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Serum concentrations of IL-1β, IL-6, and TNF-α in dogs with chronic kidney disease in uremic syndrome undergoing intermittent hemodialysis with and without bypass

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ABSTRACT

Intermittent hemodialysis (IH) is an important therapy in the context of kidney dysfunction in dogs. However, its impact on pro-inflammatory cytokines is unclear. This study assessed IL-1β, IL-6, and TNF-α serum concentrations in dogs with chronic kidney disease (CKD) undergoing one session of IH without bypass (IH group, $n=4$) and with bypass (IH + bypass group, $n=4$). The control group (CG) included four healthy dogs. Cytokine levels were measured before, during, and after the first IH session. Comparative analyses of each cytokine within each group and time point were performed, along with a global comparison between groups. No significant changes were observed in cytokines across evaluation times in the IH groups. IL-1β was significantly higher post-session in the IH and IH + bypass groups compared to CG. Globally, IL-1β and TNF-α concentrations were significantly higher in the IH (11.41 pg/mL (10–16.17) and 2 pg/mL (2–88.54), respectively) and IH + bypass groups (10 pg/mL (10–10) and 2 pg/mL (2–215.5), respectively) compared to CG (0.96 pg/mL (0–3.56) and 0 pg/mL (0–0.003), respectively). The IH group also showed elevated IL-6 concentration (0.1 ng/mL (0.1–0.5)) compared to CG (0 ng/mL (0–0.1)). Higher IL-1β and IL-6 concentrations were observed in the IH group (11.41 pg/mL (10–16.17) and 0.1 ng/mL (0.1–0.1), respectively) compared to the IH + bypass group (10 pg/mL (10–10) and 0.1 ng/mL (0.1–0.5), respectively). In conclusion, a single IH session, with or without bypass, did not increase pro-inflammatory cytokines in CKD dogs with uremic syndrome but maintained the inflammatory state. Dogs undergoing IH without bypass may receive a stronger stimulus for cytokine release.

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1. Introduction

Similar to humans, chronic kidney disease (CKD) affects a substantial number of canine patients, resulting in high mortality rates (Roura 2019). CKD involves irreversible and progressive impairment of kidney structure and or function, impacting various body functions, leading to systemic debilitation in animals (Polzin 2011; Bartges 2012).

Clinical management of CKD encompasses conservative and supportive measures aimed at slowing disease progression and maintaining the patient's quality of life (International Renal Interest Society 2023). However, depending on the severity of the disease, such treatment might prove insufficient (O'Neill et al. 2013), prompting the consideration of renal replacement therapies like intermittent hemodialysis (IH) as adjunct therapeutic options (Langston 2002).

In veterinary practice, IH is indicated for both acute and chronic nephropathies, with its application in the latter aiming to mitigate uremia-related detrimental effects, stabilize the patient, and enhance survival quality (Cowgill and Francey 2012). In contrast, the introduction of IH in human medicine is mainly performed in a chronic manner, focusing on maintaining the patient's stability until a potential transplant becomes feasible, resulting in numerous studies assessing the impact of hemodialysis in these patients (Dai et al. 2020; Ságі et al. 2020; Donadei et al. 2023).

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In humans with CKD undergoing IH, an additional inflammatory response occurs due to the procedure (Stenvinkel et al. 2005; Cohen et al. 2010), as evidenced by elevated pro-inflammatory cytokines and acute-phase proteins, particularly in cases of chronic dialytic treatment (Stenvinkel et al. 2005; Yhee et al. 2008; Vianna et al. 2011; Lee et al. 2015; Amdur et al. 2016; Maissen-Villiger et al. 2016). Even evaluations of the acute effects of IH demonstrate the same outcome (Goldstein et al. 2003), indicating that the dialytic procedure potentially contributes to patient inflammation (Bologa et al. 1998; Tarakçioğlu et al. 2003; Cohen et al. 2010; Kade et al. 2016).

However, the relatively limited application of this therapy in animals has hindered the progression of veterinary research in this context. Picelli de Azevedo et al. (2022) demonstrated increased C-reactive protein (CRP) levels in stage-four CKD dogs undergoing IH, analyzing samples collected before and after the procedure. However, some human studies have indicated that cytokines might offer better predictive applicability for outcomes than acute-phase proteins (Vincenzo Panichi et al. 2004). Furthermore, the dynamics of these inflammatory mediators might not be well elucidated through isolated analyses (Song et al. 2012; Floras et al. 2014). Meneses et al. (2021) evidenced an increase in tumor necrosis factor-alpha during IH sessions in dogs with medication-induced acute kidney injury; however, the study's sessions lasted only one hour, a condition not representative of the majority of prescriptions for this type of treatment in CKD patients.

The traditional approach to an IH session involves uninterrupted treatment lasting typically 3 to 6 h, although this can vary based on individual therapy objectives (Cowgill and Guillaumin 2013). However, in cases requiring low extracorporeal blood flow according to prescription calculations, an alternate approach involves alternating periods of effective dialysis and interruption of exchanges during the session (the 'Bypass' mode), allowing safe increases in blood flow throughout the procedure (Cowgill 2011; Dufayet and Cowgill 2021).

In the bypass mode, the exchanges interruption and dialysate flow pausing, may culminate with a larger amount of blood (proportional to the animal's size) to come into contact with the circuit. Therefore, given the fact that studies with humans reveal an increase in cytokines in patients undergoing IH and given the limited investigation into the impact of IH on the inflammatory profile of CKD dogs, especially concerning cytokine analysis and the utilization of the bypass mode, this study aimed to assess serum concentrations of pro-inflammatory cytokines in CKD dogs in uremic syndrome undergoing IH with and without bypass, becoming, as far as we know, the first study to address this issue.

2. Material and methods

The research was carried out at the Veterinary Hospital of the Faculty of Veterinary Medicine and Zootechnics (FMVZ), UNESP, Botucatu following approval by the Institutional Animal Ethics Committee under protocol number 0050/2021.

2.1. Animals

Twelve dogs were included in the study, regardless of gender or breed, with age > 1 year. The animals were selected based on the attendance to the Veterinary Nephrology and Urology Service of the Veterinary Hospital of FMVZ, UNESP, Botucatu, São Paulo. The inclusion of dogs was carried out with the owners' consent form signature.

