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Subconjunctival autologous muscle-derived mesenchymal stem cell therapy: A novel, minimally invasive approach for treating equine immune-mediated keratitis

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Abstract

Objective: To establish the safety of subconjunctival injections of autologous muscle-derived mesenchymal stem cells (mdMSCs) in healthy horses and to evaluate their effect in four horses (six eyes) with severe chronic equine immune-mediated keratitis (IMMK) that was unresponsive to medical treatments.

Methods: MdMSCs were cultured from minimally invasive muscle biopsies. In the safety group, four healthy horses received two subconjunctival injections of 2.5 and 5 million cells, respectively, at 1-month interval, to the same eye. Ocular side effects were monitored for 1 month following each injection. In the treatment group, six eyes received four to seven subconjunctival mdMSCs injections (2.5 or 5 million cells per injection) every 4 weeks, approximatively. Medical treatment was discontinued 1 week before and throughout the entire treatment period. A scoring system was used to assess the evolution of the ocular lesions.

Results: In the safety group, all horses exhibited mild to moderate chemosis and conjunctival hyperemia at the injection site, lasting 24–48 h. In the treatment group, all eyes initially responded positively to therapy, with a reduction in lesion scores observed after the first injection. Four eyes achieved control of the lesions with repeated injections during the 9.2 months of follow-up.

Conclusion: The first subconjunctival injection of mdMSCs resulted in improvement of the ocular lesions. Repeated injections were found to be safe, minimally invasive and showed promise in managing refractory cases of equine IMMK. Further studies are warranted to demonstrate the long-term benefits of these injections and to optimize the therapeutic protocol.

KEYWORDS

cellular therapy, cornea, eye, horse, immune system disease, immunomodulation

1 | INTRODUCTION

Equine immune-mediated keratitis (IMMK) is a nonulcerative keratitis of idiopathic origin commonly diagnosed in veterinary ophthalmology.[1,2] IMMK is characterized by varying degrees of corneal vascularization, opacification, and is generally nonpainful.[1–4] While the lesions are usually not globe-threatening, they can significantly impair vision if not properly controlled medically.[1] The precise immunopathogenesis of the disease remains unclear, but dysregulation of the adaptive immune responses is suspected.[2,3] On histopathology, the disease shows a predominant lymphocytic–plasmocytic infiltration with $CD4^+$ T helper cells and $CD8^+$ cytotoxic T cells.[3,4]

IMMK usually requires long-term medical treatment consisting of immunomodulatory and anti-inflammatory medications.[2,4] Therapy can be challenging, as the disease often becomes increasingly refractory to medical treatment and prone to recurrence over time.[2] In addition, prolonged use of steroidal anti-inflammatory treatment can lead to corneal degeneration.[1] Several surgical approaches have been described for treating IMMK, including episcleral cyclosporine implants[5] and lamellar keratectomy.[4,6] Episcleral cyclosporine implants address owner compliance issues but their effectiveness may be limited in cases that are unresponsive to medical treatment.[5] Lamellar keratectomy, with or without conjunctival grafting,[4,6] has shown success in certain refractory cases. However, it is more invasive and carries potential complications as secondary infection[6] and permanent fibrosis at the keratectomy site.[1] Therefore, alternative therapeutic approaches are of great interest.

Mesenchymal stem cells (MSCs) were initially known for their regenerative potential, differentiating into specialized corneal cells such as keratocytes, epithelial cells and endothelial cells, to aid wound healing and improve corneal optical properties.[7,8] However, the focus surrounding MSCs has shifted to their immunomodulatory effects, as they modulate both innate and adaptive immune system through cytokine secretion,[9–11] regulating various immune cell functions.[12] This direct immunomodulation makes MSCs a promising therapy for immune-mediated diseases.

MSCs have been extensively investigated as an alternative treatment for degenerative and inflammatory diseases, including various ocular pathologies.[13] The use of MSCs as a treatment for equine IMMK has only been reported in one study to date.[14]

Bone marrow and adipose tissue are the two most commonly exploited sources of adult MSCs.[15–17] However, these sampling methods are invasive and not easily performed.[18]

In 2017, Ceusters et al.[19] introduced a minimally invasive method to obtain multipotent mesenchymal stem cells from muscle microbiopsies (mdMSCs) in mammals. This technique requires only 10–20 mg of tissue and allows for the production of large quantities of mdMSCs. They also demonstrated the mdMSCs' ability to inhibit the proliferation of T lymphocytes, highlighting their potential as a therapy for IMMK.[19]

The objectives of this study were to establish the safety of subconjunctival injections of autologous mdMSCs, obtained through this new minimally invasive technique,[19] in healthy horses and to evaluate their efficacy in six eyes of four horses affected by severe chronic equine IMMK that did not respond to medical treatments. This study includes clinical findings, a description of the procedures performed, and outcomes including an objective scoring system used to evaluate response to treatment.

