Retrospective study: Laser excision versus combined laser, cryotherapy and intralesional 5-fluorouracil in the treatment of equine sarcoids.

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April 11, 2024

Abstract

Background Laser excision is used routinely in the treatment of sarcoids but may be ineffective in cases where complete excision cannot be achieved. A multimodal approach is warranted in these cases. 5-FU may improve the lethal effect of cryotherapy as an adjunct to laser excision. *Objectives* To compare two treatment protocols for equine sarcoids: laser excision alone versus a combination protocol of laser excision, cryotherapy, and 5-FU chemotherapy. Factors associated with sarcoid recurrence are also investigated. *Study Design* Retrospective case controlled study. *Results* Eighty-four horses with 168 histologically confirmed sarcoids were included, with a median follow up time of 39 months (IQR 21-62 months). Sarcoid recurrence at the treated site was reported in 38% of cases and in 23% of any individual sarcoids. No significant difference was demonstrated between treatment categories in either rate of sarcoid recurrence (p=0.45 for any treated horse, p=0.63 for individual sarcoids) or time to sarcoid recurrence (p=0.73). Sarcoid recurrence was higher in horses with a greater number of sarcoids (OR 1.2 (1.0-1.5), p=0.03); when treatment had been received prior to admission (OR 7.6 (2.0-33), p=0.004); for individual sarcoids [?]100mm in diameter (OR 5.6 (1.1-30), p=0.03); and for treatment under general anaesthesia (OR 5.0 (1.4-19), p=0.01). Horses with urogenital sarcoids and >1 mixed sarcoid experienced more rapid recurrence (HR 3.6 (1.3-10), p=0.02 and HR 9.9 (3.3-30), p<0.001) and recurrence was less rapid following the treatment of a horse's first sarcoid (HR 0.3 (0.1-0.7), p=0.009). *Main Limitations* Significant differences in case populations in each treatment category. Treatment selection was neither blinded nor randomised and missing data and recall bias limit the study's power. Sarcoid recurrence was owner reported. *Conclusions* When assessing the likelihood of sarcoid recurrence, characteristics of the individual patient and sarcoid(s) are likely to be more significant than selection of any individual treatment protocol.

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Running Title: Treatment of equine sarcoids

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Summary

Background

Laser excision is used routinely in the treatment of sarcoids but may be ineffective in cases where complete excision cannot be achieved. A multimodal approach is warranted in these cases. 5-FU may improve the lethal effect of cryotherapy as an adjunct to laser excision.

Objectives

To compare two treatment protocols for equine sarcoids: laser excision alone versus a combination protocol of laser excision, cryotherapy, and 5-FU chemotherapy. Factors associated with sarcoid recurrence are also investigated.

Study Design

Retrospective case controlled study.

Results

Eighty-four horses with 168 histologically confirmed sarcoids were included, with a median follow up time of 39 months (IQR 21-62 months).

Sarcoid recurrence at the treated site was reported in 38% of cases and in 23% of any individual sarcoids. No significant difference was demonstrated between treatment categories in either rate of sarcoid recurrence (p=0.45 for any treated horse, p=0.63 for individual sarcoids) or time to sarcoid recurrence (p=0.73).

Sarcoid recurrence was higher in horses with a greater number of sarcoids (OR 1.2 (1.0-1.5), p=0.03); when treatment had been received prior to admission (OR 7.6 (2.0-33), p=0.004); for individual sarcoids [?]100mm in diameter (OR 5.6 (1.1-30), p=0.03); and for treatment under general anaesthesia (OR 5.0 (1.4-19), p=0.01). Horses with urogenital sarcoids and >1 mixed sarcoid experienced more rapid recurrence (HR 3.6 (1.3-10), p=0.02 and HR 9.9 (3.3-30), p<0.001) and recurrence was less rapid following the treatment of a horse's first sarcoid (HR 0.3 (0.1-0.7), p=0.009).

