Leflunomide with prednisone or nonsteroidal anti-inflammatory drug therapy is safe and tolerated for long-term treatment of immune-mediated polyarthritis in 27 dogs

Rebecca B. Chesne, MVB*; Michael T. Doornink, DVM, DACVS; Loren S. H. Sri-Jayantha, DVM, DACVS; Bridget K. Urie, DVM, MS, DACVIM
Blue Pearl Pittsburgh North, Pittsburgh, PA
*Corresponding author: Dr. Chesne (rebecca.chesne@bluepearlvet.com)

OBJECTIVE
To retrospectively evaluate safety and tolerance of leflunomide for long-term treatment of canine idiopathic immune-mediated polyarthritis (IMPA).

ANIMALS
27 dogs with clinical signs and synovial fluid cytology supportive of IMPA with ≥ 6 months’ follow-up after starting leflunomide.

METHODS
Medical records were reviewed to identify dogs prescribed leflunomide for treatment of IMPA from February 2012 to May 2022. Initial leflunomide doses of 2 to 4 mg/kg once daily were prescribed and were titrated to the lowest effective dose with concurrent anti-inflammatory therapy. Complete blood count, serum chemistry, and clinical signs were monitored throughout the course of treatment.

RESULTS
Adverse effects potentially attributable to leflunomide noted in 9 of 27 dogs (33%) included vomiting, diarrhea, lethargy, decreased or absent appetite, polyuria and polydipsia, and secondary antibiotic responsive infection and were self-limiting or resolved with outpatient therapy. Alkaline phosphatase (ALP) and alanine aminotransferase (ALT) elevation were documented in all dogs prescribed leflunomide plus prednisone, with persistent liver enzyme elevation in 6 of 9 dogs (67%) and normalization after antibiotic therapy in 3 of 9 dogs (33%). The majority of dogs prescribed leflunomide plus NSAID (11/17 [65%] dogs) did not experience liver enzyme elevation; 2 of 17 (12%) dogs developed transient antibiotic-responsive liver enzyme elevations, and 4 of 17 (23%) dogs had persistent liver enzyme elevation.

CLINICAL RELEVANCE
Leflunomide was well tolerated for long-term management of IMPA. A significant difference in liver enzyme elevation was identified between dogs prescribed prednisone versus NSAID in combination with leflunomide. Leflunomide with NSAID therapy resulted in less hepatotoxicity compared with leflunomide with prednisone.

Keywords: leflunomide, immune-mediated polyarthritis, prednisone, NSAID, hepatopathy
includes treatment of the underlying immunologic trigger and mitigating impact of joint inflammation.\(^1\)

Dogs with IMPA are frequently managed with numerous immunomodulatory medications, corticosteroids being most common. Aside from recognized adverse side effects associated with corticosteroids, collagen catabolism negatively impacts tendons and ligaments, exacerbating arthropathy,\(^1\) and prompts the need to explore alternative, ideally prednisone-sparing, immunomodulatory therapy.

Leflunomide is a disease-modifying drug harboring anti-inflammatory and immunomodulatory properties, and is FDA approved for treatment of rheumatoid arthritis and psoriatic autoimmune arthritis in people due to the ability to limit damage to joints by helping to maintain integrity of articular cartilage and bone.\(^6\) The primary metabolite of leflunomide (A77-1726) inhibits pyrimidine synthesis through reversible selective inhibition of dihydroorotate dehydrogenase and subsequent inhibition of activated B and T lymphocytes resulting in dampened proinflammatory signaling and reduced neutrophil infiltration.\(^1,7–14\)

Leflunomide’s efficacy for management of rheumatoid arthritis is comparable to methotrexate with respect to improved clinical signs and slowing progression of joint damage.\(^15\) Although largely tolerated, adverse effects reported in people include diarrhea, liver enzyme elevation (2% to 13% of patients), nausea, headaches, skin rashes, alopecia, epidermal necrosis, myelosuppression, and interstitial lung disease.\(^16\) Hepatotoxicity is considered the most concerning and treatment-limiting adverse effect. Transient liver enzyme elevations were noted in 15% of people prescribed leflunomide, but several cases of acute liver failure resulting in death or need for urgent liver transplantation have been described.\(^16\)

