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Analytical Clinical Studies

Intra-articular treatment with triamcinolone compared with triamcinolone with hyaluronate: A randomised open-label multicentre clinical trial in 80 lame horses

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Summary

Reasons for performing study: Intra-articular (IA) injection of corticosteroids with or without hyaluronate (HA) has been used for decades in equine practice for treatment of noninfectious synovitis and osteoarthritis. However, to date, no large-scale randomised equine field trials have been reported that address the supposed superior clinical efficacy of the combination of corticosteroid + HA compared with IA injection of corticosteroid alone.
Objectives: To compare the clinical efficacy of IA triamcinolone acetonide (TA, 12 mg) compared with IA TA (12 mg) + high molecular weight HA (20 mg) in horses with clinical joint disease.
Study design: Prospective, randomised, parallel, open label, multicentre clinical trial.
Methods: Eighty client-owned horses from 13 clinics were included. Lameness and effusion scores were assessed at baseline and 3 weeks after IA treatment. A standardised telephone questionnaire was completed between the owner and consulting veterinarian at 3 months. The primary outcome parameter was clinical success rate, defined as ≥2 grades lameness reduction (on a 0–5 scale) at 3 weeks. Chi-square statistics and binary logistic regression were used to analyse data on an intention-to-treat basis for the 3 week outcome.
Results: The success rate of IA TA 3 weeks after treatment was 87.8%, while that of TA+HA was 64.1% (P = 0.01). Age >13 years was associated with a reduced success rate for the combination treatment (P = 0.004) at 3 weeks. At 3 months, half the horses in each group had returned to their previous level of performance.
Conclusions: The combination of TA with HA was associated with a lower short-term clinical success rate and a similar medium-term outcome compared with IA TA, with only half of the horses performing at their previous level of exercise after 3 months regardless of treatment group allocation.

Keywords: horse; joint; arthritis; corticosteroid; hyaluronan; randomised

Introduction

Synovitis and osteoarthritis are common conditions in both sports and pleasure horses. Joint medications are widely used for targeted treatment of one or more symptomatic joints, particularly if initial rest and anti-inflammatory therapy do not resolve lameness [1]. Corticosteroids are still among the most commonly used drugs for intra-articular (IA) treatment of noninfectious synovitis, providing potent anti-inflammatory and indirect analgesic activity [2]. Intra-articular corticosteroids reduce lameness and joint effusion in horses with synovitis and induced osteoarthritis [3,4] and decrease the expression of catabolic and proinflammatory factors in cartilage and/or synovial membrane [5,6]. However, several studies have also identified potential detrimental effects of corticosteroids on articular cartilage composition and morphology [7]. While these negative effects are now known to be related to the type and dose of corticosteroid used, the frequency of repeated administration and to joint loading after injection, this does imply IA corticosteroids should be used judiciously [2]. Results of *in vitro* and *in vivo* research indicate triamcinolone (TA), a corticosteroid with medium duration of action, has the most favourable effect in terms of equine cartilage metabolism compared with other corticosteroid preparations like methylprednisolone acetate (MPA) and betamethasone [8].

Joint injection with hyaluronate (HA) is another popular treatment that has been shown to reduce lameness and provide clinical anti-inflammatory and analgesic effects, without detriment to cartilage or synovial membrane morphology [9]. Although the mode of action of HA on articular cells has not been fully elucidated, in addition to reducing friction [10], HA has direct analgesic effects [11] and reduces synovial prostaglandin E_2 release [12], cartilage fibrillation [13] and interleukin (IL)-1 induced proteoglycan loss from cartilage [14].

Given that HA could positively affect joint function and has intrinsic analgesic and anti-inflammatory activity, a popular treatment strategy is to combine a corticosteroid with HA in a single IA injection. The premise behind this is that addition of HA might minimise any potential negative effect of the corticosteroid on cartilage metabolism and/or that the combination provides more effective alleviation of lameness than the corticosteroid alone [1]. The validity of this latter assumption has, however, not been formally addressed by a large-scale randomised clinical trial in horses. The aim of the current study was to investigate the clinical efficacy of IA TA compared with IA TA plus HA for the treatment of arthrogenic lameness in horses.

