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Tibial plateau levelling osteotomy: significance of matrix metalloproteinases in long-term monitoring of canine stifle stabilization after cranial cruciate ligament rupture

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Abstract

Cranial cruciate ligament rupture is one of the most common causes of osteoarthritis in dogs. Surgical stabilization is obligatory and tibial plateau levelling osteotomy (TPLO) is the most commonly used surgical technique. Studies on the long-term monitoring of matrix metalloproteinases (MMPs) and acute-phase proteins are limited, especially those with parallel monitoring of changes in the serum, synovial fluid (SF) and urine. We aimed to describe long-term changes in 1) MMPs: gelatinases and caseinases, 2) APPs: ceruloplasmin, haptoglobin and paraoxonase-1, and 3) the correlation of MMPs in the serum, SF and urine with lameness in dogs 2 and 6 months undergoing TPLO. From 17 dogs diagnosed with cranial cruciate ligament rupture, sera, SF and urine samples were collected preoperative 2 and 6 months after the surgery. Relative activity (RA) of MMPs was measured in all samples using zymography. Acute-phase proteins were measured in the serum and SF using spectrophotometry and agarose gel electrophoresis. Relative activity of MMPs in serum was not change at different sampling points. In SF, a marked decrease in MMPs RA was evident, however, only RA of caseinases was significantly reduced during the recovery period. In urine, RA of caseinases was positively correlated with the lameness score with decreasing activity trend during time. Serum and synovial acute-phase proteins were not changed after surgery, which was consistent with the agarose gel analyses. Decreased activity of caseinases pointed to reduced degradation of extracellular matrix after TPLO. Synovial and urine caseinases are potential biomarkers in predicting the recovery outcome following stifle stabilization.

Introduction

Cranial cruciate ligament rupture (CCLR) is the leading cause of hind limb lameness and one of the most frequently diagnosed orthopedic disorders in dogs.[1] It is characterized by knee joint instability, pain, lameness and discomfort for the dog. In most affected dogs, CCLR is a result of gradual degeneration of the ligament. Eventually, progressive osteoarthritis (OA), a common consequence of CCLR, is inevitable. Rupture of the medial meniscus could also follow. Surgery is the method of choice in the treatment of CCLR. One of the preferred surgical techniques is tibial plateau levelling osteotomy (TPLO).[1] When compared to extracapsular lateral suture stabilization procedures, TPLO results in significantly greater limb function following surgery and greater client satisfaction.[2,3] Moreover, one year after surgery, TPLO can result in limb function equivalent to that in normal dog population as reported by Nelson *et al*.[3]

The pathophysiological mechanism of OA in general is not fully understood, however, it is known that matrix metalloproteinases (MMPs) play an important role. Furthermore, one of the major acute-phase proteins (APPs), serum amyloid A and C-reactive protein in synovial fluid (SF) have shown to increase in dogs with joint disorders.[4,5] Their role in the pathophysiology of OA remains elusive.

Matrix metalloproteinases are a group of endo-peptidases that regulate the cell matrix composition and are also involved in extracellular matrix degradation.[6] They take part in both physiological and pathological processes such as tissue reconstitution, wound healing, inflammation and cancer.[7] These proteases regulate inflammatory mediators and significantly impact the

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progressive development of CCLR. Increased expression of MMP-2 and MMP-9 in joint tissue and SF has been reported in dogs with CCLR.[8] Caseinolytic MMP-3 activity in SF seems to be a sensitive marker for the local joint inflammation in dogs with CCLR due to its positive correlation with lameness.[9] Apart from the MMPs, APPs provide early detection of inflammation especially when clinical signs are absent.[10] In veterinary medicine, the determination of APPs levels has been shown to be useful in monitoring postoperative complications.[11] There are very limited long-term studies of the activity of MMPs in CCL-deficient dogs after surgery.[12] Data on potential biomarkers in predicting the recovery outcome following stifle stabilization in dogs with CCLR are also scarce. Therefore, we aimed to describe long-term changes in 1) MMPs: gelatinases and caseinases, 2) APPs: ceruloplasmin (CP), haptoglobin (HPT) and paraoxonase-1 (PON-1) in the serum, SF and urine, and 3) the correlation of MMPs in the serum, SF and urine with lameness, 2 and 6 months after surgical stabilization of CCLR in dogs which underwent TPLO.