Multiple studies support the role of leflunomide for managing canine IMPA, immune-mediated hemolytic anemia, immune-mediated thrombocytopenia, cutaneous histiocytosis, and colorectal polyps.\(^2,8,17\) Severe adverse effects were documented when administered at doses higher than 4 mg/kg/d.\(^10,12\) At doses of 3 to 4 mg/kg/d, response rates of 80% to 93% are reported for multiple immune-mediated conditions, but adverse effects were treatment limiting or prompted further dose reduction. Recent data suggest that a starting dose of 1 to 2 mg/kg/d is effective, with marked improvement in incidence and severity of adverse side effects.\(^18\) Hepatotoxicity is infrequently reported in dogs and may be influenced by concurrent medications.\(^5,10\)

The primary objectives of this retrospective study were to report long-term improvement of clinical signs and safety of leflunomide for management of canine IMPA, including incidence and comparison of hepatotoxicity with long-term administration of NSAIDs versus corticosteroids with leflunomide.

**Methods**

**Case selection**

Medical records of dogs presented to Blue Pearl Pet Hospital Pittsburgh North diagnosed with IMPA and prescribed leflunomide were reviewed. Included dogs had clinical signs, synovial fluid cytology, and negative infectious disease testing leading to clinical diagnosis of idiopathic IMPA with follow-up data for ≥ 6 months after starting treatment and responding to leflunomide.

**Review of medical records**

Signalment, presenting complaint, and physical examination findings were extracted from the medical record of dogs included in the study. Evaluated diagnostic results included CBC, serum biochemistry, urinalysis, synovial fluid cytology, bacterial culture of synovial fluid, tick-borne disease screening (4DXSNAP test; Idexx), radiographs, and abdominal ultrasound.

**Treatment**

Information regarding leflunomide treatment obtained included dose, concurrent medications, response, and duration of therapy. Based on previous studies, initial leflunomide doses of 2 to 4 mg/kg once daily were prescribed. Sato et al\(^18\) reported the starting dosage of leflunomide of 2 mg/kg/d, notably lower than the previously recommended starting dosage of 3 to 4 mg/kg/d, and the initial dose prescribed was based on this amended dosing recommendation. An individual’s daily leflunomide dose was titrated to lowest effective dose. Doses of respective anti-inflammatory medications were based on recommended doses.\(^19\) Adverse events were identified by physical examination findings, clinical signs reported by owners, and CBC or serum biochemistry results. Enrofloxacin (Baytril) was prescribed at recommended doses\(^19\) when liver enzyme elevation was noted. Repeated cytologic analysis of synovial fluid was not performed.

**Adverse effects**

Development of adverse effects potentially attributable to leflunomide were defined as, but not limited to, vomiting, diarrhea, lethargy, anorexia, polyuria/polydipsia (PU/PD), or secondary antibiotic-responsive infections within 6 months (180 days) of initiating treatment. These clinical signs were based on findings from previous studies.\(^1,3,18\)

Hepatotoxicity was defined as liver enzyme values above reference range (normal ALP, 5 to 131 IU/dL; normal ALT, 12 to 118 IU/dL) or within reference range but increased by more than 2-fold from the individual’s previous bloodwork. Grades of adverse events were categorized following criteria established by the Veterinary Cooperative Oncology Group Common Terminology Criteria for Adverse Events.\(^20\)

**Statistics**

All analyses were performed with standard software (SAS, version 9.4; SAS Institute Inc). A significance threshold of 0.05 was used. Fisher exact tests were used to test for an association between liver enzyme elevation and combination of medications used for treatments (groups separated into leflunomide alone, leflunomide with a glucocorticoid, and leflunomide with an NSAID).
Results

A total of 27 dogs diagnosed with IMPA and prescribed leflunomide between 2012 and 2022 met criteria for inclusion. The mean age of included dogs was 5.9 years (range, 3 to 11 years), with a mean body weight of 16.5 kg (range, 3.3 to 55.7 kg). There were 12 neutered male dogs, 1 intact male dog, and 13 spayed female dogs. There were 21 breeds represented. The diagnosis of IMPA was determined by cytologic analysis of synovial fluid collected via arthrocentesis. Aseptic neutrophilic inflammation or mixed inflammation in the affected joint was documented in 26 of 27 (96%) dogs.

