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Klinik für Pferde
allgemeine Chirurgie und Radiologie
des Fachbereichs Veterinärmedizin
der Freien Universität Berlin

Evidence-based review of efficacy and adverse effects of joint medication and evaluation of synovial fluid and serum markers for osteoarthritis in the horse

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Für meine Familie

Contents

1 Introduction

Osteoarthritis (OA) is one of the most common causes of lameness and poor performance in the horse (Kane et al. 2000; Caron and Genovese 2003). OA refers to a clinical syndrome of joint pain accompanied by varying degrees of functional limitation (National Animal Health Monitoring System 2000). The disease is caused by acute trauma, overload or repetitive stress and is characterised by several pathways of articular degeneration and regeneration. Chronic inflammation of the synovial membrane, progressive cartilage damage, remodelling of the subchondral bone, narrowing of the joint space and proliferation of marginal osteophytes are often associated with progressing OA (McIlwraith 1996; Wieland et al. 2005). Despite intensive ongoing research in the field of human and veterinary medicine, the knowledge about the exact pathogenesis of OA is limited (Carmona and Prades 2009; Bay-Jensen et al. 2010).

The diagnosis of OA is routinely based on physical lameness examination and diagnostic analgesia. Diagnostic imaging methods include radiography, ultrasonography, magnetic resonance imaging (MRI), computed tomography (CT) and gamma scintigraphy. Additional information might be gained by the analysis of synovial fluid or serum and also by arthroscopic examination.

Once the diagnosis of OA is established, a variety of treatment options are available. Therapeutic substances can be classified into symptom-modifying (SMOAD) and disease-modifying (DMOAD) osteoarthritic drugs (Higgins und Lees 1984; Goldberg und Buckwalter 2005; Michon et al. 2010). The intra-articular medication is common practice, since high intra-articular concentrations of the therapeutic agent can be achieved and the risk of systemic side effects can be minimised (Frean and Lees 2000; Dechant et al. 2003; Ungemach 2006).

Evidence-based veterinary medicine is the use of best relevant evidence in conjunction with clinical expertise to make the best possible decision about a veterinary patient (Straus et al. 2005). The aim of the first part of this study was to identify clinically relevant information about the efficacy and adverse effects of commonly used intra-articular joint medications such as corticosteroids, hyaluronic acid and polysulfated glycosaminoglycan, based on a systematic review of currently published *in vitro*- and *in vivo* research.

A recent development in the treatment of OA is the use of autologous, regenerative and innovative preparations to achieve restoration of articular cartilage (Clegg 2012; Innes et al. 2013; Broeckx et al. 2014). The purpose of the second part of this work was to provide the veterinary practitioner with evidence-based information about new inventions in the field of equine joint medication. The review was mainly focused on autologous conditioned serum, platelet-rich plasma, mesenchymal stem cells and gene therapy.

One major goal of research in the field of OA is the early detection of intra-articular pathology. Information about disease activity and the progression of cartilage and subchondral bone damage, or even the prediction of future levels of function and likelihood of the progression of OA, would have a huge impact on the equine industry (Dymock et al. 2014; Jackson et al. 2015).

The interleukin-1 receptor antagonist (IL-1Ra) is of interest since this cytokine occurs naturally in response to joint pathology, and is used clinically as a therapeutic agent for the treatment of OA (Textor 2011). Together with tumour necrosis factor α (TNF-α), interleukin-1β (IL-1β) is one of the most important catabolic cytokines in equine and human OA (Ross et al. 2012; Yang et al. 2015). The third part of this thesis was performed to further evaluate the levels of IL-1Ra and IL-1β in synovial fluid and serum in groups of horses with different grades and types of joint disease using equine-specific antibody ELISA kits to determine whether relevant information regarding disease severity can be obtained.

2 Scientific publications in peer reviewed journals

2.1 Wirkungen und Nebenwirkungen der intraartikulären medikamentellen Therapie beim Pferd – eine Literaturübersicht – Teil 1: Konventionelle intraartikuläre medikamentelle Therapie und Risiken der Gelenkinjektion beim Pferd

Efficacy and adverse effects of joint medication in the horse – A review of the literature – Part 1: Conventional joint medication and the risks involved with joint injection in the horse

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Pferdeheilkunde 29 (2013) 1 (Januar/Februar) 54-64
http://www.hippiatrika.com/download.htm?id=20130108

2.2 Wirkungen und Nebenwirkungen der intraartikulären medikamentellen Therapie beim Pferd – eine Literaturübersicht – Teil 2: Regenerative and innovative intraartikuläre medikamentelle Therapie beim Pferd

Regenerative and innovative joint medication in the horse – Part 2: Efficacy and adverse effects of joint medication in the horse - A review of the literature

Anna Ehrle[1], Anton Fürst[2] und Christoph Lischer[1]
Klinik für Pferde, Allgemeine Chirurgie und Radiologie, Berlin[1] und Departement für Pferde, Vetsuisse-Fakultät Universität Zürich[2]

Pferdeheilkunde 29 (2013) 2 (März/April) 212-218
http://www.hippiatrika.com/download.htm?id=20130208
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2.3 Synovial fluid and serum concentrations of Interleukin-1 receptor antagonist and interleukin-1β in naturally occurring equine osteoarthritis and septic arthritis

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Abstract

The objective of this study was to investigate the concentrations of interleukin-1 receptor antagonist (IL-1Ra) and interleukin-1ß (IL-1ß) in synovial fluid (SF) and serum (SE) of horses with different grades of osteoarthritis (OA) and septic arthritis. Based on SF analysis, radiographic, and arthroscopic scores, 40 horses were classified into three groups as follows: mild OA, advanced OA, and septic arthritis. Horses without orthopedic problems served as a control group. Equine-specific antibody enzyme-linked immunosorbent assays were used to determine the concentration of IL-1Ra and the catabolic cytokine IL-1ß in SF and SE. Results were further compared with levels of the previously described biomarkers C-telopeptide fragments of type II collagen (CTX-II) and myeloperoxidase. In the present study, the SF of healthy joints, those with nonseptic OA, and those with septic arthritis contained significantly different levels of IL-1Ra. Serum concentrations of IL-1Ra were only significantly elevated in horses with septic arthritis when compared with the control group. Different levels of IL-1ß were detected in SE of control horses compared with those with various degrees of joint disease. Synovial fluid concentrations of IL-1ß were only moderately elevated in the groups of horses with joint disease. In addition to lameness examination and standard diagnostic procedures, the determination of IL-1Ra concentration in SF, in combination with further biomarkers, might be useful to assess the extent of intrasynovial inflammation.

Keywords: Equine, Interleukin, C-telopeptide fragments of type II collagen, Myeloperoxidase, Osteoarthritis, Septic arthritis

1. Introduction

Lameness due to joint disease is a major cause of reduced performance in horses [1]. The diagnosis of joint disease is routinely based on physical lameness examination, diagnostic imaging methods, analysis of synovial fluid (SF), and diagnostic arthroscopy. The identification of parameters in SF or serum (SE) to more accurately assess intrasynovial inflammation in cases of joint disease is an active area of research [2,3].

The interleukin-1 receptor antagonist (IL-1Ra) is of particular interest because this cytokine occurs naturally in response to joint pathology, and it is used clinically as a therapeutic agent in osteoarthritis (OA) [4]. IL-1Ra is a competitive inhibitor of the interleukin (IL)-1 receptor. Although binding on the IL-1 receptor, IL-1Ra prevents multiple effects observed during the pathogenesis of OA including prostaglandin E_2 (PGE_2) synthesis in synoviocytes, collagenase production by chondrocytes, and cartilage matrix degradation [5]. Increased levels of IL-1Ra in SF of horses with experimentally induced OA have been detected [6]. The disease modifying effects of autologous conditioned SE, containing IL-1Ra, have been investigated in clinical trials using both equine and human subjects [6,7]. The enzyme-linked immunosorbent assays (ELISAs) that have been used previously for the determination of IL-1Ra concentration in equine SF use mouse or human antibodies [6]. To the authors' knowledge, no previous study of naturally occurring equine joint disease has used an ELISA with equine-specific antibodies for the determination of IL-1Ra.

