria, polydipsia, and polyphagia to more serious complications such as diabetes mellitus, urinary tract infections, pyoderma, and breakdown of collagen in tendons and ligaments. As a result, alternative or combination treatment is often sought, either to avoid complications associated with treatment with corticosteroids or for treatment of unresponsive disease.⁷

Leflunomide is an immunomodulating agent that is structurally unlike any other immunomodulating drug. It has been proven effective in experimentally induced and clinical instances of rheumatoid arthritis and other immune-mediated disease in humans^{8,9} and dogs.¹⁰ The most common adverse effects of leflunomide in humans include diarrhea, nausea, headaches, skin rashes, and alopecia. Although increases in serum liver enzyme activities have been reported in 2% to 13% of patients treated with leflunomide, these changes are typically reversed with dose reductions or discontinuation of treatment.⁸ Severe adverse effects including myelosuppression, interstitial lung disease, or toxic epidermal necrosis are reported less frequently.^{8,11–13} The incidence of serious adverse effects increases with increasing dosages of leflunomide.^{8,11} Discontinuation from treatment with leflunomide has been reported in 15.9% to 70% of humans as a result of adverse effects.^{14–16}

Confirmed use of leflunomide for treatment of IMPA in dogs is limited to a single dog within a reported case series in which the use of leflunomide was evaluated for a variety of immune-mediated diseases.¹⁰ Because substantial toxic effects of leflunomide have not been observed in dogs receiving therapeutic dosages, leflunomide may be an attractive alternative to corticosteroids for treatment of IMPA. The purpose of the study reported here was to retrospectively determine the efficacy and adverse effects of leflunomide for the treatment of naturally occurring IMPA in dogs. Our hypothesis was that treatment with leflunomide would effectively induce resolution of clinical signs of disease and that the adverse effects of leflunomide would be minimal. Our overall goal was to identify an orally administered treatment for use in IMPA in dogs with minimal treatment complications.

Materials and Methods

Case selection

Medical records from dogs admitted to the University of Wisconsin Veterinary Medical Teaching Hospital that were treated with leflunomide for any reason were reviewed. To be

included in the study, dogs had to meet the following criteria. All dogs included in the study had to have clinical signs and findings from cytologic evaluation of synovial fluid samples from ≥ 1 affected joints that were consistent with a diagnosis of IMPA. In addition, follow-up information for at least 1 week after initiation of treatment with leflunomide had to be available to assess compliance with administration and occurrence of adverse effects.

Medical records review

Age, sex, breed, weight, admitting complaint, previous medications, and physical examination findings were obtained from the records for all dogs included in the study. Complete blood cell count and results of serum biochemical analysis, urinalysis, cytologic evaluation of synovial fluid samples, radiographic findings, infectious disease screening, bacterial culture of synovial fluid, and serologic testing for autoantibodies (rheumatoid factor, antinuclear antibodies, and Coombs test) were examined if available. Dogs were classified as having immune-mediated arthritis if they had a high TNCC in synovial fluid with cytologic evidence of nonseptic neutrophilic or mixed inflammation. Results of the other diagnostic tests were examined to screen for the presence of underlying disease associated with immune-mediated arthritis.

Leflunomide treatment and complications

Information regarding leflunomide administration was obtained including duration of clinical signs of IMPA before treatment, dosage, concurrent medications, treatment duration, and response to treatment. Complications of treatment were determined on the basis of physical examination findings at follow-up and, if available, follow-up CBC and serum biochemical analysis results. In addition, all owners were contacted via telephone to determine current clinical signs of IMPA, medications, and observed adverse effects associated with leflunomide treatment.

Response to leflunomide treatment

Because only 1 dog had arthrocentesis performed on the same joint both before and after treatment with leflunomide, dogs were assessed for response to treatment subjectively on the basis of clinical signs of IMPA and physical examination findings. Dogs were classified as having no response to treatment if they had minimal or no alleviation of clinical signs of IMPA. Dogs were classified as having partial response to treatment if clinical signs of IMPA were reduced but did not allow for resumption of normal quality of life. Dogs were classified as responsive to treatment if clinical signs of IMPA were alleviated to a degree that was perceived as a normal quality of life by the owner.