2.1.1. Inclusion and exclusion criteria

Dogs diagnosed with CKD based on ultrasonographic alterations, serum biochemical profile, urinalysis, and urine protein-to-creatinine ratio, following the guidelines of the International Renal Interest Society (2023), and exhibiting uremic syndrome, characterized by systemic clinical signs (vomiting, diarrhea, anorexia, depression, lethargy, weakness) due to a significant decline in kidney function, were included in the study. Furthermore, these animals were refractory to clinical treatment.

Dogs in shock or in critically unstable condition, moderately dehydrated (dehydration > 6%), severely altered mental state, at risk of bleeding (platelet count < 80.000) since regional anticoagulation was not available, and anemic (hematocrit < 18%) were excluded from this study, even if CKD in uremic syndrome was present. The underlying cause of CKD was not considered in the inclusion and exclusion criteria, as it is typically non-specific in routine veterinary practice. Healthy dogs without any clinical signs or laboratory alterations were also selected. In this selection, dogs with a surgical history six months before and those not meeting the body weight criteria for undergoing IH procedure (<5 kg) were excluded. Details of the allocation of animals to each group are provided below.

2.2. Experimental groups and treatments

Three experimental groups were established, each comprised of four animals. Among these, two groups consisted of CKD dogs with uremic syndrome, which were assigned to receive IH. The determination of the number of animals ($n = 4$ per group) took into account a statistical model of repeated measures over time. The analysis was conducted considering the parameters: effect size (0.5), error probability (0.05), number of groups (3), and number of moments (6), with a test power of 0.8. For this purpose, the statistical package G*Power version 3.1.9.6 (Franz Faul, Germany) was utilized.

The distinction between the two groups of animals undergoing hemodialysis was whether or not the bypass was performed during the procedure. Specifically, one group underwent IH without bypass,

referred to as the 'Intermittent hemodialysis group without bypass' (IH group). The other group underwent IH with the inclusion of bypass mode, known as the 'Intermittent hemodialysis group with bypass' (IH + bypass group). Each animal was allocated to a specific group following the prescription of the hemodialysis session. This assignment considered the blood flow rate (mL/min) calculated at the end of the prescription. Since the intensity of the treatment and the quantity of whole blood processed are determinants of extracorporeal blood flow, each prescription was individually crafted. These factors distinguished the animals in each treated group. Four healthy dogs constituted the control group (CG), and they were assessed at the beginning of the study as illustrated in Figure 1.

2.2.1. Intermittent hemodialysis group without bypass (IH group)

The animals in this group were those that, based on the calculations for extracorporeal therapy prescription (urea removal rate (URR), session time, and processed blood volume), achieved an extracorporeal blood flow rate (mL/minute) capable of enabling continuous and safe extracorporeal circulation while adhering to the prescribed intensity. In alignment with the team's expertise and in accordance with the machine's acceptable minimum values, this flow rate was established as ≥ 50 mL/minute. This value facilitated the procedure to remain in effective dialysis mode throughout the entire session, without interruptions in the dialysate flow, thereby proceeding in the conventional manner (Figure 2A).

2.2.2. Intermittent hemodialysis group with bypass (IH + bypass group)

The animals allocated in this group were those whose extracorporeal blood flow rate after therapy prescription (ideal to maintain the calculated intensity) was <50 mL/minute. In this scenario, acknowledging the potential for inaccuracies and an increased risk of system coagulation, the IH approach involved the use of the bypass mode. Thus, these animals underwent calculated periods of dialysate flow interruption throughout the defined total session time. To achieve this, each hour of the session was considered a cycle (60 min), and each cycle was further divided into three moments (20 min each) (Figure 2B). Within each moment, the minutes on dialysis and the minutes without exchanges (bypass mode) were calculated. The duration of effective dialysis and bypass mode (at each moment) varied for each animal in the group, as its determination was influenced by the volume of blood to be dialyzed and the determined blood flow. This approach enabled an increase in extracorporeal blood flow rate while maintaining the intensity of the URR within the initially established safe ranges.

2.2.3. Conservative treatment and management of clinical signs

All CKD dogs included in the study received clinical treatment for a minimum of 24–48 h before the recommendation of dialytic therapy. As part of the conservative objective, the clinical treatment encompassed, when appropriate, therapeutic diet, anti-hypertensive management, anti-proteinuric treatment, antioxidant administration, management of hypoproliferative

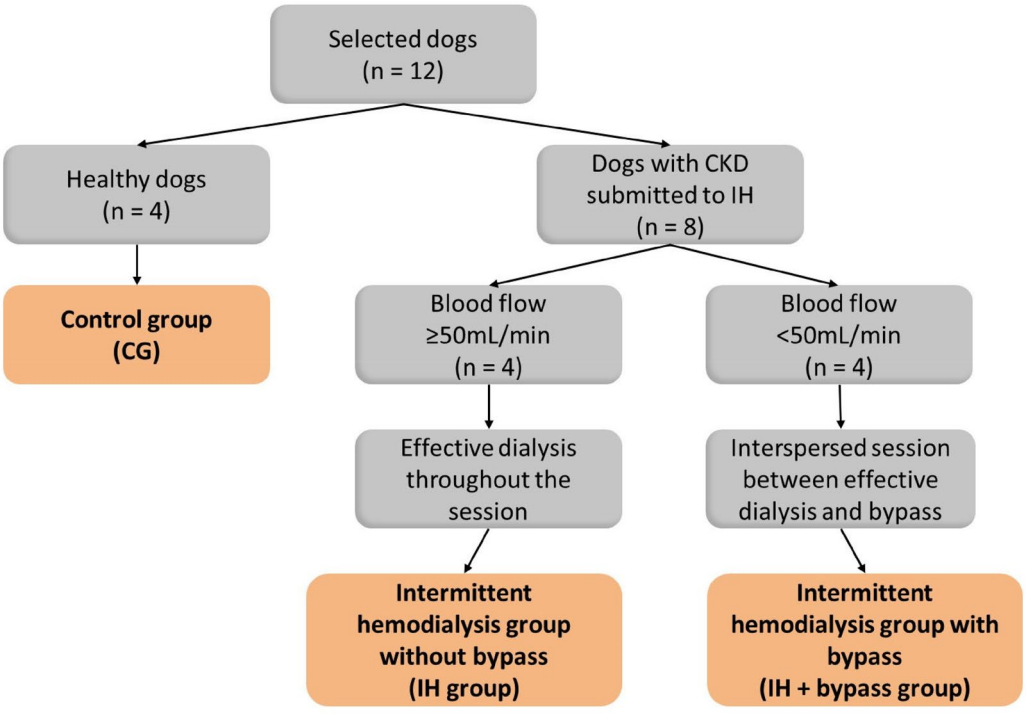

Figure 1. Flowchart of the experimental groups' composition. Chronic Kidney Disease (CKD); Intermittent hemodialysis (IH); blood flow deviation (bypass); control group (CG); Intermittent hemodialysis group without bypass (IH group); Intermittent hemodialysis group with bypass (IH + bypass group).