Together with tumor necrosis factor alpha, IL-1ß is one of the most important catabolic cytokines in equine and human OA [8,9]. Interlekin-1ß mediates joint degradation by stimulating the production of IL-6, IL-8, cyclooxygenase-2, and nitric oxide, as well as matrix metalloproteinases and PGE_2. The influence of IL-1ß leads to the destruction of synoviocytes, cartilage matrix degradation, and osteoclastic bone resorption [10,11]. In dogs with OA due to cranial cruciate ligament rupture, a significant positive correlation has been described between lameness and IL-1β activity in SF [12]. Furthermore, IL-1β activity is significantly increased in dogs with clinical hip dysplasia [13]. In previous studies, it has not been possible to demonstrate a significant difference in IL-1ß levels in equine OA of varying severity [14,15].

Type II collagen and aggrecan comprise most extracellular matrix of articular cartilage. C-telopeptide fragments of type II collagen (CTX-II) are created during articular cartilage breakdown. It has been demonstrated that the concentration of CTX-II in SE, SF, and urine is

significantly higher in horses with joint injuries, compared with preexercise and postexercise findings in uninjured horses [16– 18]. Therefore, the presence of CTX-II was determined as an additional marker to help to confirm the presence of joint disease.

Myeloperoxidase (MPO) is an antibacterial enzyme found in leukocytes that catalyzes the production of hypochlorous acid, which is proteolytic and can destroy hyaluronic acid and proteoglycans in the articular cartilage [19,20].Myeloperoxidase is known to be increased in the SF of humans and dogs with OA [21,22]. Previous equine studies have shown MPO to be a reliable marker for the presence of septic arthritis [23,24]. Myeloperoxidase was determined in this study mainly as a further parameter to confirm the presence of septic arthritis in those horses classified as group 3 (septic arthritis).

The aim of this study was to analyze the levels of IL-1Ra and IL-1β in SF and SE in groups of horses with different grades and types of joint disease using equine-specific antibody ELISA kits to determine whether relevant information regarding disease severity can be obtained.

2. Materials and Methods

2.1. Animals and Samples

Samples of SF and blood were obtained from 40 horses of different breeds, sex, and ages. Of these horses, 33 were presented with joint disease. Seven sound horses served as a control group. The diagnosis of OA was based on a comprehensive orthopedic examination including radiography (33 of 33), ultrasonography (15 of 33), aspiration of SF (33 of 33), and arthroscopy (31 of 33). Horses assigned to the control group underwent orthopedic and radiographic examination before aspiration of SF. Horses showing lameness, positive flexion tests, or radiographic evidence for OA were not included in the control group. None of the affected joints had undergone intraarticular analgesia during the month before SF aspiration. Horses selected for this study were subjected to physical examination and a hematological profile. Any horse displaying clinical signs unrelated to OA was excluded from the study. The horses with joint disease were classified into three groups based on radiographic and arthroscopic findings, as well as analysis of SF samples including nucleated cell count and total protein (TP; Table 1).

2.2. Radiographic Score

All joints used in the study were radiographed before SF sampling. There were 10 categories of radiographic changes that were each graded by two blinded surgeons from 0 to 3 to make up a total radiographic score, with a maximum score of 30. Joint space narrowing, soft tissue swelling/effusion, and subchondral bone opacity were all graded as 0 = none, 1 = mild, 2 = moderate, and 3 = severe. The number of osteophytes and enthesophytes that were present in each joint was determined and graded as 0 = none, 1 = one to two present, 2 = three to four present or 3 = less than four present. The size of the largest osteophyte or enthesiophytewas determined and graded as 0 ¼ none,1 = small (<2 mm), 2 = medium (2 to 4 mm), or 3 = large (>4 mm). Osteochondral (OC) fragments were graded according to the number of fragments present: 0 = none, 1 = 1 fragment, 2 = 2 fragments, 3 = >2 fragments. The size of the largest OC fragment was determined and graded as 0 = none, 1 = small (<3 mm), 2 = medium (3 to 6 mm), or 3 = large (>6 mm) [25].

2.3. Inflammatory Score

The following five parameters of joint inflammation were evaluated at arthroscopic examination: hyperemia, petechiation, thickening of villi and increase in their density formation of new types of villi and rice bodies, atrophy, and flattening with fibrin and adhesion formation. Each parameter was graded as 0 ¼ absent, 1 = mild, 2 = moderate, and 3 = severe and extensive. The sum of the five grades comprised the inflammatory score (0 to 15) [26].

2.4. Cartilage Degeneration Score

The cartilage damage was graded at arthroscopic examination as 0 ¼ normal, 1 = swelling and softening, 2 = superficial fibrillation, 3 = deep fibrillation down to bone, and 4 = exposure of subchondral bone [27,28].

2.5. Classification of Joint Disease

Horses were classified as being in group 1 (mild OA) when they had a radiographic score between 0 and 10, inflammatory score 0 to 5, and a cartilage degeneration score 0 to 2. Horses assigned to group 2 (advanced OA) had a radiographic score between 11 and 30, inflammatory score 6 to 10, and degenerative score 3 to 4. The assignment to group 3 (septic

arthritis) was based on a white blood cell (WBC) >10.000 cells/mL and a TP level >4 g/dL [29] in the SF of the affected joint, as well as a significantly raised MPO level (>5 mU/mL) in SF. The range of the inflammatory score was between 11 and 15 and degenerative score 1 to 4 (Table 1). In two horses with septic arthritis that were euthanized due to financial considerations, inflammatory and degenerative scoring was undertaken at postmortem examination immediately after euthanasia, rather than by arthroscopy. Control samples were taken from seven joints from seven horses (carpus [2], talocrural joint [2], and metacarpophalangeal and tarsophalangeal joints (MCP/T [3]) without any history of lameness or joint disease. The absence of locomotor disease was confirmed by physical examination and radiography. Informed owner consent was obtained, and sample collection was performed after approval of the local ethics committee (0193/12 Landesamt für Gesundheit und Soziales Berlin 12.08.2012). All SF samples from clinical cases were taken either during diagnostic procedures (19 of 33) or before arthroscopy (14 of 33) using strict aseptic technique. Jugular vein blood was sampled for SE analysis. After aspiration, samples were placed in tubes containing ethylenediaminetetraacetic acid for routine analysis (cytologic evaluation, total WBC count, TP concentration, and hematocrit). SF and SE samples were then centrifuged at 2500 g for 10 minutes at 4°C and were frozen within 20 minutes to -80°C and stored until processing.

Table 1
Classification of 33 horses with joint disease using radiographic, inflammatory, and cartilage degeneration scores.

Group	Radiographic Score	Inflammatory Score	Cartilage Degeneration Score	Initial Pathology
Group 1: mild OA (n = 17)	3.53 ± 1.5 (2–8)	1.53 ± 0.24 (0–3)	1.00 ± 0.69 (0–1)	OCD (twelve horses) OA (five horses)
Group 2: advanced OA (n = 9)	18.2 ± 3.9 (12–26)	7.4 ± 0.83 (6–9)	3.1 ± 0.74 (2–4)	OCD (three horses) OA (three horses) Intraarticular fracture (three horses)
Group 3: septic OA (n = 7)	—	13.7 ± 1.57 (11–15)	3.14 ± 0.64 (2–4)	Penetrating wound (four horses) Infection after injection (three horses)

Abbreviations: OA, osteoarthritis; OCD, osteochondrosis dissecans.
The primary pathology of the OA groups was found to be OCD, OA, or intraarticular fractures.

2.6. Equine Enzyme-linked Immunosorbent Assay for IL-1Ra, IL-1ß, and CTX-II

Commercially available ELISA kits using equine antibodies were used for the quantitative determination of IL-1Ra (E90223Eq; USCN Life Science Inc, Houston, TX), IL-1ß (E90563Eq; USCN Life Science Inc), and CTX-II (Pre-Clinical CartiLaps; IDS GmbH, Frankfurt am Main, Germany) in SF and SE. All three kits were sandwich enzyme immunoassays and were processed according to the manufacturer's instructions. The kits for the determination of IL-1Ra and IL-1ß were validated for use on equine SF and SE using standard parallel and serial dilutions. Validation assays generated consistent results in intra- and inter-assay comparison. The intra-assay coefficient of variationwas under 10%, and the inter-assay coefficientwas 12% for IL-1Ra and 13.5% for IL-1ß. The CTX-II ELISA has been validated for use in equine SE and SF samples [30,31]. Each sample was measured in duplicate.