Main Limitations

Significant differences in case populations in each treatment category. Treatment selection was neither blinded nor randomised and missing data and recall bias limit the study's power. Sarcoid recurrence was owner reported.

Conclusions

When assessing the likelihood of sarcoid recurrence, characteristics of the individual patient and sarcoid(s) are likely to be more significant than selection of any individual treatment protocol.

Keywords

Horse, sarcoid, recurrence, cryotherapy, chemotherapy

Clinical Relevance

- In a referral population consisting of horses with multiple and/or invasive sarcoids, sarcoid recurrence may be expected in 38% of cases and 23% of individual sarcoids.
- Sarcoid and patient characteristics are likely to be more significant in determining the likelihood of sarcoid recurrence than selection of any particular treatment protocol.
- A multimodal approach employing diode laser excision, cryotherapy and intralesional 5-fluorouracil may be employed in cases where complete sarcoid excision is difficult to obtain.

Introduction

The equine sarcoid is the most common equine cutaneous neoplasm, responsible for approximately 46% of neoplastic cutaneous biopsy samples. (Schaffer et al., 2013; Taylor & Haldorson, 2013) Although tumours are rarely metastatic, aggressive local invasion and secondary infection/ulceration may have a significant impact on equid welfare. (Ireland et al., 2013; Taylor & Haldorson, 2013) Currently no uniformly effective therapy for its treatment has been reported despite publication of numerous treatment protocols. (Lane, 1977; Klein et al., 1986; McConaghy et al., 1994; Carstanjen et al., 1997; Knottenbelt & Kelly, 2000; Martens et al., 2001; McCauley et al., 2002; Byam-Cook et al., 2006; Stewart et al., 2006; Theon et al., 2006; Stadler et al., 2011; Tamzali et al., 2012; Compston et al., 2016; Hollis & Berlato, 2018; Hollis, 2020; Knottenbelt et al., 2020) Laser excision, either by CO_2 or diode laser, is widely used for the surgical removal of sarcoids. (Carstanjen et al., 1997; A. Martens et al., 2001; McCauley et al., 2002; Compston et al., 2016) As with any surgical excision, one of the major limitations is that surgical margins must still be obtained. This is frequently difficult with equine sarcoids, depending on sarcoid type, size and anatomic location. (Knottenbelt, 2019) Though thermal injury secondary to laser excision extends beyond the surgical field, this is variable and difficult to predict. (Knottenbelt, 2019; Tate, 2019) A multimodal approach to sarcoid treatment has frequently been suggested to reduce sarcoid recurrence rates, (Klein et al., 1986; Spoormakers et al., 2003; Tamzali et al., 2012; Knottenbelt, 2019) likely due to greater destruction of remaining neoplastic cells in situations where complete tumour excision has not been achieved.

Cryotherapy, the freezing of cells in this case by the application of liquid nitrogen, kills cells directly due to ice crystal formation and microcirculatory failure. (Baust & Gage, 2005) Papers investigating cryotherapy as a treatment modality suggest success rates (Klein et al., 1986)may be as low as 9% when used as a sole therapy. (Knottenbelt & Kelly, 2000) Recent molecular research has focussed on tissues in the periphery of the cryotherapy treated zone where cell death is delayed and via apoptosis. (Hollister et al., 1998) It has been suggested that further therapy promoting apoptosis in this region may be beneficial in improving the lethal effect of cryotherapy. (Baust & Gage, 2005) (Clarke et al., 1999)