Materials and methods

Trial design and settings

A parallel randomised multicentre open-label comparative clinical efficacy study of IA TA compared with IA TA + HA was planned by the Department of Equine Sciences, Faculty of Veterinary Medicine, Utrecht University in collaboration with an external clinical trial monitor (Research Drive BV)[a] and 12 equine veterinary clinics in the Netherlands (Supplementary Item 1).

Patient inclusion

Horses and ponies aged 2 years and older were screened for eligibility by the consulting veterinarian based on the following 4 inclusion criteria: lameness of at least *grade 2* on a 0–5 scale [15] (Supplementary Item 2), lameness localised to one limb only, only one joint (distal interphalangeal (DIP), metacarpo- or metatarsophalangeal (MCP or MTP), middle carpal (MC) or antebrachiocarpal (AC)) to be treated intra-articularly and a positive

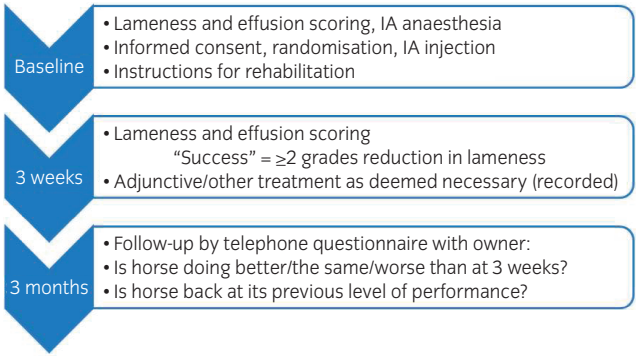

Fig 1: Flowchart of study protocol and procedures. IA = Intra-articular.

response of this joint to intra-articular anaesthesia (defined as at least 50% visible improvement in lameness within 5 min after injection of the local anaesthetic; Supplementary Item 3). Exclusion criteria for the study were IA injection in the same joint within 4 weeks prior to screening and contraindications for IA medication with corticosteroids or severe comorbidity, including joint infection, IA fractures or fissures, or laminitis. Post inclusion elimination criteria were lameness occurring in another limb between the moment of inclusion and study completion (as this would hamper lameness evaluation of the treated limb) and other medication aimed at the musculoskeletal system (such as nonsteroidal anti-inflammatory drugs [NSAIDs], polysulphated glycosaminoglycans and nutraceuticals) received by the patient between the time of study inclusion and the 3 week follow-up consultation.

Patient management and welfare

All veterinarians involved in the study worked according to the guidelines for Good Veterinary Practice (GVP) as stipulated by the Royal Netherlands Veterinary Association (KNMvD). They were instructed on study protocol and standard operating procedures by Research Drive BV and the Department of Equine Sciences of Utrecht University Faculty of Veterinary Medicine. Owners of eligible horses were provided with written information regarding the study protocol. Signed informed consent forms were obtained from all owners prior to enrolment of their horse. Horses included in the trial remained in the care of their owners and hence were housed and fed as they were accustomed to. On the day of treatment, horses were box rested.

Study protocol and interventions

A flowchart of the study is provided in Figure 1. Patients were screened for eligibility using the above mentioned criteria at presentation to the clinic (baseline, Day 0) based on lameness examination including response to IA anaesthesia. Lameness score and joint effusion scores (on a 0–4 scale; [16]) were recorded (Supplementary Items 2 and 4), with an estimated percentage response to IA anaesthesia and the time at which maximum effect of IA anaesthesia was seen (Supplementary Item 3). Prior to IA injection of the local anaesthetic, 2 ml of synovial fluid was aspirated from the joint. When owner consent was obtained, synovial fluid was centrifuged and stored at -20°C (Supplementary Item 5) and patient demographic data were entered in the online study database. The patient was then randomly allocated by the online system to one of 2 treatment groups.