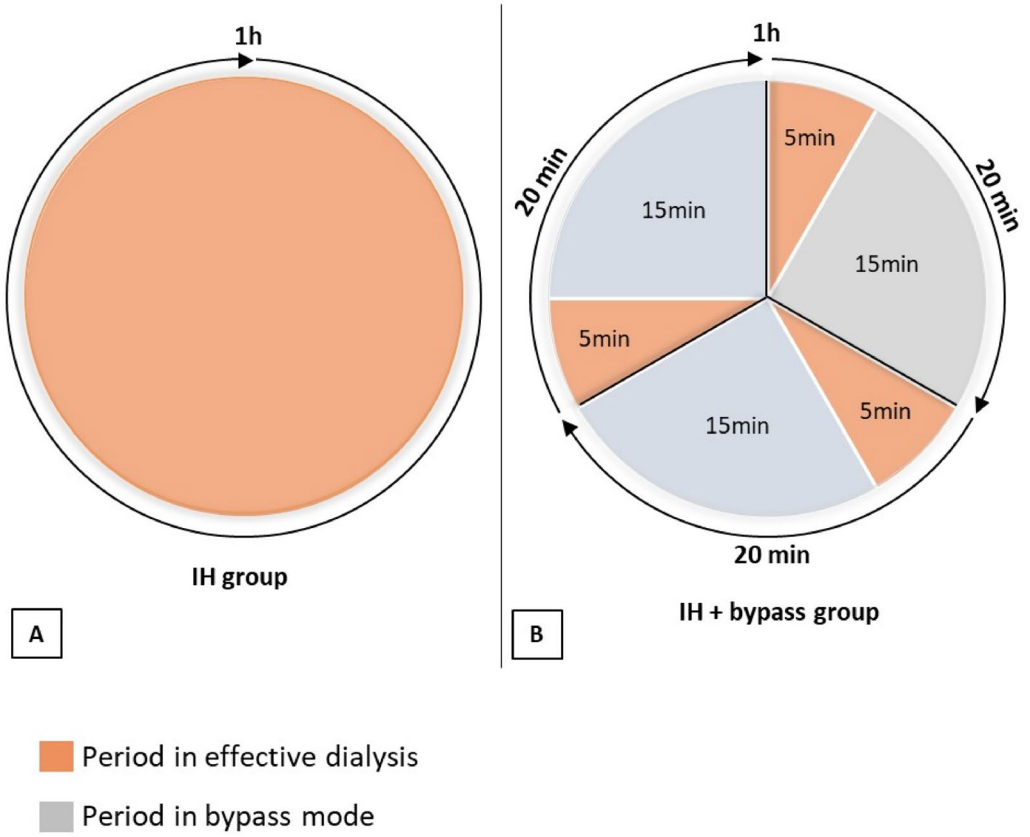

Figure 2. Schematic representation of effective dialysis prescription during intermittent hemodialysis without and with bypass. The illustration depicts a one-hour treatment (cycle) applied to the groups. (A) Intermittent hemodialysis group without bypass (IH group); (B) Intermittent hemodialysis group with bypass (IH + bypass group). It is evident that in the IH group, effective dialysis occurs throughout the treatment, whereas in the IH + bypass group, there are alternating periods of effective dialysis (exemplified in 5 min) and bypass (flow deviation without exchange occurrence – exemplified in 15 min) every 20 min (time intervals) during the one-hour cycle.

anemia, electrolyte and acid-base imbalances, and management of hyperphosphatemia. Regarding the control of clinical signs, therapy included fluid management and gastrointestinal management (antiemetic, antiacid, and mucoprotective agents).

2.2.4. Intermittent hemodialysis (IH)

The hemodialysis sessions were conducted using the 4008S hemodialysis machine (Fresenius Medical Care, Bad Homburg Höhe – Germany) with ultrafiltration control, coupled to a reverse osmosis water treatment unit (MCA.OR.PF.01, Palhoça/SC – Brazil), which provides ultrapure water (double-pass) to prevent the transfer of impurities related to the dialysate.

Capillary hemodialyzers with polysulfone membranes (Fresenius Medical Care, Bad Homburg, Germany/Fresenius Medical Care, St. Wendel, Germany) were employed in accordance with the animal's body weight (Cowgill 2011). The dialysate solution comprised 8.4% sodium bicarbonate buffer solution (Bibag, Fresenius Medical Care Ltda, Jaguariúna/SP) and an electrolyte solution with glucose (CPHD 22 G/34 with glucose, Fresenius Medical Care Ltda, Jaguariúna/SP, Brazil).

The dialytic prescription was established as described by Cowgill (2011), involving three main points: the intensity (total urea removal rate – URR, and per hour – URR/h); the session duration (number of hours to achieve total URR); and the total blood volume clearance in liters, thereby influencing the extracorporeal blood flow in mL/minute.

Considering that treatment intensity and processed blood volume directly influence the extracorporeal blood flow, each prescription was tailored individually. These factors precisely distinguished the animals within each experimental group.

2.3. Collection of biological samples

For animals in the HI and HI + bypass groups, blood samples were collected at the following time points in the first session (S1): 30 min before IH (M0), during the IH session (at the end of the first, second, third, and fourth hour of the session – M1, M2, M3, and M4), and 30 min after IH (M5) the first session (S1). Blood samples were collected from the CG animals only at the beginning of the study as shown in Figure 3.