Materials and Methods

Animals. From 2020 to 2021, 17 client-owned dogs, were referred to the Teaching Hospital at the Faculty of Veterinary Medicine, University of Belgrade, Serbia, with CCLR which was manifested as chronic lameness (2 - 8 weeks), various volumes of joint effusion, pain at stifle palpation, medial peri-articular fibrosis and muscle atrophy. The diagnosis of CCLR was reached based on clinical and orthopedic examination, radiographic assessment of the joints, and SF examination, and was confirmed during the surgery. Dog owners signed informed consent that the residual samples and the obtained results could be used for scientific purposes. The research was approved by the Ethical Committee at the Faculty of Veterinary Medicine, University of Belgrade, Serbia, and based on the Serbian Law of Animal Welfare, permission was acquired from the Ministry of Agriculture, Forestry and Water Management, Republic of Serbia (permission number: 323-07-03667/2021-05/1).

Radiological examination, anesthesia, analgesia, and surgery. Pre- and postoperative mediolateral radiographs of the stifle joint affected by CCLR were done according to standard radiological procedures.[13] Premedication included intramuscular (IM) 0.01 mg kg^{-1} medetomidine (Genera, Zagreb, Croatia) in combination with 0.20 mg kg^{-1} butorphanol (Richter Pharma AG, Wels, Austria). Intramuscular Meloxicam at the dose of 0.20 mg kg^{-1} (Boehringer Ingelheim, Ingelheim, Germany) was administered for analgesia before surgery. Anesthesia was induced with intravenous injection of 1.00 - 2.00 mg kg^{-1} propofol (Fresenius Kabi GmbH, Graz, Austria) and maintained on 3.00% sevoflurane (Sevorane®, Abbvie S.R.L. Italy) inhalation in oxygen/air. Fentanyl was used in continuous rate infusion (5.00 μg kg^{-1}, q 1 hr; Piramal Critical Care B.V., Voorschoten, The Netherlands) intra-operatively and ceftriaxone (33.00 mg kg^{-1}; Galenika AD, Beograd, Serbia) was also administered intramuscularly. All dogs with CCLR underwent TPLO surgery according to the procedure described by Slocum and Slocum, and performed by the same small animal surgeon.[14]

Laboratory analysis of blood, SF, and urine samples. Preoperatively (day 0, zero point), blood was sampled in standard hematology tubes for complete blood count using ProCyte Dx hematology analyzer (Idexx, Westbrook, USA) and biochemistry analyses using Mindray BS-240 biochemical analyzer (Shenzhen, China). Synovial fluid was collected via arthrocentesis and the smears were made and stained with Diff-Quik (Siemens Healthineers, Surrey, UK) for cytological analyses, and determination of total nucleated cell count was performed. Total proteins in SF were determined using refractometer. Urine was sampled via cystocentesis. Follow-up samples (blood, SF and urine) were taken during postoperative assessments after 2 and 6 months. The rest of the serum, SF and urine were centrifuged and the supernatants were stored at – 20.00 ˚C until use.

Zymography and agarose gel electrophoresis. Caseinolytic and gelatinolytic activity of MMPs was determined in serum, SF and urine as described previously by Spariosu *et al*.[15] Samples of SF were diluted 20.00 - fold in the loading buffer (1.00 M Tris, HCl to pH 7.50) for 2 hr at 37.00 ˚C. Serum samples were diluted 5.00 - fold in saline and urine samples were not diluted. Using 1.00 % agarose gel electrophoresis, serum and SF proteins were resolved to identify albumin, α1-, α2-, β- and γ- globulins.[16]

Acute-phase proteins. Acute phase proteins were determined in serum and SF. The concentration of CP was determined by its p-phenylenediamine oxidase activity[17] on BioTek ELx800 microplate reader (Agilent Technologies, Santa Clara, USA). Peroxidase activity of HPT-hemoglobin complex adapted to the microtiter plates was used for HPT determination following Owen *et al.*, with minor modifications.[18] Paraoxonase-1 activity was evaluated using 4-nitrophenil acetate as a substrate[19] using Cecil CE 2021 UV/VIS spectrophotometer (Select Science, Bath, UK).

Statistical analysis. Values for protein fractions were expressed as mean ± SD. Relative activity (RA) of caseinases, gelatinases and levels of CP, HPT, and PON-1 were presented with boxplots. Middle point in the boxplot represented the mean, while box and whisker represented one and two standard deviations, respectively. Normality of the distribution was tested with the Shapiro-Wilk test. Significances of the differences between measurement points were tested with the Kruskal-Wallis and Tukey post-hoc tests. The Kendall rank correlation coefficient was used to test the association of study variables with the

lameness score (ordinal variable). Statistically significant results were considered at the level of $p < 0.05$. Probabilities for the values between 0.05 and 0.10 were classified as tending to be significantly different. All analyses were performed using statistical program Tibco Statistica™ (version 14.0.0; Tibco Software Inc., Santa Clara, USA).