2.7. Myeloperoxidase Activity Assay

Myeloperoxidase activity was assessed according to Kumar et al [32] with additional modifications for equines as described by Fietz et al [23]. A specific modified o-dianisidine assay containing 4-aminobenzoic acid hydrazide (ABAH) as a potent and specific inhibitor of MPO was used. Thirty microliters of each sample were added to the assay mixture containing 0.1% Titron X-100, 0.65 mM of o-dianisidine, and 0.09 M of citrate buffer (pH 5.5). The reaction was started by 0.43 mM H_20_2, and a final volume of 300 mL was dispensed into each well of a microtiter plate. Absorbance was read on a microtiter plate reader (BioRad model 550; Germany) to obtain time 0 value. The increase in absorbance due to the oxidation of 0-dianisidine was then followed in a kinetic study over a time of 30 minutes. Volume enzyme activities were calculated. In parallel, each sample was run additionally with 30 mM of the specific MPO inhibitor ABAH. The remaining volume peroxidase activity was subtracted from the enzyme activity obtained in the absence of ABAH, and the resulting volume MPO activity was expressed as mU/mL of the sample.

2.8. Statistical Analysis

SF and SE levels of IL-1Ra, IL-1β, MPO, and CTX-II were compared between groups 1 to 3 and the control group using the software program SPSS version 18.0. Statistically significant differences in the measured parameters were determined using the Kruskal-Wallis test

followed by Dunn's post hoc test. Spearman's and Pearson's coefficient of correlation were calculated to demonstrate any correlation between parameters. P values <.05 were considered significant.

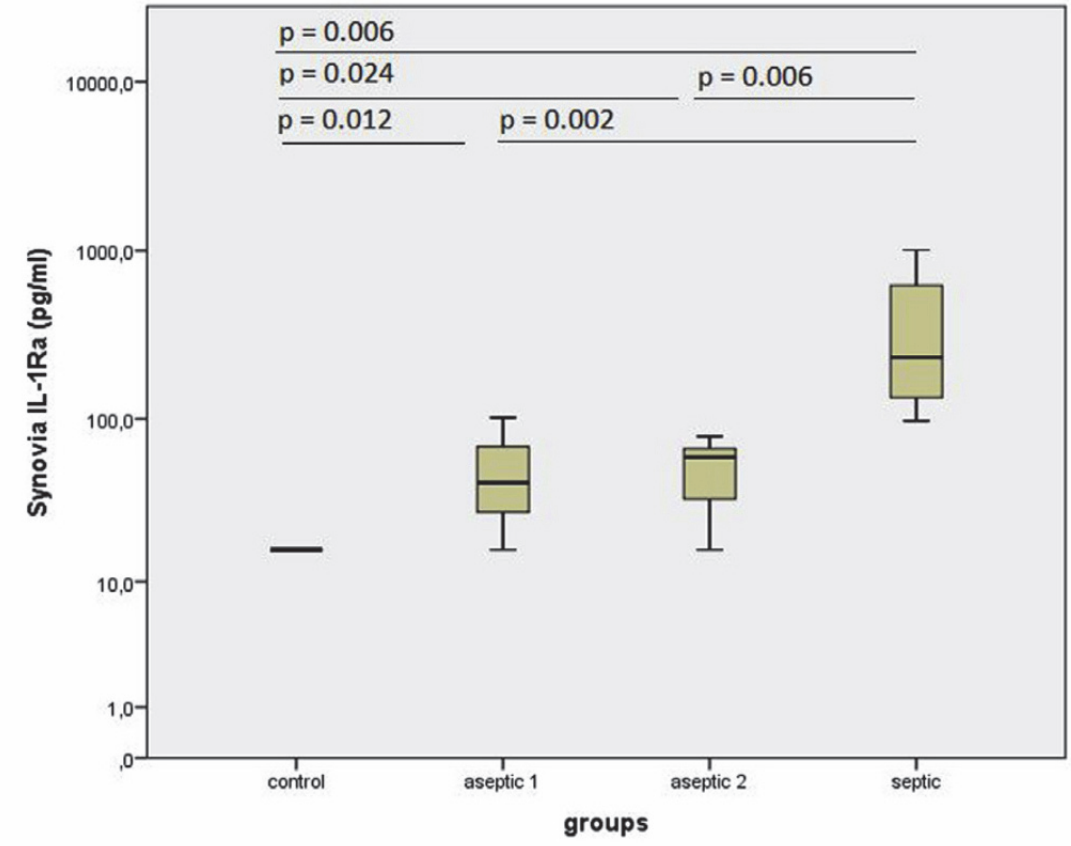
3. Results

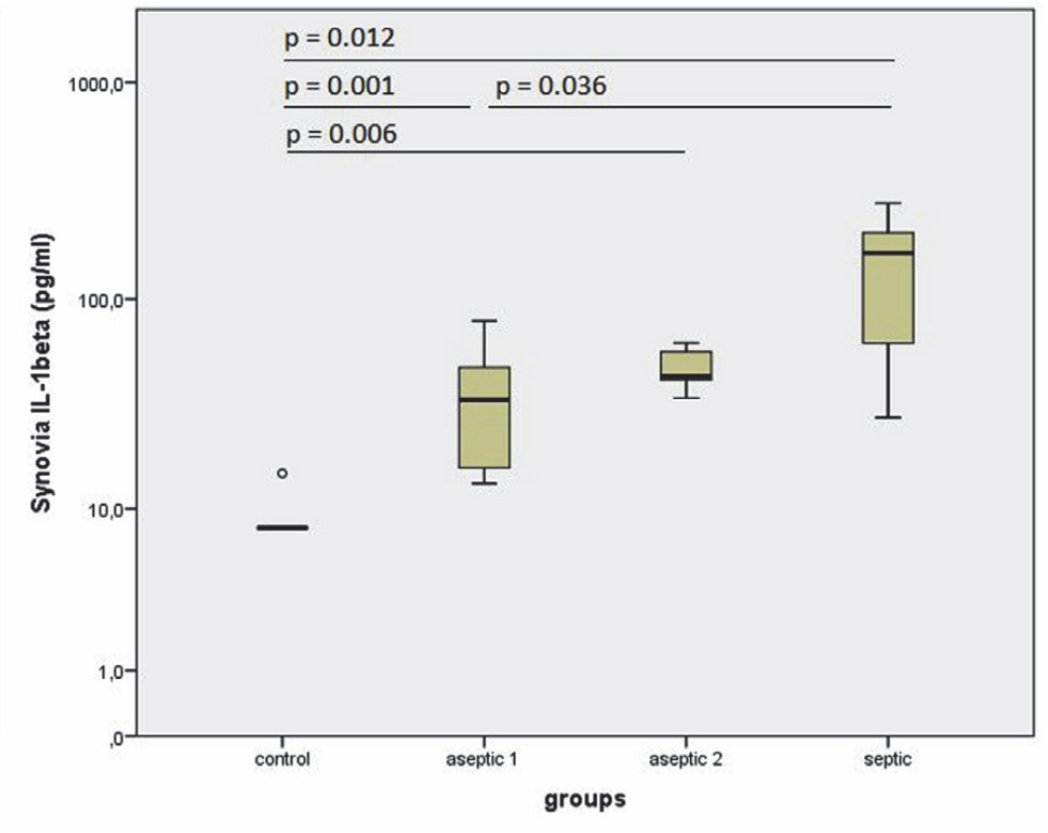
Group 1 (mild OA) comprised 17 horses (5.8 ± 2.5 years), group 2 (advanced OA) nine horses (9.8 ± 2.9 years), and group 3 (septic arthritis) seven horses (7.0 ± 3.9 years; Table 1). The control group consisted of seven sound horses (6.4 ± 2.7 years). Affected joints included carpus (2), talocrural joint (12), MCP/T (13), proximal interphalangeal joint (1), distal interphalangeal joint (3), and femorotibial joint (2). Signs of lameness were of varying duration. Cases of septic arthritis were of acute onset. The intraobserver and interobserver kappa coefficients for the applied scoring system were 0.82 and 0.64, respectively. Concentrations of the different parameters determined are demonstrated in Figs. 1, 2 and Table 2. SF concentrations of IL-1Ra were significantly different between the control group and the horses diagnosed with joint disease: group 1 (P = .012), group 2 (P = .024), and group 3 (P = .006). Furthermore, horses with septic arthritis showed significantly higher IL-1Ra concentrations in SF than horses in group 1 (P = .002) or group 2 (P = .006). Serum concentrations of IL-1Ra were only significantly elevated in horses with septic arthritis when compared with those in the control group (P = .018). In SF, a significant difference in IL-1β was detected between control horses and horses assigned to the diseased groups: group 1 (P = .001), group 2 (P = .006), and group 3 (P = .012). Within the groups 1 to 3, a significant difference in IL-1β concentration could only be found between horses with mild OA (group 1) and septic arthritis (group 3; P = .036). Levels of IL-1β in SE showed a positive correlation with the inflammatory score and were likely to reflect the severity of intrasynovial inflammation (P = .006; r = 0.47). In SE, significantly different concentrations of IL-1β were found between the groups (Fig. 2). A significant difference was detected between the two aseptic OA groups, groups 1 and 2 (P = .012).