Results

Animals

A total of 25 dogs were treated with leflunomide between September 2006 and September 2008. Of these dogs, 1 dog was treated for meningoencephalitis, 1 for immune-mediated thrombocytopenia, 1 for inflammatory bowel disease, 1 for cutaneous histiocytosis, 7 for arthritis secondary to a variety of orthopedic conditions, and 14 for IMPA. The diagnosis of IMPA was based on cytologic evidence of nonseptic neutrophilic inflammation or mixed inflammation in affected joints. For purposes of this study, data only from dogs with a diagnosis of IMPA were analyzed.

Of the 14 dogs with IMPA, 7 were neutered males, 6 were spayed females, and 1 was a sexually intact male. Breeds included mixed-breed dogs ($n = 7$), Boxer (1), Doberman Pinscher (1), Australian Shepherd (1), Weimeraner (1), Shetland Sheepdog (1), Shih Tzu (1), and Labrador Retriever (1). Mean \pm SD age of dogs was 5.6 ± 3.2 years old (range, 2 to

13 years old). Mean weight of dogs was 21.1 ± 13 kg (46.5 ± 28.7 lb) with a range of 3.9 to 36.6 kg (8.6 to 80.7 lb).

Clinical signs of IMPA

All dogs had clinical signs of IMPA that included lameness or limb stiffness. Other clinical signs of IMPA reported by owners included decreased activity, lethargy, signs of depression, difficulty rising or sitting, difficulty walking up or down stairs, carpal hyperextension, diarrhea, and drooling or gagging. There was a large variation in duration of clinical signs of IMPA before hospital admission, ranging from 1 day to 2 years with a mean \pm SD of 4.6 ± 6.3 months.

Physical examination findings

All dogs had obvious lameness with signs of pain on manipulation of the affected joints. Two of the 14 dogs had a high rectal temperature on initial hospital admission. Other physical examination findings included a cranial drawer sign in 3 dogs, patellar luxation in 2 dogs, and peripheral lymphadenopathy in 2 dogs. Carpal hyperextension was not recorded in the medical record of any dog.

Diagnostic information

Radiography was performed at the time of hospital admission for 13 of the 14 dogs. Of these 13 dogs, 11 had radiographic evidence of effusion within affected joints, and 4 had radiographic evidence of osteophytes in affected joints. No erosive changes in subchondral bone were observed in any radiographic views of affected joints.

Serologic screening assays for autoantibodies were performed for several of the dogs. Seven dogs were tested for antinuclear antibodies, of which 2 had positive results. Coombs tests were performed for 3 dogs, all of which had negative results. Six dogs were tested for rheumatoid factor, of which 2 had positive results.

Serologic screening was performed for infectious agents that might be the immunologic trigger for inflammatory arthritis. Three of 10 dogs tested were seropositive for antibodies against *Borrelia burgdorferi*; 1 of 3 dogs tested was seropositive for antibodies against *Bartonella vinsonii*. All dogs tested were seronegative for antibodies against *Ehrlichia canis* (10 dogs), *Anaplasma phagocytophila* (9 dogs), *Rickettsia rickettsii* (6 dogs), *Dirofilaria immitis* (5 dogs), *Blastomyces dermatitidis* (2 dogs), and *Neorickettsia risticii* (1 dog).

Synovial fluid samples from 5 of 14 dogs were submitted for bacterial culture of aerobic and anaerobic organisms. All synovial fluid bacterial culture results were negative. In 1 dog that was seronegative for infectious organisms and that had negative synovial fluid bacterial culture results, bacterial DNA was detected in synovial fluid cells by use of a broad-ranging 16S rRNA PCR assay.¹⁷ An uncultured *Eubacterium* sp (taxonomy identification No. 77,133) was identified on the basis of cloning and sequencing of the PCR product.¹⁷

Synovial fluid samples were available from 30 joints of the 14 affected dogs. The TNCC of synovial fluid was estimated as high in 14 joints in which there was only enough synovial fluid available for examination of a direct smear. In the remaining 16 joints, the TNCC of synovial fluid ranged from 3.5×10^9 cells/L to 147.6×10^9 cells/L with a mean \pm SD of $37.2 \times 10^9 \pm 40.5 \times 10^9$ cells/L.