Depending on group allocation horses received either 12 mg TA (Kenacort-A10)[b] or 12 mg TA in combination with 20 mg of high molecular weight HA (Hylartil vet)[c] as a single IA injection via an 18–21 gauge needle. Injection volume differed between the 2 treatments but this was allowed as treatment was intended to mirror common equine practice. Horses were sent home and restricted to box rest for the day of treatment, with a protocol for controlled walking exercise for 3 weeks (Supplementary Item 6). Owners were told no other interventions or medications aimed at the musculoskeletal system should be given for 3 weeks.

At the 3 week re-examination, lameness and effusion scores were assessed by the same veterinarian and the owner was asked about adherence to study protocol. Further treatment was initiated according to the attending clinician's judgement if lameness persisted. Details on any additional treatment received were recorded.

A standardised questionnaire (Supplementary Item 7) was conducted with the owner by telephone by the consulting veterinarian at 3 months. Adverse events were recorded in the online database whenever an owner contacted the veterinarian with concerns possibly related to treatment at any time between study enrolment and completion.

Outcomes

The primary endpoint was clinical success rate, defined as the proportion of patients that showed ≥2 grades reduction of lameness 3 weeks after treatment compared with pretreatment lameness score (i.e. at baseline). Secondary efficacy endpoints were absolute reduction in lameness and effusion scores at 3 weeks compared with baseline and proportion of horses that had returned to their previous level of performance at 3 months.

Sample size

Power analysis on the primary outcome measure was performed to establish the number of horses to be included in the trial. With an estimated 30% difference in clinical success rate between groups ($\pi1 = 0.5$, $\pi2 = 0.8$), a power of 80% ($c = 7.9$), α of 0.05 and 2-sided testing, sample size was calculated as

$$M(\text{size per group}) = c \times \left[\frac{\pi1(1-\pi1) + \pi2(1-\pi2)}{(\pi1-\pi2)^2} \right]$$

This yielded a minimal number of n = 36 patients per group (72 total). Allowing for 10% possible loss of patients to follow-up, we aimed to include 80 patients in the trial.

Randomisation

The online secure patient database software used a random number generation algorithm without restrictions for patient randomisation; patients were block-randomised within clinic by the randomisation software (which recognised clinic of enrolment based on unique patient study ID number), using a block size of 4 patients within clinic.

Blinding

The 2 products to be combined in a single injection have not been tested for physicochemical stability. Moreover, sterility of the combination after aseptic preparation and prolonged storage in the same syringe could not be guaranteed. Hence, blinding could not be performed by prefabricating identical-looking syringes for both interventions. This meant that both the veterinarian and the owner knew which treatment the horse had received. The investigator responsible for data analysis (J.C. de G.) was blinded to patient group allocation.

Data analysis

Differences between groups at baseline were assessed using unpaired *t* tests (continuous variables) and Mann–Whitney U tests (categoric variables). Primary and secondary outcome measures at 3 weeks and 3 months were compared between treatment groups using cross-tabulations with Chi-squared analysis. The 95% confidence intervals (CIs) of the difference were calculated using the modified Wald method. The outcomes 'success rate at 3 weeks' and 'return to previous level of performance at 3 months' were analysed by binary logistic regression to check for any residual confounding of the following covariates: age, duration of lameness at presentation, anatomic joint affected, study site (clinic), baseline lameness score and % response to IA anaesthesia. Computer software was used (IBM SPSS Statistics for Windows, Version 20.0)[d] and significance level set at $P<0.05$.