2 | MATERIALS AND METHODS

2.1 | Ethics statement

The protocol involving healthy horses in this study was approved by the Ethical Committee of the University of Liège (Approval No 22-2456). The institution owning the healthy horses has granted consent for their inclusion in this study, as well as for the publication of the resulting data. All affected horses were enrolled in this study as part of a clinical trial on the use of equine autologous mdMSCs at the Veterinary Teaching Hospital of the University of Liège. Prior to the study, all owners were provided information about the clinical trial and gave their informed consent for their horses to receive mdMSCs therapy. They also consented for the data and images to be published. Throughout the study, the horses remained in their home environment under the care of their owners and were only examined and treated as outpatients at the Veterinary Teaching Hospital of the University of Liège.

2.2 | mdMSCs: sampling, culture and transplantation

Muscle-derived stem cells (mdMSCs) were isolated using a patented method (Mammalian muscle-derived stem cells, WO/2015/091210) as described by Ceusters et al.[19]

To initiate the culture of multipotent mdMSCs, microbiopsy specimens (approximately 10 to 20 mg of tissue) were obtained from the long head of the triceps brachii muscle. For muscular biopsy, some horses were sedated with 2–5 μg/kg IV of detomidine hydrochloride 10 mg/mL (Domidine®, Dechra Veterinary Products) based on their behavior. The sampling procedure was performed using a semi-automatic 14-gauge microbiopsy needle. The sampling site was shaved and aseptically prepared,

and 1 mL of 2% lidocaine (Xylocaine® 2%, Astrazeneca) was subcutaneously injected. The microbiopsy was collected through a small skin puncture. Immediately after collection, each sample was placed in culture medium and maintained at 4°C until use. The culture medium (DF20) consisted of 500 mL DMEM – Ham's F12 (Gibco™, Thermo Fisher Scientific) supplemented with 100 mL heat-inactivated of Foetal Bovin Serum (FBS) from South America (Gibco™, Thermo Fisher Scientific), 5 mL of penicillin/streptomycin (Gibco™, Thermo Fisher Scientific), and 2.5 mL of amphotericin B (Gibco™, Thermo Fisher Scientific). Microbiopsies were cut into 16 pieces in a Petri dish and placed in the 16 central wells of a 24-well plate (Greiner Bio-One). To each well, 150 µL of DF20 were added, while the outer wells were filled with PBS (1 mL/well). The culture dishes were incubated at 37°C under a controlled atmosphere of 5% CO_2 and 21% O_2 until a halo of cells was visible around the muscle piece. The mdMSCs were isolated from cells obtained from the explants. The cells were harvested using TrypLE (Gibco™, Thermo Fisher Scientific), centrifuged (200 × *g*, 10 min, 37°C), and the pellet was suspended in 1 mL of HBSS (Gibco™, Thermo Fisher Scientific). The cellular suspension was then placed on a three-layer discontinuous Percoll density gradient (15%, 25%, and 35%) and centrifuged at 1250 × g (25°C, 20 min). The 15%–25% fraction was collected, washed once with HBSS and centrifuged at 200 × *g*, 10 min at 37°C. The supernatant was discarded, and the pellet was suspended in 1 mL of DF20. The cells were initially cultured in T-25 cm^2 flask (Nunc™, VWR) until 80% confluence. Subsequently, they were expanded in T-175 cm^2 flask (Nunc™, VWR) until a sufficient number of cells was obtained.

For subconjunctival injection, horses were sedated with 2–5 µg/kg IV of detomidine hydrochloride 10 mg/mL (Domidine®, Dechra Veterinary Products). A palpebral nerve block was performed using 1 mL of 2% lidocaine (Xylocaine® 2%, Astrazeneca). Additionally, half a milliliter of tetracaine hydrochloride 10 mg/mL ophthalmic solution (Bausch & Lomb) was applied topically. One milliliter of mdMSCs suspension (2.5 or 5 million cells/mL) was agitated through a 22-gauge needle into a 3 mL syringe. A 25-gauge needle was then attached to the 3 mL syringe, and the cells were injected just under the bulbar conjunctiva in the subconjunctival space.