In synovial fluid samples from 26 of 30 joints, there was a predominance of nondegenerate neutrophils. For 6 of these 26 joints, the TNCC in synovial fluid was not given, but rather was estimated as a predominance of nondegenerate neutrophils. In the remaining 20 of the

26 joints, the percentage of nondegenerate neutrophils in synovial fluid ranged from 65% to 100%, with a mean \pm SD of $88.7 \pm 9.2\%$. All but 1 of the 26 joints also contained mononuclear cells in the synovial fluid, in which the percentage of mononuclear cells ranged from 0% to 35% with a mean of $10.9 \pm 9.0\%$. In synovial fluid samples from the remaining 4 of 30 joints, there was evidence of mixed inflammation, with increased numbers of neutrophils, lymphocytes, and macrophages. Mean percentage of nondegenerate neutrophils in synovial fluid samples from these 4 joints was $24.3 \pm 13.5\%$, whereas the mean percentage of mononuclear cells was $72.3 \pm 12.3\%$. Cytologic findings for these 4 joints were interpreted as suspicious for IMPA on the basis of a higher than expected number of neutrophils within the synovial fluid.

Overall, there were synovial fluid samples from 13 joints for which a total mononuclear cell count was obtained, which ranged from 0.6×10^9 cells/L to 12.6×10^9 cells/L with a mean \pm SD of $3.8 \times 10^9 \pm 3.2 \times 10^9$ cells/L. Thus, mild to moderate mononuclear inflammation was a common finding. In the 14 synovial fluid samples for which a total protein concentration value was recorded, the range was 3.0 to 6.8 g/dL with a mean of 4.9 ± 0.8 g/dL.

Prior and concurrent medication administration

Medications administered before and concurrently with leflunomide, initiated either at the veterinary teaching hospital or by the referring veterinarian, were recorded. Nine of 14 dogs were treated with a full course of doxycycline (therapeutic doses for at least 1 month) either before or concurrently with leflunomide administration. Three of the 9 dogs were treated with doxycycline for at least 2 weeks before starting treatment with leflunomide.

Two dogs were treated with tapering immunosuppressive doses of prednisone before initiation of leflunomide administration. Both dogs initially responded well to treatment with prednisone; however, clinical signs of IMPA returned as the corticosteroid was tapered. When provided with the option to return to higher doses of prednisone versus trying leflunomide, both owners elected to switch to leflunomide because of adverse effects of prednisone. One dog had polyphagia and signs of excitement as adverse effects. The other dog had polyuria, polydipsia, excessive panting, polyphagia, and weight gain. Adverse effects in both dogs resolved upon termination of prednisone treatment. In the remaining 12 dogs, no other disease-modifying treatment for arthritis was initiated before leflunomide administration. Leflunomide was chosen as the primary form of treatment in these 12 dogs.

Twelve of the 14 dogs received an NSAID prior to, concurrent with, or after initiation of treatment with leflunomide. In 7 dogs, the NSAID was given prior to start of treatment with leflunomide and was insufficient in alleviating clinical signs of IMPA. In 3 dogs, the NSAID was administered as adjunctive treatment for pain on an as needed basis after treatment with leflunomide was initiated. The remaining 2 dogs were started on an NSAID after clinical signs of IMPA were no longer alleviated by treatment with leflunomide alone. Two dogs did not receive any NSAID during the treatment time frame. Before or concurrent with leflunomide administration, other medications given included tramadol (3 dogs), antimicrobials other than doxycycline (4 dogs), gabapentin (1 dog), and homeopathic agents (with anti-inflammatory effects) and acupuncture (1 dog).

Treatment with leflunomide

Twelve dogs received leflunomide as the primary treatment for IMPA, and 2 dogs received leflunomide after recurrence of clinical signs of IMPA following treatment with prednisone. Dosages of leflunomide ranged from 2.0 to 3.9 mg/kg (0.9 to 1.8 mg/lb) PO with a mean \pm SD dosage of 3.0 ± 0.5 mg/kg (1.4 ± 0.2 mg/lb) PO once daily. The dosage of leflunomide

for 1 dog was increased to 3.8 mg/kg (1.7 mg/lb) PO once daily (from 2.9 mg/kg [1.3 mg/lb], PO, once daily) when clinical signs of IMPA did not resolve with the initial dosage.