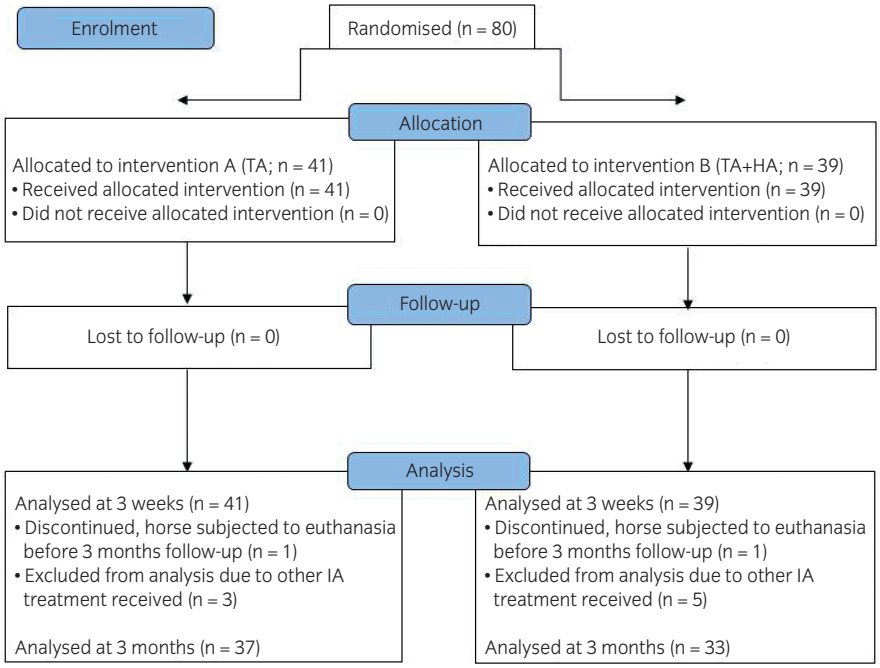

Fig 2: Participant flow diagram showing numbers enrolled, randomised, lost to follow-up and analysed at each time point. TA = triamcinolone; HA = hyaluronate; IA = intra-articular.

Results

Recruitment

Horses were recruited from May 2011 to May 2012. Re-examination by the veterinarian was at a median of 21 days (range 19–22 days) after baseline; all owners of surviving horses (n = 78) were contacted by telephone 3 months (median 92 days, range 78–96 days) after baseline.

Participant flow, losses and exclusions

A flow diagram of trial participants is provided in Figure 2. In total, 80 horses were included. Randomisation led to 41 patients being assigned to the TA group and 39 to the TA+HA group; no human error in treatment assignment was identified from printouts of the randomisation pop-up screens, thus this imbalance was computer-generated.

Baseline data

Relevant baseline data for participating horses are provided in Table 1. There were no significant differences in any of the baseline parameters between groups.

Numbers analysed

The primary analysis at 3 weeks was intention-to-treat and involved all horses (n = 80) randomly allocated to a treatment group. Analysis at 3 months was on a per protocol basis, meaning that horses with protocol

TABLE 1: Baseline patient data in 80 lame horses treated with either intra-articular 12 mg triamcinolone acetonide or 12 mg triamcinolone acetonide and 20 mg hyaluronate

Parameter	Summary statistic	Triamcinolone (n = 41)	Triamcinolone + hyaluronate (n = 39)
Age (years)	Median (range)	13 (4–20)	11 (4–20)
Use	Number of horses		
Recreation		14	20
Sports		26	19
Breeding		1	0
Duration of lameness (days)	Mean (s.d.)	35.4 (57.9)	28.1 (23.6)
	Median (range)	21 (5–365)	21 (5–120)
Joint affected	Number of joints		
Distal interphalangeal		17	21
Forelimb		17	19
Hindlimb		0	2
Metacarpophalangeal		12	14
Metatarsophalangeal		7	1
Middle carpal		1	1
Antebrachiocarpal		2	0
Unknown		2	2
Response to intra-articular anaesthesia (%)	Mean (s.d.)	87 (15)	90 (12)
Lameness score	Median (range)	2 (2–3)	2 (2–4)
Effusion score	Median (range)	1 (0–4)	1 (0–4)

TABLE 2: Outcome at 3 weeks and 3 months after intra-articular treatment with either 12 mg triamcinolone acetonide or 12 mg triamcinolone acetonide and 20 mg hyaluronate in 80 lame horses