To determine pro-inflammatory cytokines, interleukin 1β (IL-1β), interleukin 6 (IL-6), and tumor necrosis factor α (TNF-α), the samples were collected in tubes without anticoagulant (BD Vacutainer®, New Jersey, USA) to obtain serum after centrifugation (3,000 g). All samples intended for cytokine

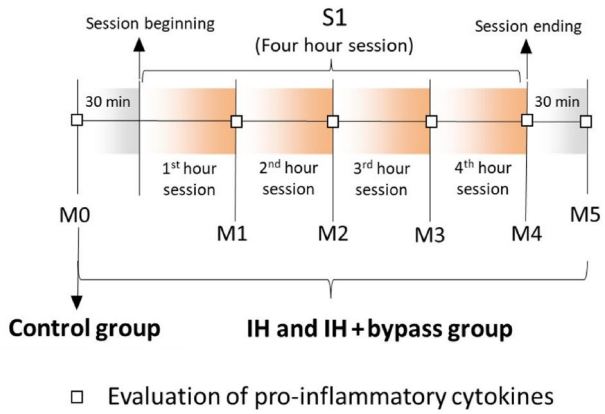

Figure 3. Experimental timeline moments for obtaining and evaluating pro-inflammatory cytokines. Control group (CG); Intermittent hemodialysis group without bypass (IH group); Intermittent hemodialysis group with bypass (IH + bypass group); first session of IH (S1); M0 (CG sample collection – 30 min before the session beginning in the IH and IH + bypass groups); M1 (at the end of the 1st hour of session); M2 (at the end of the 2nd hour of session); M3 (at the end of the 3rd hour of session); M4 (at the end of the 4th hour of session); M5 (30 min after the end of session in the IH and IH + bypass groups).

evaluation were stored in a freezer at −80 °C until the time of analysis.

2.4. Cytokine evaluation

The determination of serum concentrations of IL-1β, IL-6, and TNF-α (at each moment evaluation) was carried out using an Enzyme-Linked Immunosorbent Assay (ELISA) with specific commercial kits for dogs (RAB0572, RAB0525, RAB0526, respectively, Sigma-Aldrich Brasil Ltda., Cotia, São Paulo, Brazil), following the manufacturer's instructions. For each cytokine, two assays were performed, resulting in the following intraassay and interassay coefficients of variation: 3.6% and 3.8% for IL-1β, 3.5% and 4% for TNF-α, and 1.8% and 3 .7% for IL-6, respectively.

2.5. Clinical evaluation and session data

All animals underwent physical examination, including heart rate, respiratory rate, rectal temperature, and systolic blood pressure. In the CG, examination occurred only at M0, while animals in the IH and IH + bypass groups occurred at all moments (M0-M5). Body condition score and muscle mass index data were also collected at M0.

Regarding hemodialysis procedure-related data, the following information was collected: blood flow rate (mL/minute), blood flow rate per kilogram (mL/kg/minute), final URR, and session duration.

2.6. Statistical analysis

The Shapiro-Wilk test was used to assess the assumption of normal distribution. Data that did not exhibit normal distribution were log transformed. If the application of logarithm did not yield normal distribution, non-parametric tests were employed.

The comparison between IH and IH + bypass groups regarding session variables that did or did not achieve normal distribution, paired T-tests and Wilcoxon tests were used, respectively. Comparison of cytokine concentrations between the IH and IH + bypass groups, as well as among the different moments within each group, was conducted using the Mann Whitney test and Friedman test, respectively. To eliminate individual variation at M0, a ratio analysis was performed using the formula: concentration at moments (1,2,3,4,5)/concentration at M0. Thus, M0 was standardized and defined as the baseline point (ratio 1). Comparison of these relative values between IH and IH + bypass groups, as well as among different moments within each group, was performed using the Mann Whitney test and Friedman test, respectively.

An additional analysis was conducted by removing the moments and comparing the IH, IH + bypass groups, and CG. For this purpose, the Kruskal Wallis test followed by Dunn's test were applied.

Data analyzed using parametric and non-parametric tests are presented as mean ± standard error of the mean and median (Q1–Q3), respectively. Significant differences were considered for $p < 0.05$. Statistical analyses were performed using GraphPad Prism version 9.3.0.

3. Results

In total, 12 dogs were evaluated in the study. The CG consisted of four dogs: one male and three females with a mean age of 8.4 ± 0.89 years and mean body weight of 15.37 ± 2.62 kg. This group included two mixed-breed dogs, one Border Collie, and one Schnauzer. The IH group, also comprised of four dogs: one male and three females with a mean age of 4.2 ± 1.73 years. The breed representation in this group included two Labrador Retrievers, one German Shepherd, and one Great Dane. The IH + bypass group consisted of two males and two females, including two mixed-breed dogs, one Bull Terrier, and one English Bulldog, with a mean age of 7.8 ± 1.67 years. Concerning the mean body weight (kg) of the dogs subjected to IH, a significantly lower value was observed in the HI + bypass group (15.62 ± 1.57) compared to the HI group (36.91 ± 6.23) ($p = 0.0285$).

Considering that capillary hemodialyzers with polysulfone membranes were used based on the animal's body weight, the assigned characteristics were as follows: IH group: low-flow hemodialyzer with effective surface area of 0.8 m^2, ultrafiltration coefficient of 8 ml/h/mmHg, and blood priming volume of 51 mL ($n = 3$), and low-flow hemodialyzer with effective surface area of 1.3 m^2, ultrafiltration coefficient of 13 ml/h/mmHg, and blood priming volume of 78 mL ($n = 1$); IH + bypass group: low-flow hemodialyzer with effective

Table 1. Mean values ± standard error or median (Q1-Q3) of baseline laboratory variables (M0) for the studied groups.