Complications of treatment with leflunomide

Anorexia and vomiting were reported for only 1 dog throughout the treatment period. This dog also received doxycycline and carprofen at the same time as leflunomide, and thus it was unclear which medication caused the vomiting. Because carprofen can be associated with vomiting, anorexia, or diarrhea, it was discontinued as soon as the vomiting and anorexia were observed, and the leflunomide and doxycycline were continued for 5 additional weeks. There was no further evidence of anorexia or vomiting.

Follow-up CBC and serum biochemical analysis results were available for 5 and 4 of the 14 dogs, respectively. Mild leukopenia was observed for 2 of 5 dogs (5.5×10^3 cells/ μ L and 5.0×10^3 cells/ μ L, respectively; reference range, 6.0×10^3 cells/L to 17.0×10^3 cells/ μ L), and mild thrombocytopenia was observed for 1 of 5 dogs (fluctuating inconsistently from 125×10^3 platelets/ μ L to 173×10^3 platelets/ μ L; reference range, 175×10^3 platelets/ μ L to 500×10^3 platelets/ μ L). The dog that was thrombocytopenic was also one of the leukopenic dogs. Mild hypercholesterolemia was observed for 2 of 4 dogs (303 and 311 mg/dL, respectively; reference range, 98 to 300 mg/dL), which was not apparent on serum biochemical analysis before leflunomide administration. Anemia was not recorded for any of the dogs that had a follow-up CBC. There was no obvious correlation between age, weight, or duration of treatment and detection of hematologic or biochemical abnormalities.

Response to treatment with leflunomide

Of the 14 dogs treated with leflunomide, 1 dog did not respond to treatment. This dog was treated for 1 week. The attending veterinarian then elected to treat the dog with prednisone and discontinue leflunomide administration because there was little improvement in the dog's lameness. The dog responded well to prednisone treatment and had remission of clinical signs of IMPA. The dog had substantial polyuria and polydipsia and frequently had episodes of inappropriate elimination in the house. According to the owner, the dog also was polyphagic and lost muscle mass since starting treatment with prednisone.

Five of the 14 dogs had partial responses to treatment with leflunomide. Improvement in lameness scores, joint effusion, and activity level was observed; however, quality of life was not considered normal according to the owners. One dog was reported as having an improvement in clinical signs of IMPA at a physical therapy appointment, and 1 week later, that dog was lost to follow-up. In another 2 of the 5 dogs, a diagnosis of cranial cruciate ligament rupture with stifle joint instability was made at the time of the diagnosis of IMPA; however, the owners elected to pursue only medical treatment. For both of these dogs, clinical signs of IMPA were improved, but low-grade lameness was still evident. In the remaining 2 of the 5 dogs, improvement was observed both by the owners and by the attending veterinarian on physical examination; however, quality of life was still affected by the underlying arthropathy. In both dogs, treatment with leflunomide was discontinued. One dog received a combination of cyclosporine and firocoxib by the referring veterinarian and, according to the owner, was clinically better when receiving this combination of drugs than when receiving leflunomide. The other dog received prednisone by the referring veterinarian and was in clinical remission according to the owner.

The remaining 8 of 14 dogs had adequate clinical responses to treatment with leflunomide and resumed a normal quality of life on the basis of clinical signs of IMPA and physical examination. Three dogs had complete clinical remission, and then either they were lost to follow-up ($n = 2$) or the dose of leflunomide had not yet been altered (1). One dog had

complete clinical remission, the dose of leflunomide was tapered, and there was no further recurrence of clinical signs at 9 months after diagnosis. The remaining 5 dogs had complete clinical remission while receiving the initial dose, but then clinical signs of IMPA recurred as the dose of leflunomide was tapered or after discontinuing treatment. All 5 dogs were still receiving leflunomide at the lowest effective dose with remission of IMPA at the time of this report.