Outcome parameter	Triamcinolone	Triamcinolone + hyaluronate	Risk ratio (95% CI)	Risk difference (95% CI)	P value
Primary					
Treatment success: ≥2 degrees reduction in lameness score at 3 weeks (proportion, 95% CI)	36/41 = 87.8% (74.0–95.1%)	25/39 = 64.1% (48.4–77.3%)	1.37 (1.05–1.78)	23.7 (5.1–41)	0.02
Secondary					
Reduction in lameness score at 3 weeks (median, interquartile range)	2 (2–2)	2 (1–2)	NA	NA	0.03
Reduction in effusion score vs. baseline at 3 weeks (median, interquartile range)	1 (1–1)	1 (0–2)	NA	NA	0.6
Return to previous level of performance at 3 months (proportion, 95% CI)	19/37 = 51.4% (35.9–66.6%)	16/33 = 48.5% (32.5–64.8%)	1.06 (0.66–1.70)	2.9 (-20–26)	0.8

CI = confidence interval; NA = not applicable.

violations (n = 3 in Group TA and n = 5 in Group TA+HA) and horses that were not alive at the 3 month time point (n = 1 in each group) were excluded from efficacy analysis.

Outcomes and estimations

Results for each group at the 3 week and 3 month time points are shown in Table 2.

Significantly more horses that received IA injection of TA were classified as a treatment success after 3 weeks than horses receiving IA TA + HA (87.8 vs. 64.1%, P = 0.01; difference = 24%, 95% CI, 5–44%, Table 2). Lameness and effusion scores were lower at 3 weeks compared with baseline in both groups (P<0.0001), indicating that both treatments were effective for short-term reduction of lameness and effusion (Fig 3). However, more horses in the TA+HA group (35.9%) had no or only a partial response (i.e. improvement in lameness of 0 or 1 grade) than in the TA group (12.2%; P = 0.026, Fig 4)

At 3 months, around half the horses had returned to their previous level of performance (i.e. before they became lame) and this was comparable between groups (51.4% vs. 48.5%, P = 0.8; Table 2). The loss of 10 patients to follow-up was unlikely to have had an effect on the absence of a statistically significant difference at this time.

There was no residual confounding of age, duration of lameness, clinic, baseline lameness score or response to IA anaesthesia on success rate at 3 weeks or return to work at 3 months. A significant effect of age within treatment was detected at 3 weeks: while there were no significant differences in the proportion of horses older than 13 years between groups (the median age of the overall population), the proportion of older horses that were classified as a treatment failure was greater in the TA+HA group (7/9) than in the TA group (3/12, P = 0.004).

Adverse events

In total, 9 adverse events were reported (Table 3), 4 of which were possibly drug-related based on timing of the event and presenting clinical signs. Of these 4 patients, 3 had received TA (incidence 7.3%) and one had received TA+HA (incidence 2.6%). The incidence of adverse drug-related events was not statistically different between treatment groups.