Levels of CTX-II in SF were significantly elevated in horses with joint disease when compared with those in the control group: group 1 (P = .003), group 2 (P = .032), and group 3 (P = .007). No significant difference was detected between the different groups of joint disease (group 1 to 3) for CTX-II. Serum CTX-II concentrations were significantly different between the control group compared with those in group 1 (P = .004) and group 3 (P = .004). Serum concentrations of CTX-II in the septic arthritis, group 3 were also significantly

different from the aseptic OA groups: group 1 (P = .016) and group 2 (P = .01). A positive correlation (P = .015; r = 0.51) between the SE levels of CTX-II and the inflammatory score was detected.

Levels of MPO in SF were markedly elevated in cases of septic arthritis when compared with those in the control group (P = .006), group 1 (P = .001) or group 2 (P = .012). Furthermore, SE concentrations of MPO were significantly higher in horses with septic arthritis when compared with those in control horses (P = .018), although there was a large range of levels within the group (Fig. 2). A positive correlation between the inflammatory score and SF (P = .003; r = 0.66) as well as SE (P = .001; r = 0.55) levels of MPO was detected.

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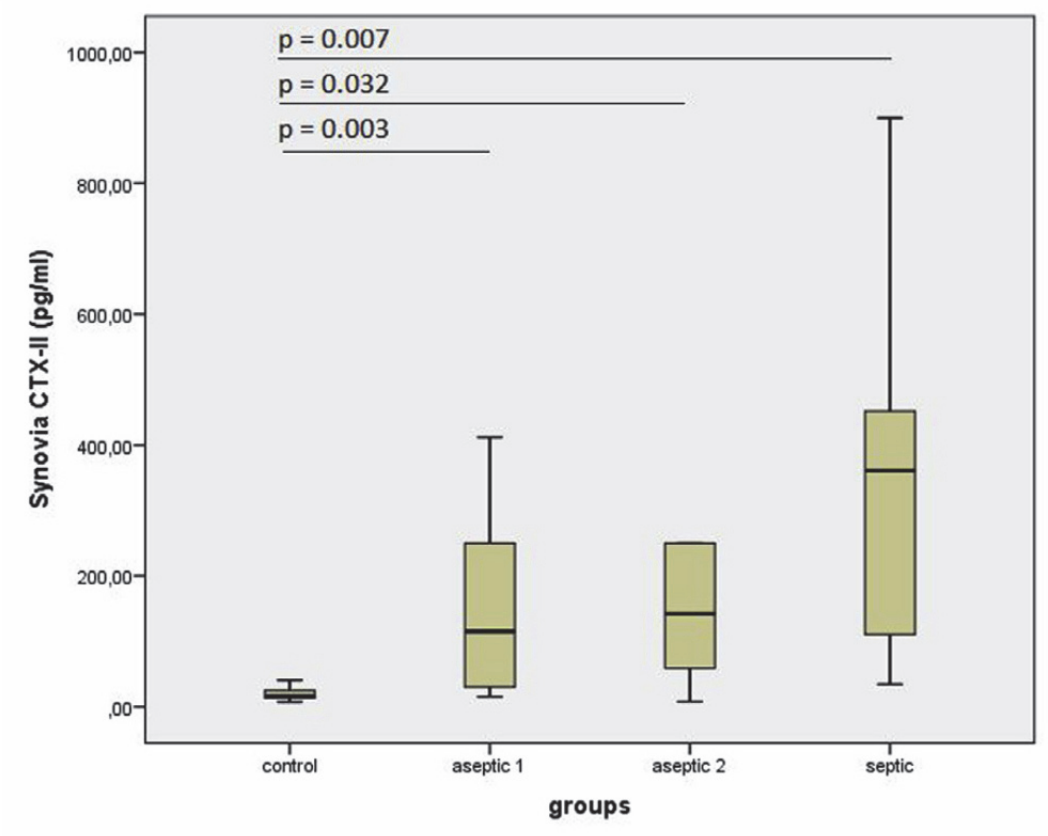
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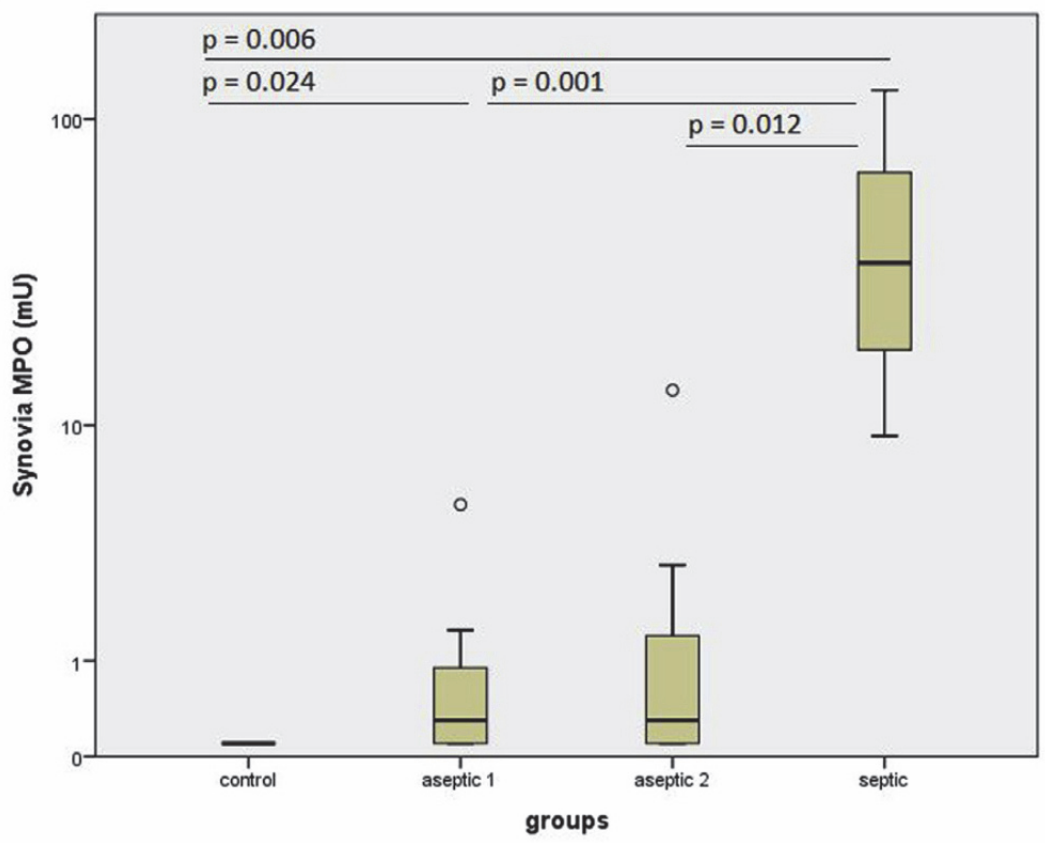
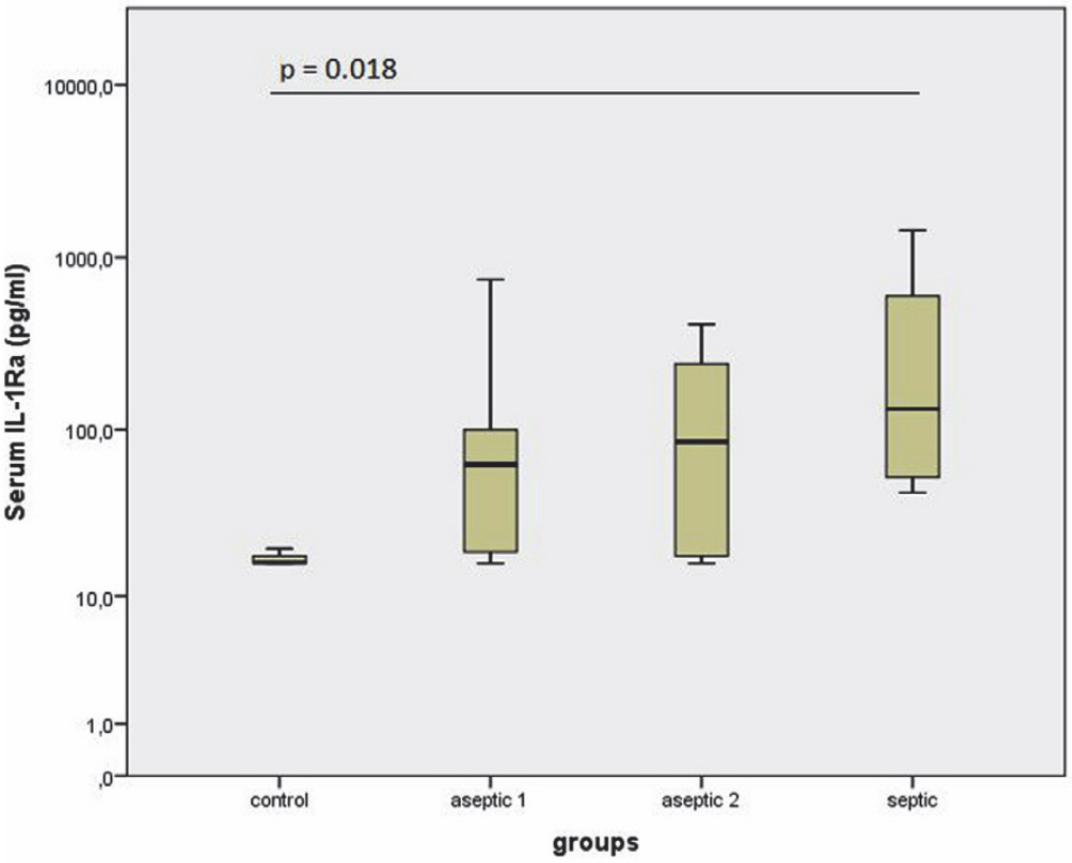