5-fluorouracil (5-FU) is a fluoropyrimidine antimetabolite used in the treatment of a range of cancers, particularly breast and colorectal cancers. (Longley et al., 2003) It exerts its effects via incorporation of the fluoronucleotides into cellular RNA and DNA, leading to subsequent cellular dysregulation and apoptosis. (Longley et al., 2003) In horses, its use has been reported in the treatment of ocular or periocular squamous cell carcinoma, (Pucket & Gilmour, 2014; Offer et al., 2022) and as a topical agent for the treatment of periocular sarcoids with variable success. (Knottenbelt & Kelly, 2000) Intralesional 5-FU alone has a reported sarcoid resolution rate of 61.5%, though rates may be lower in large or 'resistant' tumours. (Stewart et al., 2006) Its use as an adjunct to cryotherapy warrants further investigation since it has been suggested that agents, such as 5-FU, may be beneficial in promoting the apoptotic cell death at the periphery of cryotherapeutic sites. (Clarke et al., 2001; Baust & Gage, 2005)

Objectives and hypotheses

This study aimed to compare two treatment protocols for the treatment of equine sarcoids: laser excision alone versus a combination protocol of laser excision, cryotherapy, and 5-FU chemotherapy. As a secondary aim, factors associated with sarcoid recurrence were investigated. The authors hypothesise that the combination protocol will result in lower sarcoid recurrence rates than the laser excision protocol.

Materials and methods

Records were reviewed for horses referred to Glasgow Equine Hospital for the treatment of sarcoids between 2013 and 2022. Criteria for case inclusion were horses with histologically confirmed sarcoids treated either by laser excision via diode laser, or by the below combination therapy protocol. Cases were excluded if histopathological confirmation was not available, treatment was incomplete, or any other combination treatment was employed.

The standard combination therapy protocol consisted of initial excision of the mass(es) via diode laser with 1-2cm surgical margins where possible. The surgical site was then treated with 3 freeze- thaw cycles of cryotherapy followed by administration of 5-FU into the margins. Cryotherapy was performed using the CryoPro®cryosurgery unit (Cortex Technology, Denmark). The unit was held approximately 10cm from the desired site and liquid nitrogen was applied to spray freeze the site until firm. Care was taken to avoid excessive freezing of the underlying tissues. The tissues were allowed to thaw, before repeating a further 2 times. Thereafter, 50 mg/ml 5-FU (Medac) was injected into the resultant wheal to an estimated dose of 1 ml/cm^3 target tissue. Appropriate personal protective equipment (two layers of nitrile gloves, full length gown and face shield) was used during the application, and the horse handled only when wearing nitrile gloves for 3 days thereafter. The horse was re-examined between 2-5 weeks thereafter, and the cryotherapy and 5-FU application repeated. This was repeated as required by clinical progress, for a median of 3 total treatments (IQR 2-3).

Data obtained from medical records included: case history and signalment, details of the lesion(s) (sarcoid type, location, number, estimated tumour dimensions and volume), treatment protocol, associated adverse effects and duration, response to treatment and sarcoid regrowth/ new lesion occurrence. For the purpose of recording sarcoid location, the body was split into 7 anatomic regions: periocular, head and neck, dorsum (above the level of the olecranon process), ventrum (below the level of the olecranon process), upper limb (above the level of the tarsocrural/ radiocarpal joint), lower limb (below the level of the tarsocrural/ radiocarpal joint) and urogenital (affecting the penile sheath, penis, mammary glands or vulva). Sarcoid type was recorded using the currently accepted convention. (Knottenbelt, 2005) Previous histopathological findings were recorded, including the presence or absence of confirmed surgical margins. The volume of sarcoid was estimated using the ellipsoid volume formula proposed by Tomayko and Reynolds. (Tomayko & Reynolds, 1989)

Following initial correspondence, owners were contacted via telephone between August and October 2022 to determine treatment outcome. An example of the telephone questionnaire is included in Supplementary material 1. Data were collected regarding the horse's history of sarcoid growth and treatment, outcome following sarcoid treatment, regrowth of treated sarcoids and growth of any new sarcoids.

Data analysis

Data were analysed both by case, and then by individual sarcoid. Statistical analysis was performed in R studio (R version 4.2.0). (R Core Team, 2022) Normality was assessed for continuous variables using the Shapiro-Wilk test. Thereafter, univariate analysis was performed by chi-squared test or Fisher's exact test for categorical variables, Wilcoxon's rank sum for continuous variables and Cochrane Armitage test (two sided) for trend, where appropriate.