Initial diagnostics

Tick-borne disease screening—Tick-borne disease screening (4DXSNAP; Idexx) was negative for *Dirofilaria immitis* antigen and antibodies to *Borrelia burgdorferi*, *Anaplasmaphagocytophilum*, *Anaplasma platys*, *Ehrlichia canis*, and *Ehrlichia ewingii* in 26 of 27 dogs (96%). One dog was seropositive for *A. phagocytophilum*, *A. platys*, and *B. burgdorferi* and was prescribed a 30-day course of doxycycline prior to initiation of leflunomide therapy.

Cytology—Results of synovial fluid cytologic evaluation performed by an attending clinician (American College of Veterinary Surgeons board-certified diplomat) and/or board-certified clinical pathologist were available for all patients. Cellular composition of synovial fluid was described as primarily nondegenerate neutrophils in 15 of 27 dogs (56%), mixed inflammation with large mononuclear cells and nondegenerate neutrophils in 9 of 27 dogs (33%), and mixed inflammation with mainly small lymphocytes and nondegenerate neutrophils in 2 of 27 dogs (7.5%). The in-house cytology report of 1 dog (3.5%) did not contain detailed cytologic description, aside from being reported as suppurative inflammation.

Complete blood count—Thrombocytosis was documented in 6 of 27 (22%) dogs (mean elevation above reference range [RR], 161,700 platelets/µL), and thrombocytopenia was identified in 2 of 27 (7.5%) dogs (mean deviation below RR, 57,500 platelets/µL), half categorized as grade 1 and half as grade 2. Grade 1 neutrophilia was documented in 6 of 27 (22%) dogs (mean elevation above RR, 3,480 platelets/µL). Lymphopenia was identified in 1 of 27 (3.5%) dogs (mean deviation below RR, 20 platelets/µL), and eosinophilia was documented in 2 of 27 (7.5%) dogs (mean elevation above RR, 40 platelets/µL). Complete blood count was unremarkable in the remaining 10 of 27 (37%) dogs.

Serum chemistry—All 27 dogs had serum chemistry analysis included in initial screening diagnostics. Elevated ALP was documented in 7 of 27 (26%) dogs (mean elevation above RR, 205 IU/L), with elevation categorized as grade 1 in 4 of 7 dogs, grade 2 in 2 of 7 dogs, and grade 3 in 1 of 7 dogs. Elevated ALT was identified in 23 of 27 (85%) dogs (mean elevation above RR, 245 IU/L), with elevation categorized as grade 1 in 16 of 23 (70%) dogs and grade 2 in 7 of 23 (30%) dogs. Other derangements included decreased ALT in 1 of 27 (3%) dogs (mean deviation below RR, 3 IU/L), elevated GGT in 1 of 27 (3%) dogs (mean elevation above RR, 7 IU/L), and grade 1 BUN elevation in 1 of 27 (3%) dogs (mean elevation above RR, 3 IU/L).

Treatment—Based on owner observation and physical examination findings, patients were prescribed a mean leflunomide dose of 2.6 mg/kg/d (dose range, 1.1 to 4 mg/kg/d) for a mean treatment duration of 1,566 days (range, 214 to 3,478 days). Dogs received either FDA-approved leflunomide from a retail pharmacy or a compounded formulation of leflunomide using FDA-regulated leflunomide powder combined with filler. Ten of 27 (37%) dogs received capsules containing FDA-regulated leflunomide powder with microcrystalline cellulose filler from nonsterile compounding pharmacies (Porter’s Pharmacy, Jeffrey’s Drugstore, and The Medicine Shoppe). Thirteen of 27 (48%) dogs received capsules containing FDA-regulated leflunomide powder with lactulose filler from a sterile and nonsterile compounding pharmacy (Wedgewood Pharmacy). The remaining 4 of 27 (15%) dogs received FDA-approved leflunomide tablets (Zydus Pharmaceuticals). The decision to administer compounded leflunomide versus FDA-approved tablets was based on individual patient dose and available tablet sizes. Initial doses were guided by currently available recommendations and titrated to the lowest effective dose.

Nine of 27 (35%) dogs were prescribed combination leflunomide plus prednisone (dose range, 0.08 to 1 mg/kg/d; mean dose, 0.45 mg/kg/d), and 17 of 27 (65%) dogs were prescribed leflunomide plus NSAID, with 7 of 17 (41%) dogs prescribed meloxicam (dose range, 0.007 to 0.12 mg/kg, once daily; mean dose, 0.07 mg/kg, once daily) and 10 of 17 (59%) dogs prescribed carprofen (dose range, 1.1 to 2.4 mg/kg, twice daily; mean dose, 1.9 mg/kg, twice daily). One dog included in the study received single-agent leflunomide (no glucocorticoid or NSAID). As there was only 1 dog treated with single-agent leflunomide, this patient was not included in statistical analysis. Concurrent medications are outlined in Table 1.