Variable	Reference range	Groups		
		Control group	IH group	IH + bypass group
CBC				
Red Cells (10^6/μL)	5.50–8.50	7.33 ± 0.3^{A}	3.57 ± 0.2^{B}	4.37 ± 0.6^{B}
Hemoglobin (g/dL)	12.00–18.00	17.13 ± 0.4^{A}	8.7 ± 0.4^{B}	10.33 ± 1.2^{B}
Hematocrit (%)	37–55	49 ± 1.4^{A}	25.5 ± 1.1^{B}	30.25 ± 3.3^{B}
Platelets (10^3/μL)	160–430	224.75 ± 52.2^{A}	280.5 ± 71.7^{A}	233 ± 33.8^{A}
Leukocytes (10^3/μL)	6.00–17.00	6.57 ± 0.3^{A}	16.75 ± 4.3^{A}	13.10 ± 2.5^{A}
Neutrophils (10^3/μL)	3.0–11.5	4.9 ± 0.5^{A}	14.10 ± 3.4^{A}	11.57 ± 2.3^{A}
Lymphocytes (10^3/μL)	1.0–4.80	1.0 ± 0.3^{A}	0.525 ± 0.08^{A}	0.375 ± 0.1^{A}
Eosinophils (10^3/μL)	0.1–1.25	0.374 ± 0.07^{A}	0.650 ± 0.46^{A}	0.400 ± 0.18^{A}
Monocytes (10^3/μL)	0.15–1.35	0.199 ± 0.04^{A}	1.50 ± 0.87^{A}	0.700 ± 0.12^{A}
Serum biochemistry				
Urea (mg/dL)	21.40–59.92	30.10 ± 1.90^{A}	392.3 ± 55.68^{B}	369.3 ± 30.40^{B}
Creatinine (mg/dL)	0.5–1.5	0.98 ± 0.10^{A}	17.28 ± 1.3^{B}	17.70 ± 5.7^{B}
Phosphor (mg/dL)	2.60–6.20	3.37 ± 0.61^{A}	12.75 ± 2.42^{B}	16.95 ± 1.93^{B}
Total protein (g/dL)	5.40–7.10	6.67 ± 0.12^{A}	5.0 ± 0.29^{B}	5.02 ± 0.09^{B}
Albumin (g/dL)	2.6–3.30	3.37 ± 0.04^{A}	2.42 ± 0.31^{B}	2.07 ± 0.24^{B}
Globulin (g/dL)	2.70–4.40	3.3 ± 0.10^{A}	2.57 ± 0.50^{A}	2.95 ± 0.18^{A}
SDMA (μg/dL)	0.0–14.0	$10.0\ (7.5–11.7)^{A}$	$32.5\ (31.0–58.7)^{AB}$	$68.5\ (40.25–97.5)^{BB}$
Hemogasometry				
pH	7.34–7.44	7.38 ± 0.004^{A}	7.30 ± 0.02^{AB}	7.24 ± 0.04^{BB}
Base deficit (mmol/L)	−3 to 2	-3.22 ± 0.49^{A}	-10.78 ± 1.42^{AB}	-12.93 ± 3.27^{B}
HCO_3^- (mmol/L)	19–24	20.6 ± 0.5^{A}	14.20 ± 1.13^{AB}	12.90 ± 2.62^{BB}
iCa (mmol/L)	1.16–1.40	1.38 ± 0.02^{A}	0.96 ± 0.17^{A}	1.09 ± 0.09^{A}
Potassium (mmol/L)	3.5–5.0	3.85 ± 0.15^{A}	4.27 ± 0.36^{AB}	5.43 ± 0.40^{B}
Sodium (mmol/L)	144–155	148.5 ± 0.86^{A}	146.5 ± 1.25^{A}	144.5 ± 4.13^{A}
Urine parameter*				
Urinary specific gravity		1.043 ± 0.003	1.013 ± 0.0	1.011 ± 0.0
Urinary protein creatinine ratio (UPC)	<0.2	0.10 (0.09–0.12)	2.45 (2.03–2.88)	1.81 (0.80–16.23)

Intermittent hemodialysis group without bypass (IH Group); Intermittent hemodialysis group with bypass (IH + bypass group); complete blood count (CBC). Mean values followed by different superscript letters in the same row are statistically different ($p < 0.05$). When there are two superscript letters in the same group, the first corresponds (A) to the assessment for the previous group and the second (B) for the next one. *Descriptive values only.

surface area of 0.8 m², ultrafiltration coefficient of 8 ml/h/mmHg, and blood priming volume of 51 mL ($n = 3$), and high-flow hemodialyzer with effective surface area of 0.2 m², ultrafiltration coefficient of 7 ml/h/mmHg, and blood priming volume of 18 mL ($n = 1$).

The main baseline laboratory characteristics such as complete blood count (CBC), serum biochemistry, hemogasometry, and urine parameters of the evaluated dogs are presented in Table 1, and data regarding the IH session for each of the treated groups are provided in Table 2.

Although the blood flow between groups showed no significant differences, the blood flow rate (mL/kg/min) was notably higher in the IH + bypass group. Other data from the IH session, including both prescription and removal efficiency, did not exhibit significant differences between the groups.

Considering the variables of physical examination, no significant differences were observed for heart rate, respiratory rate, systemic blood pressure, body condition score, and muscle mass index among the groups in the study. However, rectal temperature displayed a higher mean in the CG (38.9 °C) compared to the IH + bypass group (37.5 °C) at M0 ($p = 0.0405$). When statistically analyzed across the moments and between groups undergoing IH, heart rate showed a significant difference. Heart rate presented a lower mean in the IH group at M2 (83 bpm) compared to the same group's at M0 ($p = 0.0389$), as well as compared to M2 in the IH + bypass group ($p = 0.0134$). Despite these findings, all physical parameters remained within the reference ranges for the species. There were no clinical complications arising from the hemodialysis procedure in any of the treated groups.

Table 2. Mean values ± standard deviation or median (Q1-Q3) regarding hemodialysis session data for IH and IH + bypass groups.

Parameter	IH group	IH + by-pass group	*p*
Session Time (minutes)	235 (218.8–240)	240 (240–262.5)	0.2857
Blood Flow (mL/min)	73.75 ± 8.98	67.25 ± 4.38	0.5397
Blood Flow Rate (mL/kg/min)	2.04 (1.80–2.37)	3.99 (3.84–5.51)	0.0286
Urea Removal Rate	0.44 ± 0.07	0.41 ± 0.03	0.7469

Intermittent hemodialysis group without bypass (IH Group); Intermittent hemodialysis group with bypass (IH + bypass group). $p < 0.05$ indicates a significant difference between groups.

Based on cytokine analysis, the comparison of each cytokine concentration within and between groups undergoing IH showed no significant differences. However, there was a significant increase in IL-1β in the IH group (14.83 pg/mL (12.95–16.93)) compared to CG (0.96 pg/mL (0–3.56)) at M5 (30 min post-session) (Figure 4).

When accounting for the elimination of individual variation at M0 (using the relationship between moments) in the treated groups, the comparative results for the assessed cytokines once again did not exhibit significant differences between the moments regardless of the group (Figure 5).