For variables of $p< 0.2$ in the univariate analysis, logistic regression was performed with sarcoid recurrence as the dependant variable. Variables with $p< 0.2$ in the univariate analysis, $n > 10$ and low collinearity with other independent variables, were used to perform multinominal logistic regression, again with recurrence as the control- dependent variable. Variables were removed from the model in a manual backwards elimination manner with the Akaike information criterion (AIC) used to assess the fit of the model at each step. Variables were retained in the final model despite not fulfilling $p< 0.2$ when their inclusion improved the AIC of the model. Variance inflation factors were used to assess final variables for multicollinearity, and binned residual plots created to assess normality of the residuals.

All variables with $p< 0.2$ in the univariate logistic regression were evaluated for inclusion in a Cox's proportional hazards model. Data were right censored at either time to recurrence of the treated sarcoid, time to follow up telephone conversation or time to death or sale of the horse. Where multicollinearity existed, the value with the lowest p value was included. Numerical covariates were transformed to categorical due to violation of the linearity assumption of the Cox's proportional hazards model. The model was refined using a manual backwards elimination approach, with threshold for inclusion in the model at $p< 0.2$ whilst maximising concordance of the model. A plot of scaled Shoenfeld residuals was used to assess the propor-

tional hazards assumptions of the model. Time to sarcoid recurrence was used to create a Kaplan-Meier plot, and differences between treatment protocols compared with a log rank test. Statistical significance was considered at $p<0.05$.

Results

One hundred and eighty equids were referred to Glasgow Equine Hospital for the treatment of sarcoids during this period, of which 84 met the inclusion criteria. Histological confirmation was available for 168 individual sarcoids in these 84 horses. Of these, 8 (5%) were categorised clinically as occult sarcoids, 26 (15%) as verrucose, 80 (48%) as nodular, 24 (14%) as fibroblastic and 26 (15%) as mixed. None were classed as 'malevolent,' and sarcoid type was not recorded in 4 cases (2%). Seventeen different breeds were represented, of which 17 horses were crossbreeds (20%), 11 Irish Sport Horses (13%), 10 warmbloods (12%), 6 Thoroughbreds (7%) and 5 cobs (6%). Four donkeys were included in the study (5%), and the remaining horses were of various breeds. Thirty-two of included equids were mares (38%), 51 geldings (61%) and 1 stallion (1%). Ages ranged from 2-20 years (median 8 years (IQR 6-14 years)). Owner follow up was available for 69/84 individuals, and regrowth ascertained from medical records in a further 4. Horses for which follow up was not available were excluded from further analysis. Sixty two horses had multiple sarcoids at the time of presentation, with a median of 3(IQR 2-7).The overall incidence of recurrence of any sarcoid on an equid at the same previously treated site was 38% (28/73) and time to follow up ranged from 0.75-132 months (median 39 months (IQR 21-62 months)). For any individual treated sarcoid, total rate of recurrence was 23% (29/128) with a median length of follow up of 48 months (IQR 24-61 months).

In total, 28 equids were treated by the combination treatment protocol (33%) and 56 by diode laser excision alone (67%). Complications occurred in 14 horses following either treatments (17%). These were short lived and comprised: oedema surrounding the treated site (5/14, 36%), owner reported delayed healing (3/14, 21%) or infection (3/14, 21%) of the treated site, and individual cases of myiasis, facial nerve neuropraxia and excessive scarring (1/14, 7% each). Overall, 60/67 owners (90%) said they were happy with the final cosmetic result of the treatment. Of the 16 horses reported to have been euthanised prior to follow-up, four of these were for sarcoid- related reasons, resulting in a fatality rate of 4/69 (5.8%) over the duration of the follow up period in this referral population.