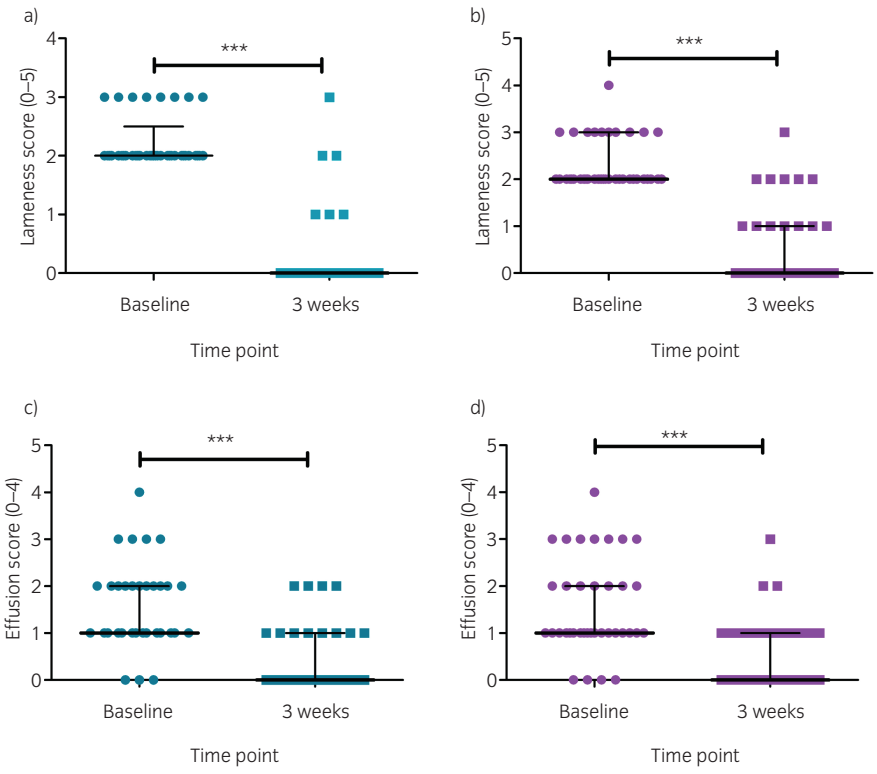

Fig 3: Baseline and 3 week lameness scores for horses randomised for intra-articular treatment with 12 mg triamcinolone acetonide only (TA, 12 mg, n = 41), (a) or 12 mg triamcinolone acetonide and 20 mg hyaluronate (TA+HA, n = 39) (b) and baseline and 3 week effusion scores for the TA group (c) and TA+HA group (d). Data are shown as median with interquartile range. ***P<0.001.

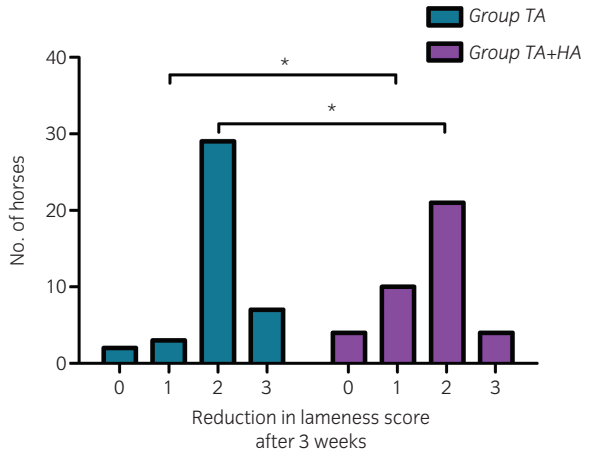

Fig 4: Number of horses with 0, 1, 2 or 3 grades reduction in lameness 3 weeks after intra-articular treatment with 12 mg triamcinolone acetonide (TA) or 12 mg triamcinolone acetonide and 20 mg hyaluronate (TA + HA). *P<0.05.

Discussion

This report describes the results of a multicentre randomised clinical trial in 80 lame horses designed to study the comparative clinical efficacy of IA treatment with 12 mg TA compared with 12 mg TA + 20 mg HA.

A greater proportion of the TA group had an improvement in lameness score of at least 2 grades at 3 weeks. This difference in short-term clinical efficacy was not only statistically significant, but may also arguably be clinically relevant given the size of the difference, albeit with wide confidence intervals. At 3 months, owners reported 5 horses in the TA group (14%) and 4 horses in the TA+HA group (12%) had deteriorated, similar proportions with each treatment. More importantly, however, only half the horses were back in full work at 3 months regardless of treatment assignment. Thus, our results indicate that there was a good initial clinical response in this patient population, particularly after injection of TA alone, but a poorer outcome for return to full athletic performance after 3 months with both treatments. It is likely that corticosteroids provide their potent anti-inflammatory and analgesic effects for a limited period of time after IA injection [7]. It has been hypothesised that addition of HA to IA corticosteroid injections and, in particular, the combination of TA with high molecular weight HA could provide additional benefits both clinically and in terms of chondroprotection [1,8,17]. As we lacked baseline radiographic data for all patients and did not collect outcome data for cartilage or synovial membrane health status, this study does not address whether joints that received the combination treatment indeed experienced chondroprotection relative to joints that received TA only. We can, however, conclude that in this patient population, there was no added benefit in terms of lameness reduction in combining TA with HA for treatment of arthrogenic lameness and that in fact fewer horses responded favourably to the combination treatment in the short term. This outcome was unexpected as it does not support the widely held view that combination of IA corticosteroids with HA would be more effective than corticosteroids alone in alleviating signs of noninfectious arthritis and synovitis [1]. Also, the current findings do not agree with a small-scale study in 24 human patients where the combination of TA+HA was clinically more successful than TA alone [18], nor with an early, underpowered report on 12 horses with traumatic arthritis [19].