Results

Out of 17 dogs that were included in this study, 5 (29.41 %) were males and 12 (70.58 %) were females. The average body mass was 21.66 ± 14.04 kg and the average age was 5.59 ± 3.10 years. No long-term changes in serum albumin and globulins were detected by agarose gel electrophoresis (Table 1, Fig. 1A). In the SF, albumin concentration tended to increase during study period with a significant difference between zero point (before surgery) and 6 months after surgery (Table 1, Fig. 1B). In the SF, no significance was detected between globulin fractions concentrations during study period (Table 1, Fig. 1B). In the serum, there were no changes in the RA of gelatinases (Table 2, Fig. 2A) and caseinases (Table 2, Fig. 2B). In contrast, in SF there were no changes noted in gelatinase activity (Table 2, Fig. 3A), only a significant decrease in caseinases RA was detected (Table 2, Fig. 3B). There were no significant differences in RA of MMPs in the urine, however, there was a clearly noticeable upward trend in gelatinase activity (Table 2, Fig. 4A) and a downward trend in caseinase activity (Table 2, Fig. 4B). The differences between preoperative and postoperative APPs concentrations in the serum and SF were not significant ($p > 0.05$; Table 3). A positive moderate correlation was observed between caseinase RA in the urine and lameness score before the surgery (zero point). Tendency towards significance was observed for correlation between serum caseinase RA and lameness scores, and between gelatinase RA in the urine and lameness scores (Table 4).

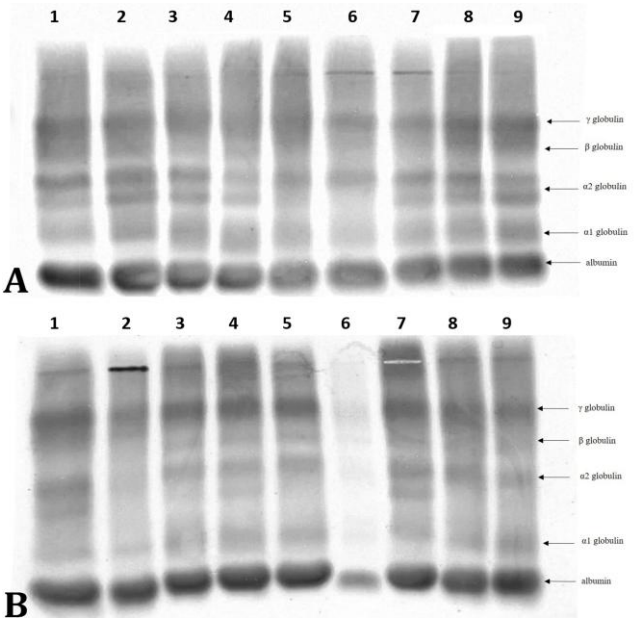

Fig. 1. Representative agarose gel electrophoresis performed from **A)** serum and **B)** synovial fluid samples. Lane 1: Sample 1 six months postoperatively, Lane 2: Sample 2 six months postoperatively, Lane 3: Sample 3 day 0 (zero point) – preoperatively, Lane 4: Sample 3 two months postoperatively, Lane 5: Sample 3 six months postoperatively, Lane 6: Sample 4 day 0 (zero point) – preoperatively, Lane 7: Sample 4 six months postoperatively, Lane 8: Sample 5 two months postoperatively, Lane 9: Sample 5 six months postoperatively

Table 1. Mean and standard deviation of albumin and globulin fractions in canine serum and synovial fluid.

Sources	Parameters	Zero point	2 months	6 months
Serum	Albumin (g L^{-1})	17.02 ± 4.45	16.78 ± 2.69	18.42 ± 2.31
	α1-globulin (g L^{-1})	11.29 ± 2.25	11.70 ± 2.43	9.70 ± 1.81
	α2-globulin (g L^{-1})	9.45 ± 2.04	9.62 ± 3.64	9.77 ± 1.53
	β-globulin (g L^{-1})	15.81 ± 2.65	16.25 ± 2.26	14.47 ± 2.07
	γ-globulin (g L^{-1})	13.32 ± 2.04	15.52 ± 3.97	14.20 ± 4.42
Synovial fluid	Albumin (g L^{-1})	9.17 ± 2.18 [a]	11.39 ± 2.96 [ab]	12.70 ± 3.42 [b]
	α1-globulin (g L^{-1})	3.10 ± 1.13	2.99 ± 0.70	2.27 ± 0.60
	α2-globulin (g L^{-1})	5.61 ± 4.99	2.64 ± 1.46	4.04 ± 5.61
	β-globulin (g L^{-1})	7.12 ± 1.49	7.61 ± 2.38	6.96 ± 1.43
	γ-globulin (g L^{-1})	3.11 ± 1.50	3.69 ± 1.26	3.29 ± 1.50

[ab] Means within the same row marked with different superscript letters differ significantly at the level $p < 0.05$.