Sarcoid recurrence at any treated site was reported in 20/47 (43%) of horses receiving laser excision alone and 8/26 (31%) treated by combination therapy for which follow up was available. For any individual sarcoid, eventual recurrence at the same site occurred in 23/95 (24%) of those treated by laser excision and 6/33 (18%) receiving the combination protocol. These differences were not statistically significant in either case ($p=0.32$ and $p=0.48$ respectively). Further differences in the case populations between treatment categories were examined to aid comparison between groups, and results are presented in Supplementary Material 2. Briefly, equids in the combination category had larger sarcoids (median width 59 mm versus 30 mm, $p<0.001$) but were less likely to have any incision closed at the time of excision ($p < 0.001$).

Postoperative complications were more frequent in horses treated by combination protocol (9/25 (36%) versus 5/45 (11%), $p=0.03$) and length of follow up significantly shorter (20months (14-25 months) versus 52 months (39-72 months), $p<0.001$).

Likelihood of Sarcoid Recurrence

A forest plot of variables significant in the logistic regression on both a whole case and individual sarcoid basis is shown in Figure 1. Full results are available in Supplementary material 3.

Recurrence was more likely if the ventrum was affected (OR 3.9 (1.5-11 $p<0.001$), particularly by verrucose (OR 1.9 (1.2-3.4), $p=0.01$) or mixed (OR 1.9 (1.3-3.5), $p=0.002$) sarcoids. Nineteen horses and 20 individual

sarcoids had received attempted sarcoid treatment prior to presentation, and this was significantly associated with subsequent sarcoid recurrence (13/19 (68%), OR 7.7 (2.5-27) p<0.001 and 9/20 (45%), OR 4.9 (1.5-16) p=0.004). A greater total number of sarcoids at presentation was also associated with sarcoid recurrence (OR 1.3 (1.1-1.5), p<0.001) as was the surgical closure of any incision at the time of initial treatment (OR 4.0 (1.5-11.6), p=0.007). Sarcoid chronicity was also associated with recurrence both for affected animals ('cases') and individual sarcoids (p=0.03 and <0.001) and any individual sarcoid treated under general anaesthesia was more likely to recur (OR 6.3 (2.5-17) p<0.001) than if treated standing under sedation.

The final multinominal logistic regression models for variables predictive of sarcoid recurrence are presented in *Table 1* . The effect of treatment category on recurrence rate whilst accounting for significant population differences between treatment category (largest sarcoid width (mm), closure of any incision, occurrence of complications following treatment and length of case follow up (days)) was examined. Treatment category remained non-significant in this model (OR 1.3 (0.14-13), p=0.82).

Time to sarcoid recurrence

Data were available regarding time to sarcoid recurrence were available for 72 horses. Variables retained as significantly associated with time to sarcoid recurrence in the Cox's Proportional Hazards model are presented in *Table 2* and the final Kaplan- Meier plot of sarcoid recurrence for each treatment category is presented in *Figure 2* . The log-rank test comparing the survival curves again indicated no significant difference between treatment categories (p=0.73).

Discussion

Treatment protocols

The rate of recurrence of any sarcoid in this study was 23%, with a median length of follow up of 48 months. There was no significant difference in recurrence between treatment category (laser 24% (23/95), combination 18% (6/33)). The lack of routinely accepted standardised outcome measure for the treatment of sarcoids makes comparison between studies very difficult. (Offer et al., 2024) Though these recurrence rates are marginally higher than those reported in the literature (sarcoid regression rates of 83- 89% with sole laser excision), (Martens et al., 2001; Compston et al., 2016) this is highly likely to be influenced by numerous factors, including greater length of follow up period in this study, and differences in case selection and treatment populations. These horses were all referred to the hospital for further evaluation and treatment, and by definition were positioned at the more severe end of sarcoid phenotype presentation.