Adverse effects: clinical signs

Adverse effects included vomiting (2/27 [7%] dogs; grade 1), diarrhea (4/27 [15%] dogs; grade 1), lethargy (2/27 [7%] dogs; grade 1), hyporexia (4/27 [15%] dogs; grade 1), PU/PD (2/27 [7%] dogs), and secondary antibiotic responsive infection (1/27 [3%] dogs; grade 2). No other adverse clinical signs were documented. All clinical signs were self-limiting or resolved with or responded to outpatient therapeutic intervention. Clinical signs, treatment, and initial leflunomide dose are provided in Supplementary Table S1.

Adverse effects: hepatotoxicity

One dog in the study received single-agent leflunomide (no glucocorticoid or NSAID) and experienced grade 1 ALP elevation unresponsive to...
antibiotic treatment. All dogs prescribed leflunomide plus prednisone developed ALP elevation; 1 dog had only ALP elevation, and the remainder developed both ALP and ALT elevations. This distribution differed significantly ($P = .004$) from dogs prescribed leflunomide plus NSAID, as only 6 of 17 (35%) developed elevated liver enzymes. Findings are outlined in Table 2.

### Table 1—Outline of leflunomide doses and anti-inflammatories prescribed to all 27 dogs included in this study and diagnosed with immune-mediated polyarthritis. Mean dosage and dose ranges reflect prescribed doses throughout the study period (February 14, 2012, to May 4, 2022).

<table>
<thead>
<tr>
<th>Medication</th>
<th>No. of dogs</th>
<th>Average dose</th>
<th>Dose range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leflunomide</td>
<td>27</td>
<td>2.6 mg/kg/d</td>
<td>1.1–4 mg/kg/d</td>
</tr>
<tr>
<td>Anti-inflammatory</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>1</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Prednisone</td>
<td>9</td>
<td>0.45 mg/kg/d</td>
<td>0.08–1 mg/kg/d</td>
</tr>
<tr>
<td>Meloxicam</td>
<td>7</td>
<td>0.07 mg/kg, q 24 h</td>
<td>0.007–0.12 mg/kg, q 24 h</td>
</tr>
<tr>
<td>Carprofen</td>
<td>10</td>
<td>1.9 mg/kg, q 12 h</td>
<td>1.1–2.4 mg/kg, q 12 h</td>
</tr>
</tbody>
</table>

### Table 2—Number and percentage of dogs that experienced ALP, ALT, or both ALP and ALT elevation.

<table>
<thead>
<tr>
<th></th>
<th>Leflunomide+ (n = 27)</th>
<th>Leflunomide + NSAID (n = 17)</th>
<th>Leflunomide + prednisone (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LE elevation</td>
<td>16/27 (60%)</td>
<td>6/17 (35%)</td>
<td>9/9 (100%)</td>
</tr>
<tr>
<td>ALP elevation</td>
<td>14/27 (52%; includes patient with no anti-inflammatory)</td>
<td>5/17 (24%)</td>
<td>9/9 (100%)</td>
</tr>
<tr>
<td>Grade 1</td>
<td>3/27 (11%)</td>
<td>2/17 (12%)</td>
<td>0/9 (0%)</td>
</tr>
<tr>
<td>Grade 2</td>
<td>3/27 (11%)</td>
<td>1/17 (6%)</td>
<td>2/9 (22%)</td>
</tr>
<tr>
<td>Grade 3</td>
<td>8/27 (30%)</td>
<td>1/17 (6%)</td>
<td>7/9 (78%)</td>
</tr>
<tr>
<td>ALT elevation</td>
<td>10/27 (37%)</td>
<td>2/17 (12%)</td>
<td>8/9 (89%)</td>
</tr>
<tr>
<td>Grade 1</td>
<td>3/27 (11%)</td>
<td>2/17 (12%)</td>
<td>1/9 (11%)</td>
</tr>
<tr>
<td>Grade 2</td>
<td>6/27 (22%)</td>
<td>0/17 (0%)</td>
<td>6/9 (67%)</td>
</tr>
<tr>
<td>Grade 3</td>
<td>1/27 (3.7%)</td>
<td>0/17 (0%)</td>
<td>1/9 (11%)</td>
</tr>
<tr>
<td>ALP and ALT elevation</td>
<td>10/27 (37%)</td>
<td>2/17 (12%)</td>
<td>8/9 (89%)</td>
</tr>
<tr>
<td>Antibiotic responsive</td>
<td>5/27 (19%)</td>
<td>2/17 (12%)</td>
<td>3/9 (33%)</td>
</tr>
<tr>
<td>Persistent LE elevation</td>
<td>12/27 (44%)</td>
<td>4/17 (23%)</td>
<td>6/9 (67%)</td>
</tr>
</tbody>
</table>