Fig. 1. SF analysis of concentrations of IL-1Ra, IL-1β, CTX-II (pg/mL), and MPO (mU/mL). Boxplots represent interquartile range of control, aseptic 1 (mild OA, group 1), aseptic 2 (advanced OA, group 2), and septic (septic arthritis, group 3); black lines represent median. Whiskers represent values outside the IQR. B indicates extreme value. The value across the top of each graph represents significant differences; P values between the bars. IL-1β, interleukin 1 beta; IL-1Ra, interleukin-1 receptor antagonist; IQR, interquartile range; MPO, myeloperoxidase; OA, osteoarthritis; SF, synovial fluid.

Table 2
Serum and synovial fluid concentrations of IL-1Ra, IL-1β, MPO, and CTX-II in control horses, horses with mild and advanced osteoarthritis, and horses with septic arthritis.

Concentrations	Control		Group 1		Group 2		Group 3	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
IL-1Ra SE pg/mL	17.1	16.0–19.7	131.0	16.0–744.4	133.31	16.0–410.7	551.73	42.5–1462.6
IL-1Ra SF pg/mL	Below limit	16.0	47.9	16.0–101.8	51.53	16.0–78.4	542.56	97.3–1465.8
IL-1β SE pg/mL	Below limit	8.0	32.6	8.7–100.6	62.18	35.2–72.3	477.63	69.9–1467.3
IL-1β SF pg/mL	9.0	8.0–15.0	35.3	15.0–70.6	47.40	34.5–62.5	329.91	27.9–1406.8
MPO SE mU	4.8	0.0–33.8	19.3	3.2–34.9	26.28	1.0–48.9	114.11	18.9–263.3
MPO SF mU	Below limit	0.1	0.8	0.1–5.2	2.14	0.1–13.2	46.65	18.0–123.4
CTX-II SE pg/mL	37.0	6.3–74.0	147.5	6.3–250.0	89.78	6.3–250.0	617.4	111.0–900.0
CTX-II SF pg/mL	20.1	7.2–40.5	150.1	15.1–292.5	141.72	7.6–250.0	371.48	34.1–900.0