Employing a multimodal approach to the treatment of sarcoids has been suggested by previous authors as a method to improve sarcoid regression rate, (Knottenbelt, 2019) and was highlighted by this author's recent systematic review as likely to be advantageous over single treatment modalities. (Offer et al., 2024) There are several confounding factors that may have prevented the demonstration of a significant difference between treatment categories in this retrospective study. Firstly, there exist several significant differences in the case populations between treatment groups. Sarcoids treated by the combination protocol were significantly larger (p<0.001) and were less likely to have been treated under general anaesthesia (p=0.04). Both these factors may prevent attainment of an adequate surgical margin, depending on the location of the sarcoid and the temperament of the horse. Sarcoids in the combination treatment category were also on significantly older horses. Whilst rare, spontaneous remission does occasionally occur in younger horses, and so this may have further confounded the results. (Berruex et al., 2016) Conversely, equids treated by laser therapy alone had a significantly longer time to follow up than those treated by the combination protocol (median 52 months versus 20 months) due to the distribution of cases and historical clinician preference. Longer term recurrence may therefore be underestimated in the combination protocol treatment category.

However, when a multivariable model was constructed to account for differences in case population between categories, treatment category remained non-significant in the model (p= 0.82). Since sarcoid recurrence is

dependent on numerous variables, (Knottenbelt, 2019) case selection is crucial in determining its likelihood. A true comparison of these treatment techniques would therefore require a fully randomised and blinded controlled trial.

Risk factors for recurrence

The total number of sarcoids on the individual at the time of presentation was significant in both the univariate and multivariate logistic regression. This supports increased susceptibility of certain individuals to the development of sarcoids. This is well recorded in the literature- the presence of multiple sarcoids has been reported by several authors as predictive of an increased likelihood of sarcoid recurrence, (Lane, 1977; Compston et al., 2016) and it has been shown by numerous authors that the susceptibility to sarcoids has at least some genetic basis. (Broström et al., 1988; Lazary et al., 1994; Christen et al., 2014) The presence of equine leukocyte antigen (ELA) W13 allele has been correlated with susceptibility to sarcoid growth, although horses without the allele may also develop sarcoids. (Goodrich et al., 1998) Candidate genes within specific chromosomal regions also have been associated with increased susceptibility to sarcoid growth, (Jandova et al., 2012) and a polygenic mode of inheritance with 21% heritability has been demonstrated. (Christen et al., 2014) Presentation at the time of first sarcoid growth was similarly retained in the survival analysis as predictive of an increased time to subsequent sarcoid regrowth, i.e. those individuals with increased susceptibility to sarcoid growth who present with multiple sarcoids spanning several years have a shorter time to regrowth than those with solitary sarcoids at first presentation.

Treatment of any sarcoid prior to presentation was associated with sarcoid recurrence and was retained in the whole case multivariable model. The reason for this is two-fold; presentation following prior treatment indicates that any previous intervention has failed to resolve the sarcoid. The sarcoid itself is therefore likely to be highly locally invasive and/or anatomically difficult to remove in its entirety. In addition, any traumatic intervention to a sarcoid may cause accelerated growth or increased malignancy, leading to subsequent difficulty in achieving full sarcoid resolution. (Knottenbelt et al.) Similarly, recurring sarcoids are likely to be more aggressive and infiltrative than those treated on first presentation. (Taylor & Haldorson, 2013)