Considering the absence of differences between moments, a comprehensive analysis of the concentration of each cytokine (median of all results) was conducted for each group. The aim was to highlight the potential for changes between the studied IH approaches. In this assessment, a notable increase in IL-1β and TNF-α concentrations was observed in both IH groups (IH 11.41 pg/mL (10–16.17) and 2 pg/mL (2–88.54) respectively; IH + bypass group 10 pg/mL (10-10) and 2 pg/mL (2–215.5) respectively) compared to CG (0.96 pg/mL (0–3.56) and 0 pg/mL (0–0.003) respectively), demonstrating potential inflammatory state of CKD dogs in uremic syndrome undergoing intermittent hemodialysis. Furthermore, in the IH group, IL-6 concentration was significantly higher (0.1 ng/mL (0.1–0.5)) than in the CG (0 ng/mL (0–0.1)).

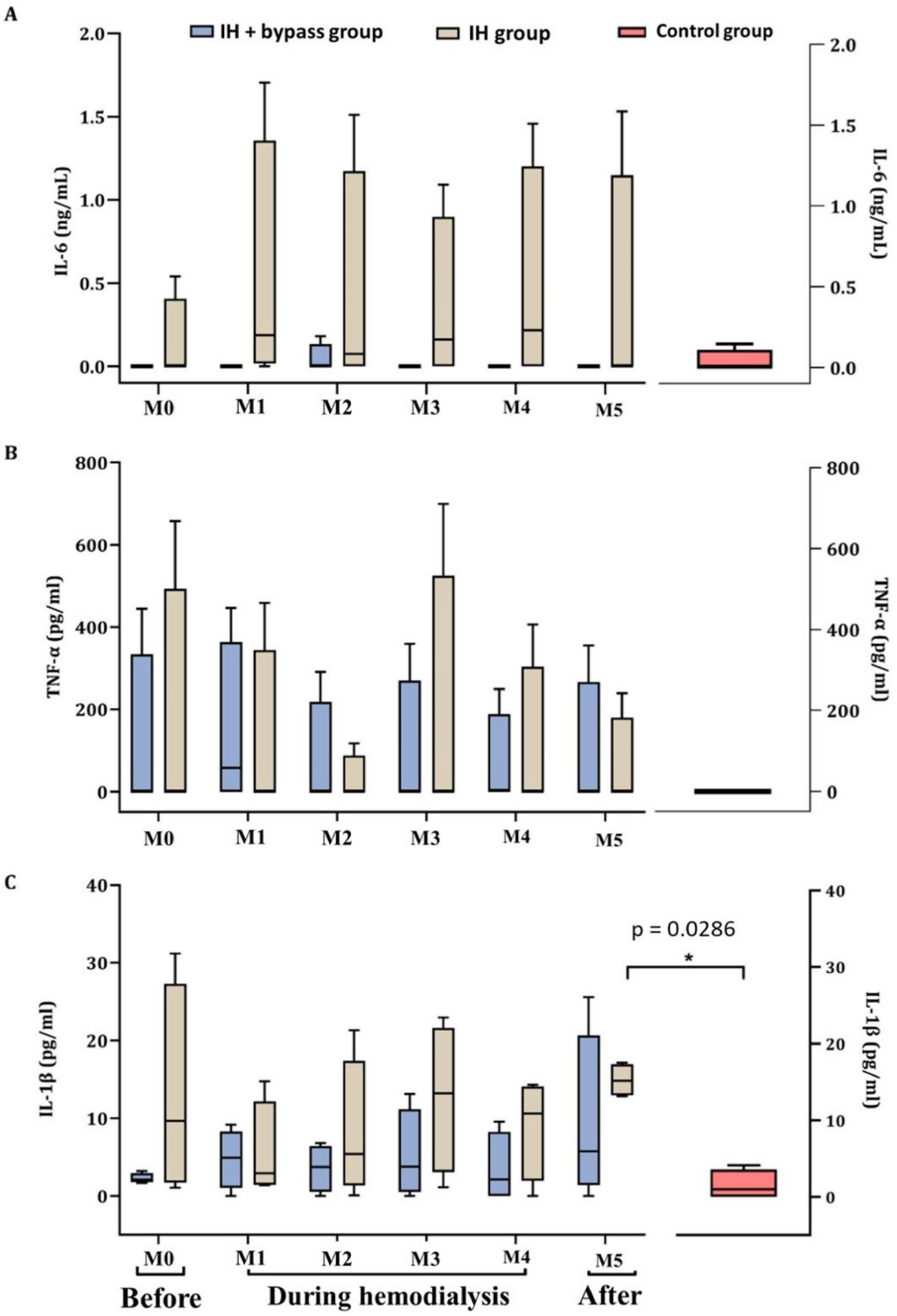

Figure 4. Boxplots illustrating the serum cytokine concentrations across the dogs' groups and moments in the study. The boxplots depict the median (line within the box), first and third quartiles (top and bottom of the box), and range (whiskers). A) Serum concentration of IL-6 (ng/mL). B) serum concentration of TNF-α. C) Serum concentration of IL-1β. Intermittent hemodialysis group with bypass (IH + bypass group); Intermittent hemodialysis group without bypass (IH group). *Indicates significant difference ($p < 0.05$).