Table 2. Mean and standard deviation of relative activity (RA) of gelatinase and caseinase in canine serum, synovial fluid and urine.

Sources	Parameters	Zero point	2 months	6 months
Serum	RA gelatinase	30.91 ± 24.64	35.83 ± 25.82	49.00 ± 33.94
	RA caseinase	48.78 ± 33.50	52.87 ± 32.85	77.00 ± 34.28
Synovial fluid	RA gelatinase	23.03 ± 11.91	24.25 ± 12.74	14.30 ± 14.80
	RA caseinase	67.71 ± 56.21 [a]	53.69 ± 34.64 [a]	20.78 ± 14.77 [b]
Urine	RA gelatinase	2.86 ± 1.13	4.02 ± 1.92	4.76 ± 2.78
	RA caseinase	32.63 ± 22.69	24.05 ± 12.66	7.84 ± 4.59

[ab] Means within the same row marked with different superscript letters differ significantly at the level $p < 0.05$.

Table 3. Mean and standard deviation of ceruloplasmin (CP), paraoxonase-1 (PON-1) and haptoglobin (HPT) in canine serum and synovial fluid.

Sources	Parameters	Zero point	2 months	6 months
Serum	CP (mg dL^{-1})	5.67 ± 3.07	4.46 ± 2.79	5.92 ± 2.69
	PON-1 (kU L^{-1})	6.58 ± 2.13	6.67 ± 1.91	7.16 ± 1.69
	HPT (mg dL^{-1})	147.22 ± 40.32	136.07 ± 43.68	147.44 ± 64.27
Synovial fluid	CP (mg dL^{-1})	6.46 ± 3.74	6.51 ± 5.28	6.42 ± 5.39
	PON-1 (kU L^{-1})	3.84 ± 1.40	3.85 ± 1.14	3.34 ± 1.59
	HPT (mg dL^{-1})	98.47 ± 49.75	68.22 ± 33.41	55.78 ± 40.38

No significant differences were noted ($p > 0.05$).

Table 4. Correlation between lameness degree and matrix metalloproteinases relative activity (RA) in serum, synovia and urine.

Variables	Zero point		2 months	
	Correlation coefficient	*p*-value	Correlation coefficient	*p*-value
RA of gelatinase in serum	– 0.39	0.189	– 0.37	0.217
RA of caseinase in serum	– 0.47	0.089	– 0.29	0.336
RA of gelatinase in synovia	0.09	0.771	0.16	0.586
RA of caseinase in synovia	0.31	0.287	0.16	0.573
RA of gelatinase in urine	– 0.54	0.089	0.12	0.687
RA of caseinase in urine	0.57	0.042	0.12	0.677

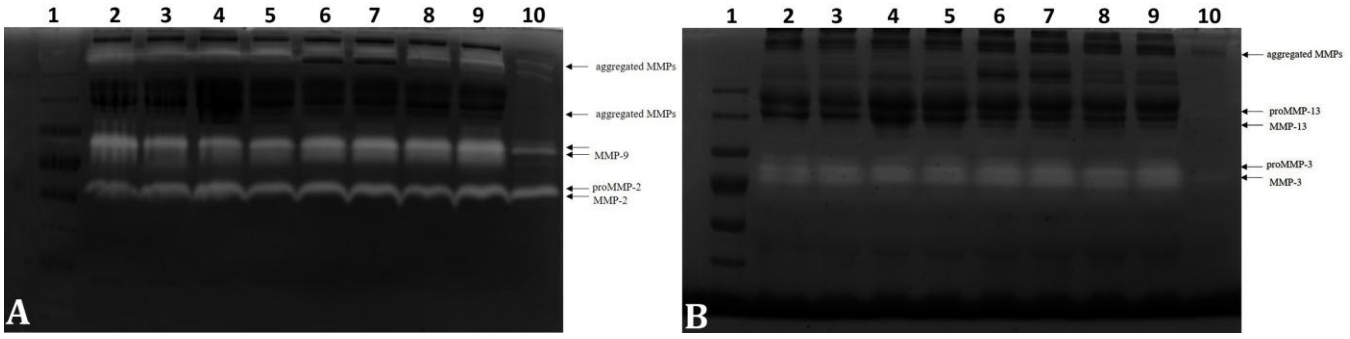

Fig. 2. Representative **A)** gelatin and **B)** casein zymogram performed from serum samples. Lane 1: Perfect™ Color Protein Ladder, Lane 2: Sample 1 day 0 (zero point) – preoperatively, Lane 3: Sample 1 two months postoperatively, Lane 4: Sample 2 day 0 (zero point) – preoperatively, Lane 5: Sample 2 two months postoperatively, Lane 6: Sample 2 six months postoperatively, Lane 7: Sample 3 day 0 (zero point) – preoperatively, Lane 8: Sample 3 two months postoperatively, Lane 9: Sample 3 six months postoperatively, Lane 10: Fetal calf serum, loading control