TABLE 3: Reported adverse events in 80 lame horses treated with either intra-articular (IA) 12 mg triamcinolone acetonide (TA) or 12 mg triamcinolone acetonide and 20 mg hyaluronate (TA+HA)

Patient ID	Treatment	Reported event	Possibly drug-related
11–17	TA	Lymphangitis, swelling and redness over IA puncture site, urticarial reaction, fever 3 days after IA treatment	Yes
20–56	TA	Subcutaneous swelling around injected fetlock	Yes
22–42	TA+HA	Urticarial reaction	Yes
6–25	TA	Urticarial reaction	Yes
8–32	TA+HA	Pastern dermatitis	No
18–13	TA+HA	Excitable behaviour	No
18–15	TA	Hoof abscess	No
18–16	TA+HA	Pododermatitis	No
22–63	TA	Colic	No

A clinical trial is designed to assess whether there are differences in clinical efficacy between treatments in a clinical population, not to address the mechanisms behind any such discrepancy. The reason for the observed difference in short-term clinical efficacy of TA compared with TA+HA thus remains to be determined. Baseline parameters such as severity or duration of lameness, joint affected or use of the horse were not different between treatment groups. Importantly, bias on the part of participating veterinarians could have influenced outcome evaluation at 3 weeks. However, this seems fairly unlikely as different veterinarians at multiple clinics were involved and outcome data were analysed after study closure and were highly comparable for all clinics. Bias in treatment evaluation might have been expected to have favoured the combination treatment, as its widespread use is based on perceived or assumed clinical superiority to that of the steroid alone. Nevertheless, we cannot rule out the possibility of bias having influenced study results.

We can only speculate as to potential causes for the observed lower reduction in lameness with the combination therapy compared with TA alone. These could include hitherto unknown drug interactions (physicochemical or pharmacodynamic), retrograde drug loss from the needle used to perform both injections consecutively, dilution or joint distension caused by the greater volume injected with the combination treatment, or subclinical synovitis triggered either by HA itself or by needle manipulation upon injection of the second drug. As the study was performed in a prospective randomised fashion, any difference in severity of joint disease at baseline between groups would have been due to chance only, and hence this is unlikely to explain the observed discrepancy. A previous retrospective study in 128 lame horses found an effect of several covariates, including age, duration of lameness at baseline and response to IA anaesthesia on success rate of IA therapy [20]. Potential confounding baseline variables in the current study (age, duration of lameness, type of anatomical joint affected, clinic, percentage improvement on IA anaesthesia) were analysed and no residual confounders were identified for success rate at 3 weeks nor for return to work. While groups did not differ with regards to patient age, an interaction of age and treatment was detected for success rate at 3 weeks, with a lower proportion of horses receiving the combination therapy classified as treatment success. Although the biological reason for this discrepancy remains to be determined, it suggests advanced age to be a negative predictor for clinical response to the combination therapy.

Previous reports on clinical efficacy of IA treatment with corticosteroids with or without HA in horses are scarce and data vary widely between studies. Importantly, no large-scale prospective randomised clinical trial on the IA use of a steroid with or without HA had been performed in horses to date. One retrospective study on 51 horses with distal tarsal pathology found short-term improvement in 58% of horses at initial re-examination but a relapse in 90% of horses at a median of 56 days thereafter [21]; that report did not differentiate between corticosteroid injection alone or injection of corticosteroid + HA. More than 2 years after treatment, 38% had an owner-reported positive outcome. Another retrospective study of 45 horses with lameness referable to the DIP joint found only 30% of cases responding to treatment by repeated IA HA injections [22]. In another retrospective study, only 36% of 28 horses with DIP joint pathology and a positive response to DIP joint anaesthesia had a successful outcome 5 weeks after a single injection of MPA [20]. A prospective study in 169 lame horses found 68% to have recovered from lameness with IA steroid + HA at the re-examination 3–4 weeks after treatment [23]. That study used 12 mg betamethasone and 20 mg of the same hyaluronate product used in the current study.