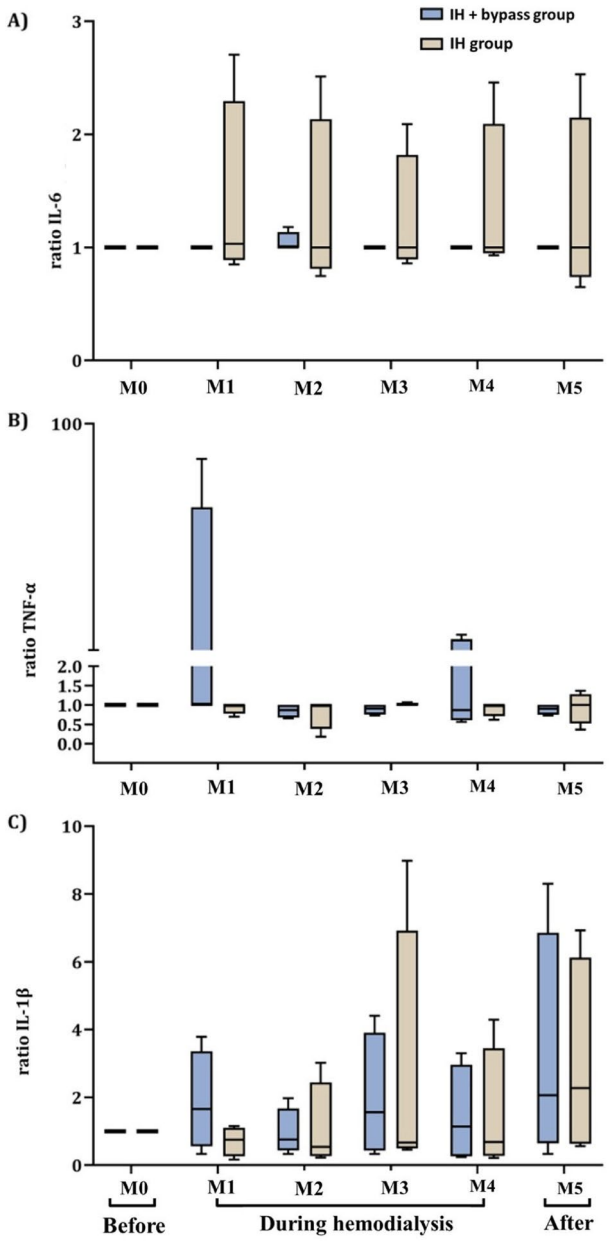

Figure 5. Boxplots illustrating the relationship between serum cytokine concentrations at different moments in relation to M0 within the IH groups. The boxplots depict the median (line within the box), first and third quartiles (top and bottom of the box), and range (whiskers). (A) Ratio IL-6 (ng/mL). (B) Ratio TNF-α (pg/mL). (C) Ratio IL-1β (pg/mL). Intermittent hemodialysis group with bypass (IH + bypass group); Intermittent hemodialysis group without bypass (IH group).

However, upon comparing the overall cytokine concentrations between the groups undergoing IH, the IH group displayed higher values for both IL-1β and IL-6 (11.41 pg/mL (10–16.17) and 0,1ng/mL (0.1-0.1) respectively) compared to IH + bypass group (10 pg/mL (10-10) and 0.1 ng/mL (0.1–0.5) respectively) (Figure 6).

4. Discussion

In veterinary medicine, scientific literature regarding the evaluation of inflammatory cytokines in dogs undergoing intermittent hemodialysis, particularly in the context of bypass mode, is extremely scarce. Therefore, it is believed that this study represents the first to introduce and comparatively address intermittent hemodialysis without and with bypass in dogs in uremic syndrome, in addition to be the first study in which this comparison involves the concentration of inflammatory cytokines. As known, intermittent Hemodialysis has been implicated as potentially inflammatory due to the activation of this process, primarily through blood contact with the extracorporeal circuit and or potential diffusion of contaminants from the dialysate into the blood. However, assessing this effect specifically is highly complex (Jacobs et al. 2004; Jofré et al. 2006; Panichi et al. 2000; Tzanatos et al. 2000). Given the sensitivity and challenges inherent in cytokine analysis in patients undergoing hemodialysis, conflicting results both supporting this premise (Caglar et al. 2002; Rysz et al. 2006; Cilan et al. 2012; Ghobrial et al. 2013; Sági et al. 2020) and opposing it have been published in humans (Grooteman et al. 1997; Tarakçioğlu et al. 2003; Dheda et al. 2022).

The results of this study demonstrated that IH did not alter the serum concentrations of IL-1β, TNF-α, and IL-6 in CKD dogs in uremic syndrome, neither during nor after the procedure. Additionally, when the cytokine ratio assessment was applied, the results again revealed an absence of an increase or decrease in these mediators throughout the study. In contrast to these findings, which suggest inflammation in CKD dogs undergoing IH, Picelli de Azevedo et al. (2022) reported an increase in CRP in their studied animals. However, the lack of significant changes in cytokines throughout our assessments does not necessarily indicate the absence of an additional inflammatory process, as the results may have been impacted by cytokine production speed, release, and disappearance (Tarakçioğlu et al. 2003; Song et al. 2012), as well as receptor expression, potentially rendering cytokines unavailable for quantification (Tarakçioğlu et al. 2003). It's also important to note that in the study by Picelli de Azevedo et al. (2022), the authors evaluated the effect of at least three hemodialysis sessions, unlike our methodology which involved one single session.

It is crucial to emphasize that the inflammatory response to a stimulus is influenced by individual and genetic factors that modulate its presentation (Girndt et al. 2001; Tarakçioğlu et al. 2003). Additionally, there is no linear activation of monocytes, making it possible for different responses to occur in the same organism even when subjected to identical hemodialysis approaches (Jofré et al. 2006). This fact corroborates with the results presented here, as each evaluation group exhibited significant variation in cytokine concentrations according to the individual, justifying the wide intervals found within the groups.

Interpreting cytokine concentrations still lacks a control group or a baseline point in each experimental design, mainly because specific reference ranges have not been described. Thus, a control group provides a basis for comparison to highlight changes