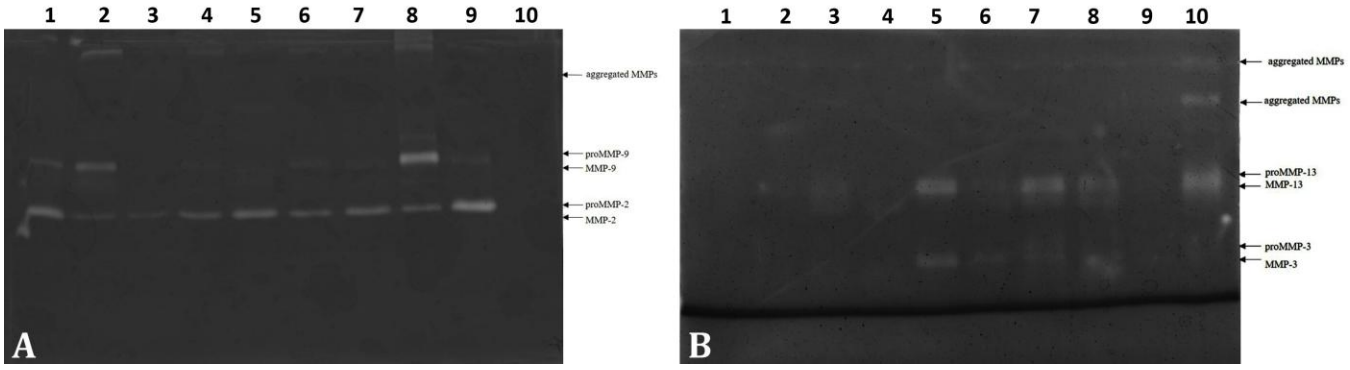

Fig. 3. A) Representative gelatin zymogram performed from synovial fluid samples. Lane 1: Fetal calf serum, loading control, Lane 2: Sample 1 day 0 (zero point) – preoperatively, Lane 3: Sample 1 two months postoperatively, Lane 4: Sample 2 day 0 (zero point) – preoperatively, Lane 5: Sample 2 two months postoperatively, Lane 6: Sample 2 six months postoperatively, Lane 7: Sample 3 day 0 (zero point) – preoperatively, Lane 8: Sample 3 two months postoperatively, Lane 9: Sample 3 six months postoperatively, Lane 10: Sample buffer, loading control. **B)** Representative casein zymogram performed from synovial fluid samples. Lane 1: Sample buffer, loading control, Lane 2: Sample 1 six months postoperatively, Lane 3: Sample 2 six months postoperatively, Lane 4: Sample 3 six months postoperatively, Lane 5: Sample 1 two months postoperatively, Lane 6: Sample 2 two months postoperatively, Lane 7: Sample 3 two months postoperatively, Lane 8: Sample 1 day 0 (zero point) – preoperatively, Lane 9: Sample 2 day 0 (zero point) – preoperatively, Lane 10: Fetal calf serum, loading control.