Abbreviations: IL-1Ra, interleukin-1 receptor antagonist; IL-1β, interleukin 1 beta; MPO, myeloperoxidase; SE, serum; SF, synovial fluid.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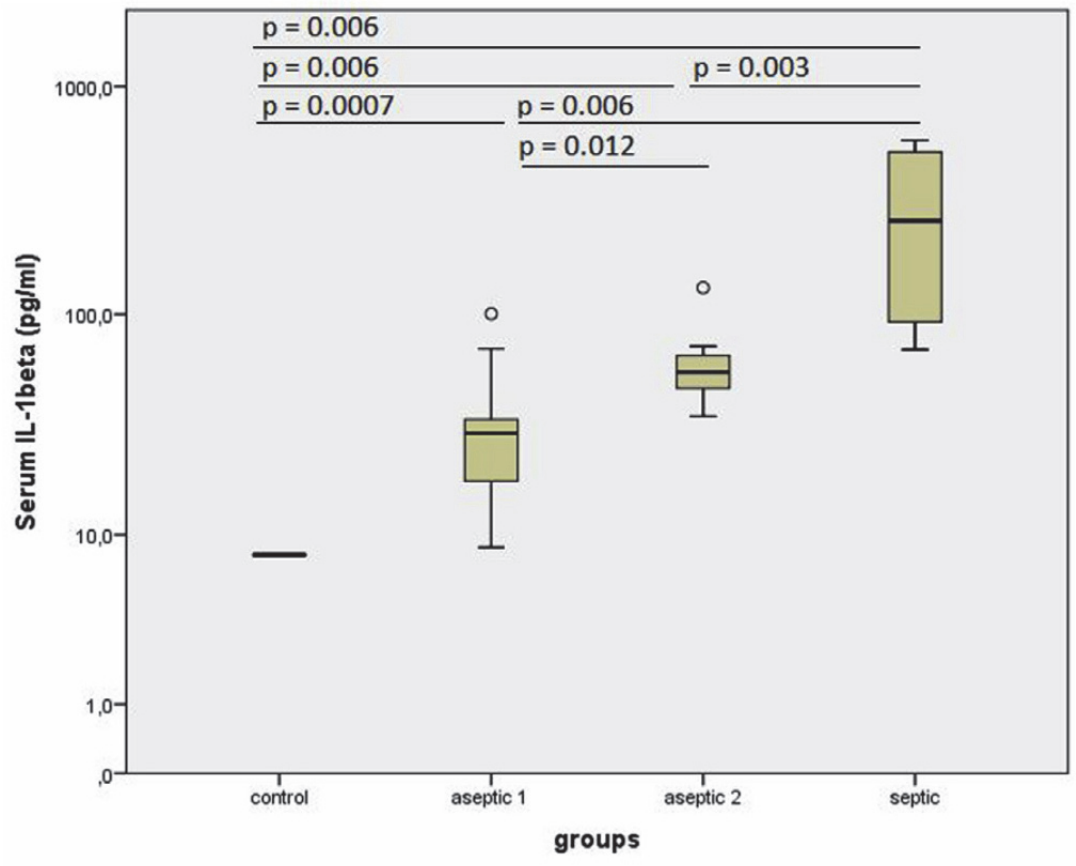
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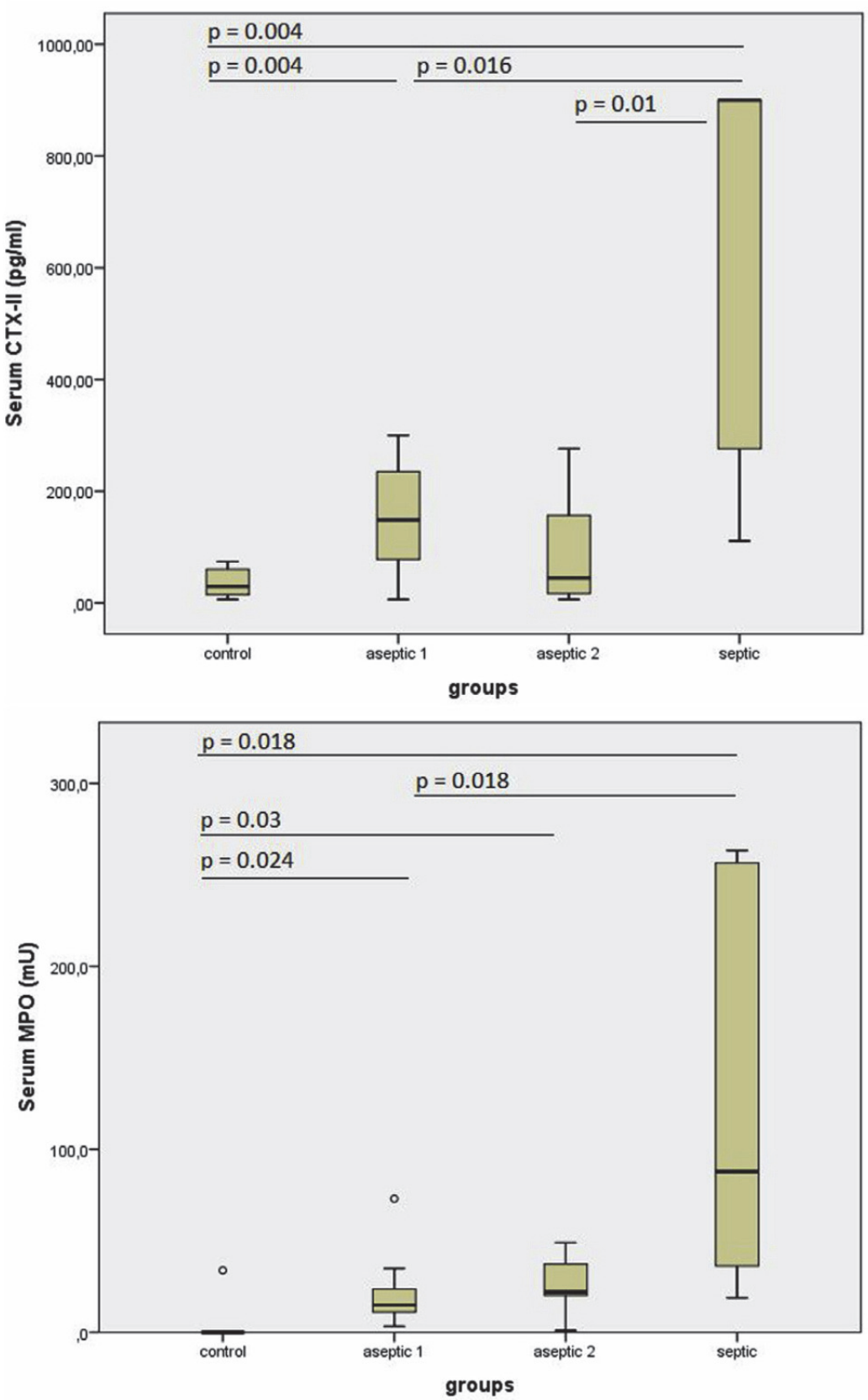

Fig. 2. SE analysis of concentrations of IL-1Ra, IL-1β, CTX-II (pg/mL), and MPO (mU/mL). Boxplots represent interquartile range of control, aseptic 1 (mild OA, group 1), aseptic 2 (advanced OA, group 2), and septic (septic arthritis, group 3); black lines represent median. Whiskers represent values outside the IQR.
B indicates extreme value. The value across the top of each graph represents significant differences; P values between the bars. IL-1Ra, interleukin-1 receptor antagonist; IL-1β, interleukin 1 beta; IQR, interquartile range; MPO, myeloperoxidase; OA, osteoarthritis; SE, serum.

4. Discussion

Using the equine-specific ELISA for the determination of IL-1Ra, low concentrations of this cytokine were found in SF of control horses, significantly higher concentrations in horses affected with OA, and the highest concentrations in those suffering from septic arthritis. In previous studies, concentrations of IL-1Ra were measured in SF of horses with experimentally induced OA by using mouse anti–IL- 1Ra antibodies. Interleukin-1 receptor antagonist concentrations were typically higher in OA-affected joints compared with sham-operated joints but the difference was not significant [6]. Human articular chondrocytes have been shown to produce IL-1Ra in response to IL-1β and IL-6 exposure [33]. A relative deficiency of IL-1Ra compared with IL-1ß has been presumed to be a predisposing factor for the initiation and perpetuation of rheumatoid arthritis [34,35]. However, in the present study, IL-1Ra levels in SF were significantly higher in horses with non–septic OA and septic arthritis compared with the control samples. In vivo experiments have revealed that to inhibit IL-1ß activity, the rise in IL-1Ra levels must be 100 to 2000 times the rise in IL-1ß [36]. The necessity for a large excess of IL-1Ra over IL-1ß is likely to explain the finding that, even though a high level of IL-1Ra is found in SF of horses with OA, there could be a deficit of IL-1Ra relative to IL-1ß within the joint which facilitates the progression of OA [5]. Naturally occurring levels of IL-1Ra in SF of horses with different grades of joint disease have not been studied before. Monitoring of IL-1Ra concentrations as a marker for intrasynovial inflammation or to indicate response to therapy would seem logical as this anabolic cytokine is released in response to synovial pathology [4,5]. Further work is required to help establish whether IL-Ra concentration, in combination with other biomarkers, can be used as an indicator for the severity of OA in equine patients.

In the present study, IL-1ß levels in SE were reflecting the severity of intrasynovial inflammation. It was the only parameter which showed significantly different levels not only between the control group and the OA and septic arthritis groups but also between the two aseptic OA groups. Although these results in SE are significant, it has been shown that this cytokine is raised in SE in cases of inflammation involving the respiratory tract, the gastrointestinal tract, muscular tissue, and other body systems [37,38]. It is unlikely that other systemic inflammation has affected our results because we excluded any horse with abnormal findings on physical examination or haematological abnormality not explained by OA. Because blood collection is less invasive than synoviocentesis, research on biomarkers for use in OA aims to find substances that can be determined in SE for the evaluation of joint

disease. In a human study, the expression of IL-1β in peripheral blood leukocytes from patients with OAwas increased more than twofold in patients who had higher pain scores, decreased function, and were at a higher risk of radiographic progression of OA [39]. However, IL-1ß in SE is not a sufficiently reliable marker because levels rise in the event of any inflammatory process within the body. Although IL-1ß plays a prominent role in the pathogenesis of OA [10,11], our study confirms previously published results stating that IL-1ß levels in SF are not a reliable indicator for the severity of joint disease [14]. This may be related to the short half-life of IL-1ß or maybe explained by an overexpression of IL-1ß in damaged cartilage without a corresponding rise in SF protein levels [15,40].