It appears that while the medium-term success rate of approximately 50% found in the current study with either treatment is roughly in line with previous reports, the short-term clinical success rate for IA TA in our study (88%) is quite high compared with other studies. The observed treatment response at 3 weeks may have been amplified by the rest and restricted walking protocol that was recommended, as a placebo-controlled IA therapy study demonstrated that many horses in the placebo group (IA saline) also improved with rest over time [9]. We did not include a placebo-treated group as it was deemed both ethically inadvisable and practically unfeasible to get owners to consent to withholding treatment for their moderately to severely lame horses. As an alternative explanation for the relatively high short-term success rates, it could be that horses in the current study suffered from less advanced joint disease than those in other studies [20–22]. Although joint radiography and ultrasonography were obtained for many of the horses, we did not include the results of diagnostic imaging in our inclusion criteria as we did not want to limit the recruitment of horses into the study and we chose to give higher priority to severity of lameness and a positive response to IA anaesthesia as determinants of patient eligibility for IA therapy. Our higher short-term treatment success rate than in some other studies may also have been due to the type of joints affected: 47.5% of our study population consisted of DIP joints and 42.5% were MCP or MTP joints, compared with 100% DIP joints in the study by Dyson [22]. The study on tarsal joint pathology [21] may have included some horses that in fact had proximal suspensory ligament pathology, which would not be expected to recover after distal tarsal joint injection, thus limiting treatment efficacy in that study [8].

Several limitations to this study must be noted. The lack of blinding is an obvious major shortcoming, which may only be partially overcome by the multicentre nature of the trial (as single investigator bias might be 'diluted out'). Secondly, outcome was assessed by the clinician at re-examination of the horse after 3 weeks, but the 3 month outcome data were obtained from the owners by telephone questionnaire; arguably, owner-reported outcome is a less reliable parameter than clinician-assessed outcome. While lameness scoring is a subjective assessment and hence less reliable than more objective lameness evaluations (kinetics, kinematics), the intra-observer reliability of lameness scoring is good [24] and hence pre- and post treatment scores assigned by the same clinician should be a reliable indicator of treatment success. The duration of maximum follow-up was 3 months meaning this does not constitute a long-term study. As a final limitation, the absence of baseline and follow-up data on structural joint damage (e.g. radiography, ultrasonography, arthroscopy) means we could not stratify patients according to severity of joint disease, nor determine the presence or absence of possible chondroprotective effects of HA addition to TA.

In conclusion, more patients that received IA TA had a reduction in lameness of 2 grades or more (88%) than patients receiving the combination treatment (64%) 3 weeks after IA treatment. Approximately half the horses were back in full work at 3 months regardless of treatment assignment. This study does not provide any evidence that a combination of TA with high molecular weight HA is more effective than TA alone for treatment of arthrogenic lameness in horses.

Authors' declaration of interests

No competing interests have been declared.

Ethical animal research

Horses included in the study were client-owned and informed consent was obtained from all owners prior to patient enrolment. As this study does not constitute animal experimentation under Dutch national law, no formal ethical committee approval was sought.

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Authorship

J.C. de Grauw contributed to study design, data analysis and interpretation, and preparation of the manuscript as well as final approval thereof. M. Visser-Meijer, F. Lashley and P. Meeus contributed to study design, study execution, data interpretation and manuscript drafting and final approval. P.R. van Weeren contributed to study design, data analysis and interpretation, and preparation of the manuscript as well as final approval thereof.

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[d]IBM Corp, Armonk, New York, USA.

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Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Supplementary Item 1: List of participating veterinary clinics.
Supplementary Item 2: Standard operating procedure lameness scoring.
Supplementary Item 3: Standard operating procedure intra-articular anaesthesia.
Supplementary Item 4: Standard operating procedure effusion scoring.
Supplementary Item 5: Standard operating procedure synovial fluid sampling, processing and storage.
Supplementary Item 6: Rehabilitation protocol.
Supplementary Item 7: Owner telephone questionnaire for 3 months follow-up.