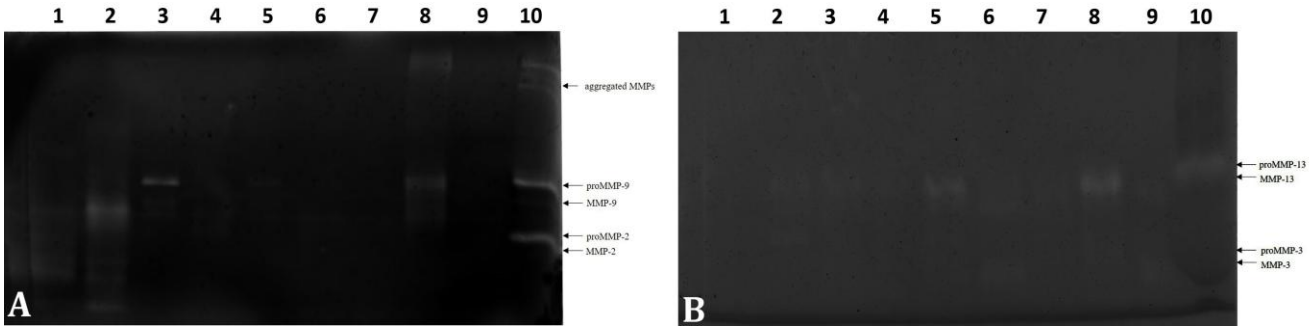

Fig. 4. A) Representative gelatin zymogram performed from urine samples. Lane 1: Sample 1 day 0 (zero point) – preoperatively, Lane 2: Sample 1 two months postoperatively, Lane 3: Sample 1 six months postoperatively, Lane 4: Sample 2 day 0 (zero point) – preoperatively, Lane 5: Sample 2 two months postoperatively, Lane 6: Sample 2 six months postoperatively, Lane 7: Sample 3 day 0 (zero point) – preoperatively, Lane 8: Sample 3 two months postoperatively, Lane 9: Sample 3 six months postoperatively, Lane 10: Fetal calf serum, loading control. **B)** Representative casein zymogram performed from urine samples. Lane 1: Fetal calf serum, loading control, Lane 2: Sample 1 day 0 (zero point) – preoperatively, Lane 3: Sample 1 two months postoperatively, Lane 4: Sample 1 six months postoperatively, Lane 5: Sample 2 day 0 (zero point) – preoperatively, Lane 6: Sample 2 two months postoperatively, Lane 7: Sample 2 six months postoperatively, Lane 8: Sample 3 day 0 (zero point) – preoperatively, Lane 9: Sample 3 two months postoperatively, Lane 10: Sample buffer, loading control.

Discussion

Joint trauma can cause arthritis which results in a complex immune response reflected by changes in the number of immune cells and protein concentration in the serum and synovia.[20] We aimed to describe how the levels of MMPs and APPs in the serum, SF and urine were influenced by surgery during the postoperative period of 6 months. Research into the inflammatory responses in human orthopedic injury showed that markers of inflammation were anticipated and were indicators of progressive changes in tissue structures following joint injury and during recovery, however, in veterinary medicine, data on this topic are very scarce.[21] We found that, in this research, preoperative serum CP, HPT and PON-1 in dogs with CCLR were not significantly altered compared to postoperative ones which were confirmed by the analysis of serum protein fractions. This result was consistent with previous finding in dogs following surgery.[22] The absence of APPs variation in serum throughout the recovery period indicated that there was no systemic inflammatory response after surgery. Also, synovial APPs did not exhibit significant changes 2 and 6 months after surgery in dogs with CCLR. However, the level of synovial albumin, analyzed by electrophoresis, was increased significantly during the postoperative period. Given that albumin is a negative APP, it indicates a decrease in inflammatory response after surgery.[23]

Caseinases (MMP-3 and MMP-13) are involved in the degradation of a wide array of matrix molecules such as collagen types II, IV, IX, laminin, osteonectin and proteoglycans. Matrix metalloproteinase-3 takes a significant part in cartilage matrix destruction.[24] In dogs with CCLR, the activity of MMP-3 and MMP-13 were shown to be higher in comparison with healthy dogs.[8,9] In this study, a significantly decreased activity of synovial caseinase was evident during the recovery from TPLO. Therefore, the overall activity of caseinases was on a downward trend after surgery. The obtained results indicated that stabilization of CCL contributed to reduced activity of caseinases. In addition, MMP-3 production was upregulated by the pro-inflammatory cytokines (interleukin (IL)-1β, tumor necrosis factor, interferon-γ, and IL-17A). In agreement with our findings, Fujita *et al*. found decreased inflammatory cytokine activities in SF in CCL-deficient dogs during the postoperative period of 6 months.[12] Meanwhile, a non-significant increase in the activity of postoperative caseinases in the serum was noticed. A similar response was remarked with respect to gelatinases activity. This was consistence with the results obtained in a study by Garner *et al*., who reported increased MMP-2 and MMP-3 8 to 12 weeks after stabilization in dogs with CCLR.[25]

Gelatinases are capable of degrading collagen, proteoglycans and aggrecan in the cartilage matrix.[6] Matrix metalloproteinase-9 is triggered in response to tissue injury and inflammation. Results of previous studies indicated that gelatinases activity in SF was high in CCL-deficient joints compared to the control.[26,27] In our study, the activity of synovial gelatinases was declined insignificantly 6 months after the surgery in comparison with that before TPLO. In dogs that underwent surgical stabilization, Malek *et al*. reported significant decreases in synovial biomarkers (IL-8, KC, and MCP-1) in the postoperative period (1 and 3 months) which indicated an impaired inflammatory response after the surgery.[28] The same researchers evaluated MMPs response in the serum and found that only MMP-2 had 2.40 - fold higher activity 1-month post-treatment.[28] We could only speculate on how the response would have differed, had it been monitored in SF.