CTX-II is considered a useful biomarker of human and equine OA [16,41]. In the present study, levels of CTX-II in SF were significantly elevated in horses with OA when compared with those in the control group. Age and joint type have both been reported to affect CTX-II concentrations in SF and SE of horses and dogs [17,42]. In the present study, however, this parameter was chosen to confirm the presence of joint disease and to further investigate CTX-II levels in horses with septic arthritis. CTX-II concentrations in SF have been reported to increase rapidly at the onset of symptoms and to peak at up to 30 times baseline values in humans suffering from septic arthritis [43]. No significant difference in CTX-II concentration in SF could be detected between those horses with septic arthritis and horses with mild or advanced OA in our study. CTX-II concentrations in SE were markedly elevated in horses with septic arthritis but showed a wide range of concentrations.

In humans, MPO was reported to be elevated in patients suffering from knee OA [44]. Using the described specific modified o-dianisidine assay, significantly elevated SF levels of MPO were also detected in canine OA [42,45]. Myeloperoxidase levels in humans and dogs were described to be elevated especially in the acute and early stages of OA; lower levels were measured as the disease progresses [43,45]. Investigation of naturally occurring OA in equine patients frequently occurs after the acute phase of the disease. This might explain why MPO levels were only mildly elevated in the SF of horses in group 1 and 2 in this study. Myeloperoxidase in equine SF has previously been shown to help establish the differentiation between infective and noninfective joint disease in horses. As in other recent studies, MPO levels in SF were mildly elevated in OA joints and could not be used as a reliable indicator of the severity of OA, except in the presence of septic arthritis [23,24,46]. Routine SF analysis is not always conclusive for the diagnosis of septic arthritis, and up to 45% of the samples

may show no growth on culture. For this reason, measurement of MPO levels in SF is valuable for aiding the diagnosis of septic arthritis [24,47].

The present study had limitations related to inherent variability of individual horses when evaluating naturally occurring OA. Although the reliability of scoring systems in the evaluation of the degenerative status of joints has been validated, a low level of interobserver and intraobserver variation remains in the clinical scoring, the radiographic interpretation, and the level of synovial damage as assessed by arthroscopy [27,28,48]. Interobserver and intraobserver variation may account for the number of values obtained which appear to lie outside of the expected ranges. In an attempt to minimize such variation, the same experienced surgeons performed all the evaluations.

4.1. Conclusions

The concentration of IL-1Ra in SF appears to reflect the severity of intrasynovial inflammation. It was found that the SF of healthy joints, those with OA and those with septic arthritis, contained significantly different levels of IL-1Ra. Modern equine-specific ELISA tests have improved our capability to detect levels of biochemical parameters and allow us to correlate them more accurately with the severity of clinical OA. Despite this advance, there remains a great deal of further experimentation to be undertaken to develop a testing system, which will reliably indicate the severity of joint disease in horses.

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3 Discussion

The objective of the 'Review of the literature – part 1 and part 2', published in *Pferdeheilkunde* was to provide the German speaking veterinary practitioner with an up-to-date source of information which considers the efficacy and adverse effects of joint medication in the horse.

Evidence-based medicine is the conscientious explicit and judicious use of the current best available scientific research in making decisions about the care of individual patients (Sackett et al. 1996). The practice of evidence-based medicine means integrating one's individual clinical expertise with the best available external clinical evidence from systematic research (Sacket 1997). The evidence-based approach to clinical care is well established in human medicine and institutions like the 'Evidence-based Veterinary Medicine Association' (EBVMA) or the 'Centre for Evidence-based Veterinary Medicine' (CEVM) aim to promote the use of evidence-based practice in the field of veterinary medicine.

A vast amount of published scientific data on the subject of equine joint medication is available. A review of the literature is time consuming and published research might include conflicting information. It has been shown that the choice of joint medication used by the equine practitioner is most commonly based on personal experience of efficacy and response to therapy. A significant difference in the usage of medication has also been shown to be related to the primary discipline of the treated horses and the treating veterinarian's number of years in practice (Caron et al. 1996; Ferris et al. 2009).

The objective of this study was to provide a summary of the current clinically relevant *in-vitro* and *in-vivo* studies, highlighting research with a high level of evidence. The database 'CAB Abstracts' has been shown to have a high percentage of journal coverage for veterinary literature and was therefore primarily used in the search for clinically relevant studies (Grindlay et al. 2012). Publications were assigned to different levels of evidence based on the grading system provided by the 'Agency of Health Care Policy and Research' (AHCPR). The highest level of evidence is reached by randomized clinical trials, systematic reviews and meta-analysis (Holmes and Ramey 2007). The availability of this type of study in the field of equine joint medication is extremely limited. Several symptom- or disease-modifying drugs have been evaluated using the standardised *in-vivo* 'Equine Osteochondral Fragment Exercise Model' (EOFEM) (Frisbie et al. 1997). Further *in-vitro* studies have been shown to provide

valid information about the efficacy and adverse effects of medication on synovial membrane, cartilage and subchondral bone (Nixon et al. 1992; Frean et al. 1997; Schaefer et al. 2009).

Based on the evidence currently available, joint medication is considered to be a low-risk-procedure, as long as aseptic technique and adequate dosages are employed and the equine patient is not suffering from an underlying medical condition. The development of regenerative and innovative joint medication in the horse has the potential to provide improved efficacy in the restoration of the joint integrity.

This literature review supports the relevance of the interleukin-1receptor antagonist (IL-1Ra) as a therapeutic agent for the treatment of equine osteoarthritis (OA). Therefore the authors decided to further investigate the synovial fluid (SF) and serum (SE) concentrations of IL-1Ra and the catabolic cytokine interleukin-1β (IL-1β) in naturally occurring equine OA and septic arthritis. They hypothesised that concentrations may show a positive correlation with the severity of OA symptoms.

Increased levels of IL-1Ra in SF of horses with experimentally induced OA have been detected (Frisbie et al. 2007). The enzyme-linked immunosorbent assays (ELISAs) that have been used previously for the determination of IL-1Ra concentration in equine SF use mouse or human antibodies. In the present study equine specific ELISA systems were utilised to determine levels of IL-1Ra and IL-1β in SF and SE of horses with different grades of joint disease. Results were compared with levels of the previously described biomarkers C-telopeptide fragments of type II collagen (CTX-II) and myeloperoxidase (MPO). Using the equine specific ELISA for the determination of IL-Ra, SF of healthy joints, those with non-septic OA and those with septic arthritis contained significantly different levels of IL-1Ra. In the present study IL-1β levels in SE were reflecting the severity of intra-synovial inflammation. A significant difference was detected between the control group, the OA groups and the septic arthritis group.

The determination of a biomarker that accurately detects the severity of joint disease is an active area of research (McIlwraith and Clegg 2014; Jackson et al. 2015). The early detection of joint damage and information on disease activity and progression of joint disease would have great benefit for the management of horses suffering from OA. A reliable marker for the activity and severity of OA could potentially be utilised for the monitoring of treatment success and prediction of future level of athletic function. Currently there is no marker

readily available that is both precise and reliable enough to be used in clinical practice. The use of a combination of biomarkers is more likely to provide sufficient information on the severity of OA (McIlwraith 2005). In addition to lameness examination and standard diagnostic procedures, the determination of IL-1Ra concentration in SF, in combination with further biomarkers, might be useful to assess the extent of intra-synovial inflammation. Data obtained from a larger number of horses is required to fully validate the benefit of the use of equine specific ELISA kits.

4 Summary

Osteoarthritis (OA) continues to be one of the most common causes of equine lameness. Although several therapeutic approaches have been shown to be beneficial in the treatment of OA, intra-articular medication remains one of the most effective treatment options. Since OA and its successful treatment is the subject of intensive ongoing research a vast quantity of scientific data regarding effectiveness, dosage and mechanism of action for joint medications is available.

Evidence-based medicine is the judicious use of the current best available scientific research in making decisions about the care of individual patients. The practice of evidence-based medicine means integrating one's individual clinical expertise with the best available external clinical evidence from systematic research. Communicating evidence-based information about a clinical condition and the treatment options available helps the owner to make informed decisions about their animal.

Based on a systematic review of the current literature, relevant information about commonly used intra-articular joint medications as well as regenerative and innovative medications was summarised. Joint medication was shown to be a low-risk procedure when performed according to the standards of good veterinary practice.