Discussion

In the present study, no breed or sex predilection for IMPA was evident in the 14 dogs. Most dogs were mixedbreed dogs, with equal numbers of male and female dogs. Arthrocentesis with cytologic evaluation of synovial fluid is central in the diagnosis of IMPA. A high TNCC in synovial fluid with a predominance of nondegenerate neutrophils is considered diagnostic for IMPA. Most laboratories use a reference range limit for the TNCC in synovial fluid of $< 2.5 \times 10^9$ cells/L to 3.0×10^9 cells/L.^{18,19} The TNCC in synovial fluid of dogs with IMPA is highly variable, ranging from 3.2×10^9 cells/L to 106.3×10^9 cells/L and from 3.7×10^9 cells/L to 130×10^9 cells/L.^{2,5} The actual magnitude of an increase in the TNCC in synovial fluid, however, does not correlate with severity of IMPA or treatment outcome.^{2,5} The range of the TNCC in synovial fluid found in affected dogs in the present study, 3.5×10^9 cells/L to 147.6×10^9 cells/L, was similar to the previously reported values.^{2,5}

The TNCC in synovial fluid may consist of lymphocytes, monocytes, macrophages, neutrophils, and a few synovial cells; normally, neutrophils represent no more than 5% to 12% of the total nucleated cell population. In dogs with IMPA, nondegenerate neutrophils are commonly the predominant cell type.^{2,4,5} It is interesting that a mild to moderate increase in the mononuclear cell population in synovial fluid is also common in dogs with IMPA. In a study by Clements et al⁵ on dogs with type I IMPA, the mean mononuclear cell count in synovial fluid was 6.0×10^9 cells/L and was high for approximately 50% of affected dogs. Similarly in a study by Jacques et al⁴ on dogs with polyarthritis, in addition to high numbers of neutrophils in synovial fluid, mononuclear cells were the predominant cell type in synovial fluid of 27.5% of affected dogs and represented half of the inflammatory cells in another 12.5% of affected dogs. In the present study, mononuclear cells were the predominant inflammatory cell type in synovial fluid samples from 4 of 30 affected joints (representing 3 of the 14 dogs with IMPA). All 3 dogs responded to immunosuppressive therapy with leflunomide or prednisone. In the 13 joints in which a total mononuclear cell count was obtained, the number of mononuclear cells alone was greater than the reference limit for the TNCC in synovial fluid.

Because the magnitude of increase in mononuclear cells is typically less than that of neutrophils, little attention has been given to the role mononuclear cells serve in the pathogenesis of IMPA. It is known that IMPA is primarily a type III hypersensitivity disorder, the result of immune complex deposition in the joints. Although it is clear that this mechanism is important in the pathogenesis of IMPA, the role of T and B lymphocytes in development of joint inflammation may be underappreciated. Both T and B lymphocytes are commonly found in arthritic joints of dogs,^{20,21} and T lymphocytes also have a pivotal role in the pathogenesis of arthritis.²²

Occasionally, ragocytes (neutrophils containing phagocytosed droplets of nucleoprotein) or lupus erythematosus cells (neutrophils containing phagocytosed bare nuclei) are found in synovial fluid of dogs with IMPA, but detection of these cells is rare.¹⁸ Two of the 14 dogs in our study had cells resembling ragocytes in the synovial fluid samples evaluated.

The reference range for total protein concentration in synovial fluid of dogs varies depending on the method of quantification, but has been reported as 1.8 to 4.8 g/dL with synovial samples from most unaffected joints of clinically normal dogs containing 1.5 to 3.0 g/dL.¹⁸ In the present study, synovial fluid total protein concentration was quantified in samples from 13 of 30 affected joints (representing 9/14 dogs with IMPA) and had a mean protein concentration of 4.98 g/dL.

Leflunomide is an immunomodulating agent that has been proven effective in treatment of experimentally induced arthritis, rheumatoid arthritis, other immune-mediated diseases, and renal allotransplantation or xenotransplantation.^{8-10,23} As a prodrug, leflunomide is metabolized in the intestinal mucosa and liver after oral administration, where leflunomide is converted to the active soluble metabolite A77-1726, a malononitriloamide.^{9,12} Leflunomide and other malononitriloamide analogues inhibit T- and B-lymphocyte proliferation, suppress immunoglobulin production, and interfere with leukocyte adhesion and diapedesis. Many targets of the active metabolite A77-1726 have been described, with inhibition of tyrosine kinases being the primary mechanism of immunomodulation.^{10,12,13,23-25} Tyrosine kinase signaling is important for activation of the T-lymphocyte receptor and several cytokine receptors, including the interleukin-2 receptor.^{26,27} A77-1726 also inhibits the mitochondrial enzyme dihydroorotate dehydrogenase, an enzyme necessary for de novo pyrimidine synthesis. Subsequent depletion of nucleotides leads to cell cycle arrest in proliferating lymphocytes.^{13,25}