Alpha-2-globulin has the ability to selectively neutralize only active protease and, in addition, it removes damaged extracellular proteins.[29]

One of the main strengths of this research is monitoring the MMPs in the urine of CCL-deficient dogs during recovery. The advantage of urine analysis is that, unlike SF, it can be collected using a non-invasive procedure. Urine biomarkers may reflect cartilage activity at the bone cartilage interface.[30] To the best of our knowledge, the activity of gelatinases and caseinases in urine has not been reported in dogs with CCLR following surgery. According to Wigner *et al*., MMP-9 and MMP-13 in urine could be potential markers to monitor the progression of fracture healing.[31] In urine MMPs analysis, non-significant changes were observed between the sampling points. However, an interesting pattern of MMPs activity after the stabilization of CCLR was noticed. In particular, caseinases in urine tended to decline over the recovery period, whereas, gelatinases had the reverse pattern. Since urine caseinases follow a pattern that is similar to that of SF caseinases, it can be said that the former represents the response to the surgery better than serum caseinases do. This claim is supported by our observation that there is a positive correlation between the degree of lameness and the activity of caseinases in urine. Thus, the response of MMPs, especially caseinases in SF and urine, pointed to reduced degradation of extra-cellular matrix after stabilization by TPLO, however, the results were not clearly expressed. It is important to note that our long-term study was conducted on a group of dogs of various breeds, some of which, but not all, were predisposed to CCLR. We assumed that this heterogeneity resulted in the high range of MMPs dispersion which could have affected the final results.

In conclusion, the activities of synovial caseinases and gelatinases are potential biomarkers in predicting the recovery outcome following stifle stabilization by TPLO technique. Urine caseinases are a promising biomarker in monitoring response to surgery and warrant further investigation into clinical cases of CCL-deficient dogs.

Acknowledgments

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Conflict of interest

The authors declare that they do not have any financial or personal conflicts of interest that could bias the study.

References

1. Nečas A, Zatloukal J, Kecová H, et al. Predisposition of dog breeds to rupture of the cranial cruciate ligament. Acta Vet Brno 2000; 69(4): 305-310.
2. Gordon-Evans WJ, Griffon DJ, Bubb C, et al. Comparison of lateral fabellar suture and tibial plateau leveling osteotomy techniques for treatment of dogs with cranial cruciate ligament disease. J Am Vet Med Assoc 2013; 243(5): 675-680.
3. Nelson SA, Krotscheck U, Rawlinson J, et al. Long-term functional outcome of tibial plateau leveling osteotomy versus extracapsular repair in a heterogeneous population of dogs. Vet Surg 2013; 42(1): 38-50.
4. Kjelgaard-Hansen M, Christensen MB, Lee MH, et al. Serum amyloid A isoforms in serum and synovial fluid from spontaneously diseased dogs with joint diseases or other conditions. Vet Immunol Immunopathol 2007; 117(3-4): 296-301.
5. Boal S, Miguel Carreira L. Serum and synovial fluid C-reactive protein level variations in dogs with degenerative joint disease and their relationships with physiological parameters. Vet Res Commun 2015; 39(3): 163-169.
6. Cabral-Pacheco GA, Garza-Veloz I, Castruita-De la Rosa C, et al. The roles of matrix metalloproteinases and their inhibitors in human diseases. Int J Mol Sci 2020; 21(24): 9739. doi: 10.3390/ijms21249739.
7. Mustafa S, Koran S, AlOmair L. Insights into the role of matrix metalloproteinases in cancer and its various therapeutic aspects: a review. Front Mol Biosci 2022; 9: 896099. doi: 10.3389/fmolb.2022.896099.
8. Muir P, Danova NA, Argyle DJ, et al. Collagenolytic protease expression in cranial cruciate ligament and stifle synovial fluid in dogs with cranial cruciate ligament rupture. Vet Surg 2005; 34(5): 482-490.
9. Fujita Y, Hara Y, Nezu Y, et al. Proinflammatory cytokine activities, matrix metalloproteinase-3 activity, and sulfated glycosaminoglycan content in synovial fluid of dogs with naturally acquired cranial cruciate ligament rupture. Vet Surg 2006; 35(4): 369-376.
10. Alves JC, Santos A, Jorge P, et al. The influence of IL-1 and C-reactive protein levels in synovial fluid of companion dogs with bilateral hip osteoarthritis on various clinical disease parameters. Am J Vet Res 2022; 83(11): 1-10.
11. Löfqvist K, Kjelgaard-Hansen M, Nielsen MBM. Usefulness of C-reactive protein and serum amyloid A in early detection of postoperative infectious complications to tibial plateau leveling osteotomy in dogs. Acta Vet Scand 2018; 60(1): 30. doi: 10.1186/s13028-018-0385-5.
12. Fujita Y, Hara Y, Nezu Y, et al. Biomarkers in dogs surgically treated for ruptured cranial cruciate ligaments. Vet Rec 2012; 171(17): 426. doi: 10.1136/vr.100801.