Further variables associated with sarcoid recurrence in this study may be associated with the ability to achieve surgical margins at initial laser excision. Sarcoid chronicity and size are likely correlated and affect the ability to achieve complete tumour excision. There is likely to be conscious reluctance to create huge skin deficits in horses with the largest sarcoid masses. Similar difficulties occur when removing sarcoids on the ventrum, particularly when attempted under standing sedation, as limited by patient tolerance and staff safety. Verrucose and, to a lesser extent mixed sarcoids (often with verrucose boundaries), have often been reported to have a poorer outcome than other sarcoid morphological types. (Knottenbelt & Kelly, 2000; Compston et al., 2016) It has been hypothesized that these sarcoids may extend further into the dermal layer than other types, and gross margins are less defined, potentially influencing the attainment of surgical margins. (Martens et al., 2000; Compston et al., 2016) Complete excision of neoplastic cells logically is often stated as a significant factor contributing to sarcoid resolution, (Carstanjen et al., 1997; Knottenbelt, 2019) though the desirable surgical margins for equine sarcoids have not been well defined. A margin of between 1 and 2cm is often stated as desirable as BPV DNA has been demonstrated in 33% of cases with a surgical margin of 16mm, (Martens et al., 2001; Compston et al., 2016; Knottenbelt, 2019) and histopathological evidence of sarcoid infiltration has been demonstrated at 2cm from the sarcoid margin removed via laser excision. (Mair & Fews, 2016) Coagulative necrosis extends beyond the surgical site when laser excision is employed but this may be insufficient to prevent sarcoid recurrence in verrucose or mixed sarcoids where neoplastic cells extend beyond this region of necrosis. (Knottenbelt & Kelly, 2000; Compston et al., 2016) However, the presence of papillomaviral DNA within the surgical margin has inconsistently been associated with sarcoid recurrence, as has any association between width of surgical margin and recurrence. (Martens, et al., 2001)

The treatment of a sarcoid under general anaesthesia was identified as a factor positively associated with recurrence in this study. Whilst general anaesthesia and patient recumbency allow for greater and more

precise access to the sarcoid sites, this is confounded in this study by case selection and clinician preference. This should therefore be interpreted with extreme caution, and restraint via general anaesthesia should remain the preferred option for equids presenting with numerous, large, or infiltrative masses, or where patient temperament does not allow surgical excision whilst standing.

Limitations

There are a number of limitations to this study. Firstly, as with any retrospective study, missing data, and loss of cases to follow up limits its power. Owner recall bias may skew results, though answers were corroborated with written clinical records where possible. The data described here apply only to a referral hospital with one case population. With the treatment of equine sarcoids, case selection plays a significant role in the likelihood of tumour recurrence. Though populations in each treatment group were compared, they were not identical and treatment assignation was neither randomised nor blinded. Similarly, there was no untreated or placebo control population in this study, and so findings should be interpreted with caution. A significant limitation with the survival analysis was the differing follow up times between treatment categories, although it should be noted that both exceeded the 12-month period used in many previous studies. This may have prevented any demonstration of differences in time to sarcoid regrowth between groups.

Conclusion

No significant difference in sarcoid recurrence rate or time to sarcoid recurrence has been demonstrated with the use of a cryotherapy/chemotherapy protocol following laser excision versus laser excision alone, though this is limited by available time to follow up and differences in case selection. When assessing the likelihood of sarcoid recurrence, characteristics of the individual patient and sarcoid(s) are likely to be more significant than selection of treatment protocol. Regardless of treatment protocol, approximately 25% sarcoid recurrence may be expected at 24 months following hospital discharge.

Declarations

Authorship

K. Offer was responsible for data collection and analysis. Both authors were involved in preparation of the manuscript.

Authors declarations of interest

The authors have no competing interests to declare.

Source of funding

No funding was required for the study.

Ethical animal research

Ethical approval was obtained from the University of Glasgow Research Ethics Committee (reference EA42/21).

Acknowledgements

The authors would like to acknowledge the Joana Devesa and Amie McGarva for their help in data gathering.