Further the concentration of interleukin-1 repector antagonist (IL-1Ra) and IL-1β was determined in the synovial fluid (SF) and serum (SE) of horses with different grades of joint disease using equine specific ELISA systems. The concentration of IL-Ra in SF and IL-1β in SE appear to reflect the severity of intra-synovial inflammation. Modern equine specific ELISA tests have improved our capability to detect levels of biochemical parameters in SF and SE of horses with clinical joint disease. IL-1Ra in SF in combination with further biomarkers might be useful to assess the extent of intra-synovial inflammation. These results require a larger number of clinical cases to be utilized in order to confirm the benefit of using the equine specific ELISA. There remains a great deal of further experimentation to be undertaken to develop a testing system, which will reliably indicate the severity of joint disease in the horse.

5 Zusammenfassung

Evidenz-basierte Literatur- Übersicht zu Wirkungen und Nebenwirkungen der intraartikulären medikamentellen Therapie und Untersuchung von Biomarkern in Synovialflüssigkeit und Serum zur Diagnose von Osteoarthritis beim Pferd

Die unter dem englischen Begriff Osteoarthritis (OA) zusammengefassten degenerativen Veränderungen von Gelenken sind die häufigste Ursache für Lahmheit beim Pferd. Obwohl für die Therapie der OA verschiedenste Wege der Applikation beschrieben sind, wird die intraartikuläre Injektion in der Praxis bevorzugt angewendet. Die Pathophysiologie und Therapie der OA sind Gegenstand aktueller Forschung. Eine Vielzahl von wissenschaftlichen Studien beschäftigt sich mit Wirkmechanismus, Effektivität, Dosierung und Nebenwirkungen der intraartkulären Therapie. Das Ziel der Literaturübersicht ist es, die Wirksamkeit der gängigsten Präparate zur Behandlung der OA des Pferdes anhand von aktuellen *in-vitro* und *in-vivo* Studien evidenzbasiert zu beschreiben.

Die evidenzbasierte Medizin beschreibt den gewissenhaften, ausdrücklichen und vernünftigen Gebrauch der gegenwärtig besten externen, wissenschaftlichen Evidenz für die Entscheidung in der medizinischen Versorgung individueller Patienten. Die Praxis der evidenzbasierten Medizin bedeutet die Integration individueller klinischer Expertise mit der besten verfügbaren, externen Evidenz aus systematischer Forschung. Die Kommunikation evidenzbasierter Information bezüglich der klinischen Erkrankung und den zur Verfügung stehenden Therapiemöglichkeiten erlaubt dem Besitzer eine informierte Entscheidung über die Behandlung seines Tieres.

Anhand einer systematisch durchgeführten Literaturrecherche wurde eine Übersicht der publizierten Literatur erstellt. Wirkungen und Nebenwirkungen konventioneller intraartikulärer Therapeutika sowie regenerativer und innovativer Medikamente wurde in einem zweiteiligen Review zusammengestellt. Die Arbeit zeigt, dass die intraartikuläre Behandlung mit einem geringen Risiko für Nebenwirkungn verbunden ist, wenn die Behandlung dem veterinärmedizinischen Standard entsprechend durchgeführt wird.

Des Weiteren wurde die Konzentration von Interleukin-1 Rezeptor Antagonist (IL-1Ra) und Interleukin-1β (IL-1β) in Synovialflüssigkeit (SF) und Serum (SE) von Pferden mit OA unterschiedlichen Schweregrades mit Hilfe eines pferdespezifischen ELISA Systems

bestimmt. Die Konzentration von IL-1Ra in SF und IL-1β in SE war indikativ für den Grad der intrasynovialen Entzündung.

Moderne, pferdespezifische ELISA Systeme erlauben die genauere Bestimmung biochemischer Parameter in SF und SE von Pferden mit klinischer OA. IL-1Ra in SF in Kombination mit der Messung weiterer Biomarker könnte die Evaluierung intrasynovialer Entzündungsreaktionen erleichtern. Eine weiterführende Studie mit einer größeren Anzahl an klinischen Fällen ist indiziert um die Genauigkeit des pferdespezifischen ELISA weitergehend zu untersuchen. Eine Vielzahl wissenschaftlicher Studien wird notwendig sein bis ein Testsystem für die verlässliche Bestimmung des Schweregrades der OA des Pferdes entwickelt ist und klinisch angewendet werden kann.

6 References for Introduction and Discussion

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7 Oral presentation

Ehrle, A. et al. ‘Synovial fluid and serum concentrations of Interleukin-1 receptor antagonist and interleukin-1β in naturally occurring equine osteoarthritis and septic arthritis’ German Equine Veterinary Association (GEVA) congress, 6.-9. November 2014, Munich

8 List of publications

Ehrle, A.; Fürst, A.; Lischer, C. (2013) 'Wirkungen und Nebenwirkungen der intraartikulären medikamentellen Therapie beim Pferd – eine Literaturübersicht – Teil 1: Konventionelle intraartikuläre medikamentelle Therapie und Risiken der Gelenkinjektion beim Pferd' *Pferdeheilkunde* 29(1); pp. 54-64

Ehrle, A.; Fürst, A.; Lischer, C. (2013) 'Wirkungen und Nebenwirkungen der intraartikulären medikamentellen Therapie beim Pferd – eine Literaturübersicht – Teil 2: Regenerative and innovative intraartikuläre medikamentelle Therapie beim Pferd' *Pferdeheilkunde* 29 (2); pp. 212-218

Ehrle, A.; Lischer, C.; Lasarzik, J.; Einspanier, R.; Bondzio, A. (2015) 'Synovial fluid and serum concentrations of Interleukin-1 receptor antagonist and interleukin-1β in naturally occurring equine osteoarthritis and septic arthritis' *Journal of Equine Veterinary Science* 35; pp. 815-822

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10 Declaration of own research activity

10.1 Wirkungen und Nebenwirkungen der intraartikulären medikamentellen Therapie beim Pferd – eine Literaturübersicht – Teil 1: Konventionelle intraartikuläre medikamentelle Therapie und Risiken der Gelenkinjektion beim Pferd

Authors: Anna Ehrle; Anton Fürst, Christoph Lischer

Year: 2013

Journal: *Pferdeheilkunde* 29 (1); pp. 54-64

	Anna Ehrle	**Anton Fürst**	**Christoph Lischer**
Study design	50%	10%	40%
Data collection	80%	10%	10%
Study execution	-	-	-
Data analysis and interpretation	60%	20%	20%
Preparation of the manuscript	60%	20%	20%

10.2 Wirkungen und Nebenwirkungen der intraartikulären medikamentellen Therapie beim Pferd – eine Literaturübersicht – Teil 2: Regenerative and innovative intraartikuläre medikamentelle Therapie beim Pferd

Authors: Anna Ehrle; Anton Fürst, Christoph Lischer

Year: 2013

Journal: *Pferdeheilkunde* 29 (2); pp. 212-218

	Anna Ehrle	**Anton Fürst**	**Christoph Lischer**
Study design	50%	10%	40%
Data collection	80%	10%	10%
Study execution	-	-	-
Data analysis and interpretation	60%	20%	20%
Preparation of the manuscript	60%	20%	20%

10.3 Synovial fluid and serum concentrations of Interleukin-1 receptor antagonist and interleukin-1β in naturally occurring equine osteoarthritis and septic arthritis

Authors: Anna Ehrle; Christoph Lischer; Juliane Lasarzik; Ralf Einspanier; Angelika Bondzio

Year: 2015

Journal: *Journal of Equine Veterinary Science* 35; pp. 815-822

	Anna Ehrle	**Christoph Lischer**	**Juliane Lasarzik**	**Ralf Einspanier**	**Angelika Bondzio**
Study design	50%	20%	-	-	30%
Data collection	60%	-	20%	-	20%
Study execution	60%	-	10%	-	30%
Data analysis and execution	60%	10%	10%	10%	10%
Preparation of the manuscript	70%	10%	-	10%	10%

10.4 Selbstständigkeitserklärung

Hiermit bestätige ich, dass ich die vorliegende Arbeit selbsständig angefertigt habe. Ich versichere, dass ich ausschliesslich die angegebenen Quellen und Hilfen in Anspruch genommen habe.

Neston UK, den 16. Oktober 2015

Anna Ehrle