This table compares all 27 dogs in the study (dogs receiving leflunomide plus NSAID [17/27], dogs receiving leflunomide plus prednisone [9/27], and $P$ value). Grades of adverse events were categorized following criteria established by Veterinary Cooperative Oncology Group Common Terminology Criteria for Adverse Events. 20

LE = Liver enzyme.

### Adverse effects: myelosuppression

Myelosuppression was defined as RBC count, overall WBC count, or specific WBC line (neutrophils, eosinophils, lymphocytes, macrophages) below reference ranges or decreasing by more than 2-fold during the course of treatment. Complete blood count was regularly monitored for all dogs, and myelosuppression was not identified in any dog included in this study.

### Discussion

The mean treatment duration for 27 dogs in this study was 1,566 days, which is longer than reported elsewhere, and numerous findings echoed those in previous studies. Based on observation and examination findings, all dogs in this study experienced improved clinical signs after starting leflunomide, similar to response rates (80% to 93%, 13 93.3%, 6 92.9%) reported by others. Hematologic adverse effects were not encountered throughout the duration of our study, consistent with studies 12,21 where leflunomide was dosed < 4 mg/kg/d; this fact suggests that the incidence of myelosuppression is influenced by dose but not impacted by duration of therapy.

Liver enzyme elevation was documented in 16 of 27 (60%) of all dogs in this cohort: 9 of 16 (56%) dogs were treated with a combination of leflunomide plus prednisone, and 6 of 16 (37.5%) dogs were treated with a combination of leflunomide plus NSAID (1 dog did not receive an anti-inflammatory). A statistically significant difference in development of liver enzyme elevation and medication coadministered with leflunomide was identified. More specifically, 9 of 9 (100%) dogs treated with leflunomide plus prednisone developed elevated liver enzymes, in contrast with 6 of 17 (35%) dogs treated with leflunomide plus NSAID. Additionally, patients prescribed prednisone with leflunomide had higher incidence of suspected antibiotic-responsive liver enzyme elevation (3/9 dogs receiving leflunomide plus prednisone, in contrast with 2/17 dogs receiving leflunomide plus NSAID; $P = .004$). Our results supported other studies speculating that concurrent medications, prednisone specifically, contributed to liver enzyme elevation observed in dogs prescribed leflunomide. 8

In our study, adverse effects potentially attributable to leflunomide were noted in 9 of 27 dogs (33%) within 6 months after starting treatment. Documented side effects were similar to those previously reported and included vomiting, diarrhea, lethargy, anorexia, PU/PD, and presumptive secondary bacterial infections, all of which were self-limiting or resolved with outpatient therapeutic intervention. The
incidence of adverse effects largely mirrored the previously reported range (7% to 73%).

Our study had several limitations, most attributable to retrospective design. Comparison between treatment groups would be more accurate with a uniform leflunomide dosing regimen, the same anti-inflammatory prescribed to all patients, and contemporaneous control groups consisting of patients treated with leflunomide or anti-inflammatories alone. Abdominal ultrasound and sampling at detection of elevated liver enzymes would have been beneficial to characterize hepatic insult. A randomized, blinded, controlled clinical trial would be ideal to compare the effectiveness and safety of single-agent leflunomide, leflunomide plus prednisone, and leflunomide plus NSAID.

Our data suggested that leflunomide is safe and well tolerated for long-term management of canine IMPA at the established dose of 2 mg/kg/d. Dogs treated with leflunomide and concurrent prednisone may be predisposed to develop hepatic insult based on ALT elevation compared to dogs prescribed leflunomide and NSAID.

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References

Supplementary Materials
Supplementary materials are posted online at the journal website: avmajournals.avma.org.