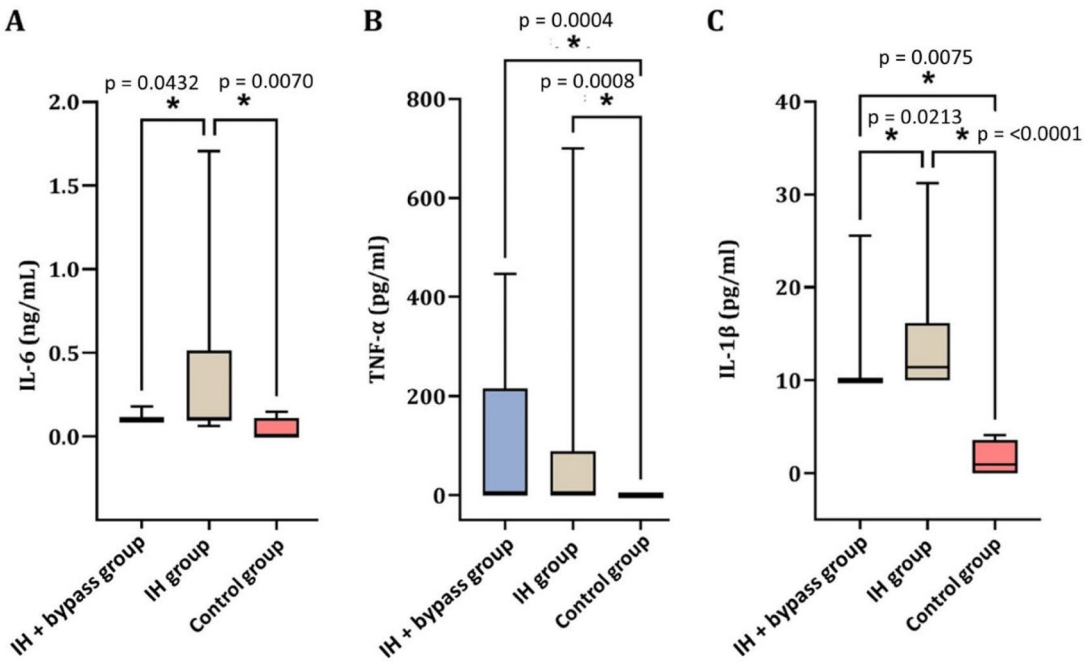

Figure 6. Boxplots illustrating the overall serum cytokine concentrations among groups. The boxplots depict the median (line within the box), first and third quartiles (top and bottom of the box), and range (whiskers). (A) Serum concentration of IL-6 (ng/mL). (B) Serum concentration of TNF-α (pg/mL). (C) serum concentration of IL-1β (pg/mL). Intermittent hemodialysis group with bypass (IH + bypass group); Intermittent hemodialysis group without bypass (IH group); control group (CG). * Indicates significant difference ($p < 0.05$).

arising from the studied condition or treatment (Caglar et al. 2002; Kostic et al. 2019; Dheda et al. 2022; Ren et al. 2023). Therefore, the results of the comparison between moments within the hemodialysis groups (IH group and IH + bypass group) and CG in our study suggest a potential inflammatory increase in the IH group in the post-session, as indicated by IL-1β. However, this increase was subtle enough to not be different from other moments within the group. IL-1β is one of the first cytokines to be released after stimulation, triggering the release of various others, such as IL-6 (Ceciliani et al., 2002; Medzhitov 2008; Soller et al. 2007). This kinetic profile likely justifies the observed IL-1β increase and aligns with data from other studies that noted an increase in IL-1β after a hemodialysis session but not in IL-6 (Tzanatos et al. 2000 and Goldstein et al. 2003).

Given the absence of differences in cytokine concentration and ratio over time for both treated groups (IH group and IH + bypass group), the overall assessment of cytokine concentrations among the three studied groups isolated the potential effect of each hemodialysis approach on the results. The inflammatory potential of CKD dogs was then highlighted by the higher concentration of IL-1β and TNF-α in both treated groups, with the IH group also presenting higher concentration of IL-6. It is well known that kidney dysfunctions, including CKD, are inflammatory conditions affecting the organism. Studies in dogs have shown increased cytokine concentration (Tavener et al. 2022) and mRNA expression of pro-inflammatory cytokines (Nentwig et al. 2016), as well as other inflammatory mediators (Raila et al. 2011), which supports our results.

Although the hemodialysis approach using bypass was an adaptation of previously described prescriptions (Dufayet and Cowgill 2021), it proved to be similar to the non-bypass mode in terms of blood flow, session duration, and final URR, rendering it equally effective. Consequently, the lack of difference in blood flow between the groups and the lower body weight attributed to the bypass group could explain the significant increase in blood flow rate (mL/kg/h) in this group. In this context, it can be postulated that a larger blood volume, proportional to body weight, was in extracorporeal circulation in this group during the same duration of the procedure. Interestingly, beyond the higher concentrations of IL-1β and IL-6 in the IH group compared to CG, this result was also observed when comparing IH group and IH + bypass group, suggesting a higher inflammatory stimulus in animals not subjected to bypass during the dialytic procedure. This finding is highlighted, as blood contact with the extracorporeal circuit is a major stimulus for inflammation during hemodialysis (Memoli 1999). The hypothesis would be that differences between hemodialysis approaches would reveal higher cytokine concentrations in the IH + bypass group, as described earlier. However, the obtained result dismisses this hypothesis.

Therefore, considering that the primary difference between the approaches is that in the IH without bypass, no interruption of dialysate flow occurs, resulting in effective dialysis throughout the treatment duration (Dufayet and Cowgill 2021). A possible explanation for this result could involve the dialysate itself, as it represents a potential source of inflammation during hemodialysis (Suzuki et al.

2011). Even with the use of ultrapure water and consequently markedly lower levels of endotoxins and bacteria, the dialysate is still not a sterile solution (IV. 1 2002). Moreover, while the use of ultrapure dialysate might reduce inflammatory activation during hemodialysis (Arizono et al. 2004; Susantitaphong et al. 2013), it does not entirely prevent activation of inflammation (Bommer and Jaber 2006; Lamas et al. 2006). For this reason, to the best of the author's knowledge, this is the first study to compare the inflammatory profile of dogs undergoing two IH approaches. It is possible to speculate that maintaining dialysate flow throughout the session might enhance the potential diffusion of endotoxins and bacteria present in the dialysate solution into the blood, thereby impacting increased cellular activation and cytokine release.

Although our results are novel and thought-provoking, the interpretation should take into consideration potential limitations of the study. The small sample size, despite statistical power considerations, might have contributed to variations being missed in cytokine analysis. Additionally, the heterogeneous population, including initial disease severity, might also have influenced the results. Another issue involves the absence of a group composed of dogs with CKD that did not undergo hemodialysis, which could provide more specific comparative results. It should also be noted that one of the animals in this study used a high-flux hemodialyzer, which features an advanced polysulfone membrane. While it is not possible to determine the actual influence of these characteristics on the study's results, no discrepant values of individual cytokine concentrations were observed in this animal compared to the other animals in the group. Finally, emphasize that this study only represents the acute effects of the procedure is important, as only the first hemodialysis session was evaluated. Some methodological strengths of this study include the use of a well-accepted and validated technique and assay for the species, evaluation of three important pro-inflammatory cytokines, and sampling at multiple time points.

5. Conclusion

Our findings suggest that a single session of IH, with or without bypass, does not have a significant additional effect on inflammation in CKD dogs in uremic syndrome based on pro-inflammatory cytokine concentrations. However, the extracorporeal procedure contributes to the maintenance of the inflammatory state in patients. Furthermore, dogs undergoing IH without bypass tend to receive a stronger stimulus for the release of the studied cytokines. Moreover, both IH approaches are safe and effective.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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