Leflunomide treatment has successfully increased the survival rate of dogs undergoing experimental renal transplantation and can prevent acute allograft rejection when combined with cyclosporine administration.^{28,29} Leflunomide administered at 4 mg/kg (1.8 mg/lb) has also been clinically effective in dogs for treatment of various immune-mediated conditions, including the cutaneous and nasal form of systemic histiocytosis, immune-mediated thrombocytopenia, immune-mediated hemolytic anemia and Evans syndrome (ie, acquired hemolytic anemia and thrombocytopenia), multifocal nonsuppurative encephalitis and meningomyelitis, and immune-mediated polymyositis, polyarthritis, and pruritic skin disease.^{10,30} Of the 14 dogs in the present study with IMPA, 8 had complete initial resolution of clinical signs, 5 had partial response to treatment, and 1 had no response to treatment. It is important to mention that the dog with no alleviation of clinical signs of IMPA was given leflunomide for only 1 week. The half-life of leflunomide is unknown in dogs, but if it is similar to that in humans (ie, 15 to 18 days),^{8,11,12} 1 week of treatment may not have been long enough to achieve steady-state serum leflunomide concentrations. Of the 5 dogs that had only partial resolution of clinical signs of IMPA, 2 had a positive cranial drawer sign on physical examination. The owners elected not to pursue surgical treatment for stifle joint instability. Thus, stifle joint instability was likely a contributing factor to these dogs' continued signs of discomfort. A third dog with only partial resolution of clinical signs was lost to follow-up after 1 week of treatment. There were 2 dogs that were reported to have patellar luxation at the time of the initial diagnosis. One dog had full resolution of lameness following leflunomide treatment, and thus the patellar luxation was likely an incidental finding. The other dog also had a positive cranial drawer sign and only had partial response to treatment with leflunomide. Given these observations, the true response rate to treatment with leflunomide in dogs with IMPA may have actually been higher than reported in the present study. Having an objective measurement of therapeutic response (ie, cytologic evaluation of synovial fluid samples) would help allow for better estimation of the true response to treatment with leflunomide. Because the TNCC does not always correlate with severity of clinical signs of IMPA or treatment outcome,^{2,5} cytologic evaluation of synovial fluid as a method of monitoring the response to leflunomide treatment may be warranted.

Previously reported adverse effects of leflunomide in dogs include a dose-dependent anemia at dosages above 4 mg/kg PO once daily and severe inanition when administered at a dosage of 16 mg/kg (7.3 mg/lb) PO once daily.²⁹ When leflunomide is administered at ≤ 4 mg/kg PO once daily, clinically evident complications of treatment have not been observed.^{10,28,29} In the present study, dosages of leflunomide ranged from 2.0 to 3.9 mg/kg PO once daily without the development of complications. Although a mild thrombocytopenia (1/5 dogs), leukopenia (2/5 dogs), and hypercholesterolemia (2/4 dogs) was identified on follow-up CBC and serum biochemical analysis, no clinical signs were associated with these findings. Because a follow-up CBC and serum biochemical analysis were performed for only 5 of the 14 dogs in the present study, no conclusions can be reached regarding the relative risk of treatment with leflunomide and development of hypercholesterolemia or blood dyscrasias.

This study had several weaknesses inherent to a retrospective case series, the most notable of which was a lack of a consistent treatment regimen. It was impossible to control for previous or concurrent medications that were administered in addition to the leflunomide. Although 12 of the 14 dogs had not been receiving any other immunosuppressive medication either before or during treatment with leflunomide, medications other than leflunomide were commonly given. Doxycycline was the most consistent medication given concurrently with leflunomide for empirical treatment of underlying infectious disease. It is possible that doxycycline may also have had direct disease-modifying effects on arthritis. In vitro, doxycycline can inhibit degradation of type XI collagen in articular cartilage, with reductions in active collagenase in cartilage and inhibition of mRNA for inducible nitric oxide synthase (an enzyme responsible for secretion of matrix metalloproteinases by chondrocytes).³¹⁻³⁴