13. Coulson A, Lewis N. An Atlas of interpretative radiographic anatomy of the dog and cat. 2nd ed. Iowa, USA: Blackwell Publishing 2008; 78-83.
14. Slocum B, Slocum TD. Tibial plateau levelling osteotomy for repair of cranial cruciate ligament rupture in the canine. Vet Clin North Am Small Anim Pract 1993; 23(4): 777-795.
15. Spariosu K, Janjić F, Radaković M, et al. Low serum levels of promatrix metalloproteinase-2 and -9 occur during acute *Babesia canis* infection in dogs. Vet Parasitol 2021; 300: 109612. doi: 10.1016/j.vetpar. 2021.109612.
16. Milanović Z, Ilić A, Andrić JF, et al. Acute-phase response in *Babesia canis* and *Dirofilaria immitis* co-infections in dogs. Ticks Tick Borne Dis 2017; 8(6): 907-914.
17. Sunderman FW Jr, Nomoto S. Measurement of human serum ceruloplasmin by its p-phenylenediamine oxidase activity. Clin Chem 1970; 16(11): 903-910.
18. Owen JA, Better FC, Hoban J. A simple method for the determination of serum haptoglobins. J Clin Pathol 1960; 13(2): 163-164.
19. Dantoine TF, Debord J, Charmes JP, et al. Decrease of serum paraoxonase activity in chronic renal failure. J Am Soc Nephrol 1998; 9(11): 2082-2088.
20. Gautreaux MA, Tucker LJ, Person XJ, et al. Review of immunological plasma markers for longitudinal analysis of inflammation and infection in rat models. J Orthop Res 2022; 40(6): 1251-1262.
21. Markus DH, Hurley ET, Mojica ES, et al. Concentration of synovial fluid biomarkers on the day of anterior cruciate ligament (ACL)-reconstruction predict size and depth of cartilage lesions on 5-year follow-up. Knee Surg Sports Traumatol Arthrosc 2023; 31(5): 1753-1760.
22. Schmidt EMS, Rubio CP, Thomas F, et al. Acute phase proteins in bitches subjected to conventional and minimally invasive ovariohysterectomy. Pesq Vet Bras 2018; 38(11): 2124-2128.
23. Shahid M, Manchi G, Brunnberg L, et al. Use of proteomic analysis to determine the protein constituents of synovial fluid samples from the stifle joints of dogs with and without osteoarthritis secondary to cranial cruciate ligament rupture. Am J Vet Res 2018; 79(4): 397-403.
24. Evans-Pickett A, Longobardi L, Spang JT, et al. Synovial fluid concentrations of matrix metalloproteinase-3 and interluekin-6 following anterior cruciate ligament injury associate with gait biomechanics 6 months following reconstruction. Osteoarthritis Cartilage 2021; 29(7): 1006-1019.
25. Garner BC, Stoker AM, Kuroki K, et al. Using animal models in osteoarthritis biomarker research. J Knee Surg 2011; 24(4): 251-264.
26. Volk SW, Kapatkin AS, Haskins ME, et al. Gelatinase activity in synovial fluid and synovium obtained from healthy and osteoarthritic joints of dogs. Am J Vet Res 2003; 64(10): 1225-1233.
27. Rabillard M, Danger R, Doran IP, et al. Matrix metalloproteinase activity in stifle synovial fluid of cranial cruciate ligament deficient dogs and effect of postoperative doxycycline treatment. Vet J 2012; 193(1): 271-273.
28. Malek S, Weng HY, Martinson SA, et al. Evaluation of serum MMP-2 and MMP-3, synovial fluid IL-8, MCP-1, and KC concentrations as biomarkers of stifle osteo-arthritis associated with naturally occurring cranial cruciate ligament rupture in dogs. PloS One 2020; 15 (11): e0242614. doi: 10.1371/journal.pone.0242614.
29. Vandooren J, Itoh Y. Alpha-2-macroglobulin in inflammation, immunity and infections. Front Immunol 2021; 12: 803244. doi: 10.3389/fimmu.2021.803244.
30. Bay-Jensen AC, Mobasheri A, Thudium CS, et al. Blood and urine biomarkers in osteoarthritis - an update on cartilage associated type II collagen and aggrecan markers. Curr Opin Rheumatol 2022; 34(1): 54-60.
31. Wigner NA, Kulkarni N, Yakavonis M, et al. Urine matrix metalloproteinases (MMPs) as biomarkers for the progression of fracture healing. Injury 2012; 43(3): 274-278.