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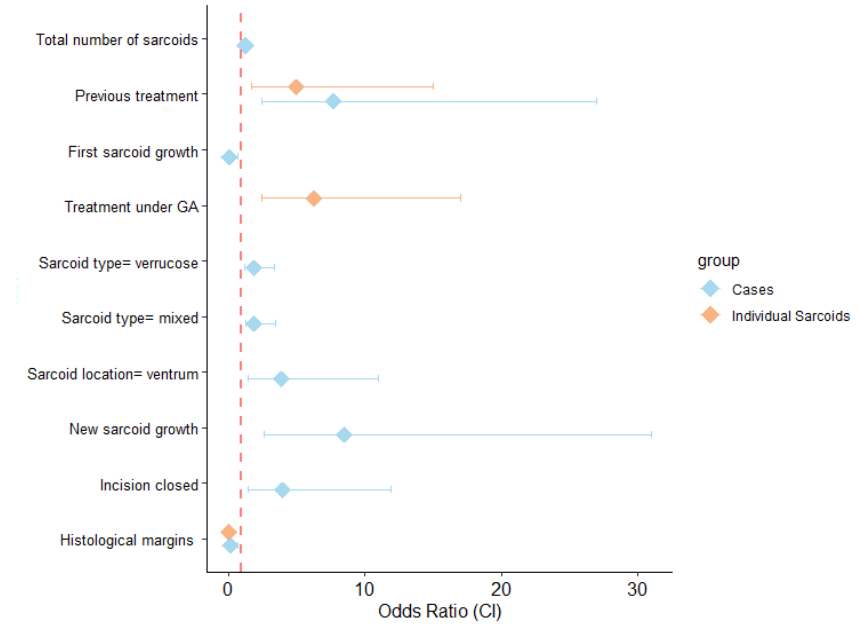
Figure/ Table Legends

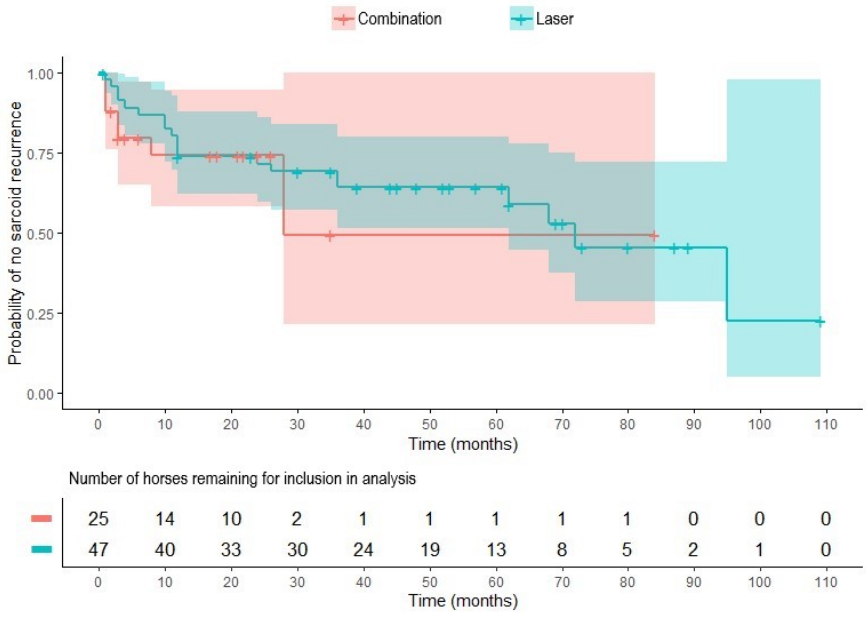
Table 1: Multinominal logistic regression models for variables associated with sarcoid recurrence in 84 horses with histopathological confirmation of sarcoid(s).

Table 2: Cox's proportional hazards model of variables associated with time to sarcoid recurrence showing Hazard Ratios (HR) and 95% confidence intervals [CI] for included variables.

Figure 1: Forest plot showing Odds Ratios (OR) and 95% confidence intervals for variables significantly associated with likelihood of sarcoid recurrence within an individual and for individual treated sarcoid lesion. The red dashed line indicates an OR of 1.

Figure 2: Kaplan-Meier plot showing time to sarcoid recurrence in both treatment categories. The table shows the number of included horses remaining with no sarcoid recurrence at each time point and in each treatment group, and therefore remaining for inclusion in analysis.





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