The initial dose of leflunomide given in the present study was variable. Administration of leflunomide at 3 to 4 mg/kg was recommended as a starting dose on the basis of previous reports of adverse effects observed at doses of > 4 mg/kg.²⁹ However, in the present study, the initial dose of leflunomide ranged from 2.0 to 3.9 mg/kg, with a mean \pm SD dosage of 3.0 ± 0.5 mg/kg PO once daily. Duration of treatment and time until dose reduction were inconsistent. Most dogs were treated for 4 to 6 weeks before reduction of the leflunomide dose. However, there were several dogs in which the dose was reduced before this period on the basis of resolution of clinical signs of IMPA. To the best of our knowledge, there is no information establishing the half-life of leflunomide in dogs. Given the long half-life of the drug in humans,^{8,11,12} future studies establishing the half-life of leflunomide in dogs and the time until steady state is achieved would be useful in guiding treatment.

In humans, the standard dose recommendation for patients with rheumatoid arthritis had been to start patients on a loading dose of 100 mg PO once daily and then maintain them at 20 mg PO once daily for the duration of treatment. This dose recommendation is attributable to the long half-life of the drug and the need to induce remission quickly in patients with debilitating disease. Recently, however, there has been a confirmed decrease in the number of patients that are initially administered a loading dose. This correlates with a decrease in the incidence of severe adverse effects.³⁵⁻³⁷ To our knowledge, use of a loading dose in dogs has not been explored. It is our preference to induce dogs with a tapering immunosuppressive course of corticosteroids while concurrently starting a maintenance dose of leflunomide if rapid induction is necessary for debilitating disease. Because time until steady state of leflunomide has not been determined for dogs and adverse effects have been reported at doses > 4 mg/kg,²⁹ a loading dose of leflunomide in dogs is not recommended at this time.

In the present study, serial cytologic analyses of synovial fluid samples was not used to determine treatment recommendations, although it is recommended to monitor treatment.⁷

Lack of serial synovial fluid analyses may relate to financial concerns, concerns regarding the risk of multiple arthrocenteses in a dog receiving immunosuppressive therapy, or inability to convince clients to return for follow-up visits. It is possible that when reducing leflunomide dose on the basis of clinical signs of IMPA alone, there is still detectable inflammation within the joints. The presence of residual inflammation could lead to relapse in clinical signs of IMPA once the medication has been decreased enough to allow proliferation of inflammatory cells.

Although clinical adverse effects associated with leflunomide treatment were not observed in dogs of the present study, only 5 of the 14 dogs had a follow-up CBC and serum biochemical analysis. More consistent follow-up laboratory data are necessary to determine whether the mild leukopenia and hypercholesterolemia observed in this study are true risks of treatment with leflunomide in dogs.

In summary, it was our purpose to retrospectively analyze patients treated with leflunomide for IMPA to determine whether leflunomide could be used as an alternative to drugs such as corticosteroids and cytotoxic drugs that have a high incidence of adverse effects. Immune-mediated polyarthritis can be a challenging disease to treat, and thus it is beneficial to have many drugs available that can be used as alternative treatments. On the basis of our findings, an initial starting dosage of leflunomide at 3 to 4 mg/kg PO daily appears to be both safe and efficacious for treatment of IMPA in dogs. On the basis of the available information regarding the half-life of leflunomide in humans, this initial dosage should be continued for at least 6 weeks before making adjustments unless adverse effects are observed. Adjustments in dogs should be based on clinical signs of IMPA as well as follow-up cytologic evaluation of synovial fluid samples. As with other immunosuppressive drugs, lifelong treatment with leflunomide at the lowest effective dose may be necessary. Although substantial toxic effects of leflunomide were not observed in this population of dogs, a follow-up CBC and serum biochemical analysis should be performed to monitor for adverse effects of treatment. Combination treatment with other disease-modifying drugs, such as corticosteroids or cyclosporine, may be indicated if the initial response to leflunomide is inadequate.

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ABBREVIATIONS

IMPA	Immune-mediated polyarthritis
TNCC	Total nucleated cell count

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