2.3 | Safety study

A preliminary preclinical study was conducted to assess the safety of subconjunctival autologous mdMSCs injection. The horses received an initial injection of 2.5 million cells, followed by a second injection of 5 million cells after 1 month, both administered to the same eye under the dorsal bulbar conjunctiva. Ophthalmological examinations were conducted twice a day for 1 week following the injections, and then weekly for 1 month, to monitor any potential ocular side effects. Briefly, a score was assigned to each potential side effect according to its severity, with a total score ranging from 0 (normal) to 36 (highest score). A power analysis was performed to determine the required sample size for assessing the safety of repeated mdMSCs injections. Using a standard deviation score of 3, an alpha level of .05, and a power of 80%, the calculation indicated that four animals would be required.

2.4 | Cases selection

Horses diagnosed with IMMK and admitted to the Veterinary Teaching Hospital of the University of Liège were included in this study. The inclusion criteria required a minimum duration of pathology of 1 year and a non- or limited response to the currently recommended treatment which consisted of topical glucocorticoids and topical cyclosporine. The treatment had to be administered for at least 6 months before considering the case as refractory. Horses with other ocular conditions such as lacrimal deficiency, infection, glaucoma, and systemic conditions were excluded. Corneal ulcers associated with IMMK were not excluded from the study.

Collected data included breed, age, sex, date of disease onset, medical treatment history, and IMMK classification (epithelial, anterior, mid or deep stromal). Diagnosis of IMMK was based on clinical signs including corneal opacification with active vascularization and cellular infiltration. Topical and systemic anti-inflammatory or immunomodulatory treatments were discontinued at least 1 week before the stem cell therapy and throughout the entire treatment period.

The injection site was selected based on the severity of the corneal lesions, targeting the bulbar area closest to the most severe lesions. An initial dose of 2.5 million cells per injection was administered, which was later increased to 5 million cells per injection in cases where there was a partial response or deterioration. Injections were repeated approximately every 4–6 week, with a total of 4–7 injections.

A scoring system used employed to evaluate ocular comfort, conjunctival hyperemia, severity of corneal opacification (including cellular infiltration and fibrosis), calcific degeneration, and corneal vascularization. The SPOT System Section for the Anterior Segment[20] had to be modified (Table 1) and adapted to suit our specific cases: specifically regarding scoring of corneal vascularization and degeneration that had to be adjusted. The

TABLE 1 Scoring System extrapolated and adapted (*) from SPOT System for anterior segment.[13]

Observation/lesion	Score	Description
Discomfort* (blepharospasm, lacrimation)	0	Normal. No blepharospasm, no lacrimation
	1	Eyelashes downwards, no lacrimation
	2	Partially closed eye, no lacrimation
	3	Totally closed eye, lacrimation
Conjunctival hyperemia	0	Bulbar conjunctiva is normal. Small, pale pink vessels may be observed, primarily at, or adjacent to, the limbus
	1	Pink-to-red bulbar conjunctival vessels with minimal branching are visible extending 1–3 mm posteriorly from the limbus toward the conjunctival fornix
	2	Prominent red bulbar conjunctival vessels with multiple branches are visible extending from the limbus to the conjunctival fornix
	3	Red-to-dark red, engorged bulbar conjunctival vessels with extensive branching and/or tortuosity are visible extending from the limbus to the conjunctival fornix. The conjunctiva between large vessels may have a flushed pink-to-red appearance
Corneal opacity severity (cellular infiltration/ fibrosis)	0	Normal cornea. Appears with the slit lamp as having a bright gray line on the epithelial surface and a bright gray line on the endothelial surface with a marble-like gray appearance of the stroma
	1	Minimal loss of corneal transparency. With diffuse illumination, the underlying anterior segment structures are clearly visible, although corneal opacity is apparent to an experienced observer
	2	Mild loss of corneal transparency. With diffuse illumination, the underlying anterior segment structures are visible, although there is a reduction in the ability to appreciate their detail
	3	Moderate loss of corneal transparency. With diffuse illumination, there is a greater inability to see the details of the underlying anterior segment structures than with a score of 2, but the observer is still able to score aqueous flare, iris vessel congestion, observe for pupillary response, and note lenticular changes
	4	Severe loss of corneal transparency. With diffuse illumination, the underlying anterior segment structures cannot be seen so that the evaluation of aqueous flare, iris vessel congestion, pupillary response, and lenticular changes is not possible
Corneal vascularization*	0	Normal or ghost vessels
	1	Inactive blood vessels
	2	Localized, active blood vessels
	3	Diffuse, active blood vessels
Calcific degeneration*	0	Normal cornea with no area of corneal calcification
	1	Rare, focal mineralization
	2	Diffuse mineralization
	3	Dense plaque of mineralization

effectiveness of the treatment was assessed based on the changes observed in the scoring system.

2.5 | Statistical analysis

Statistical analysis was performed using MEDCALC® STATISTICAL Software. A Friedman test followed by a Wilcoxon test were used to compare the total score lesion before stem cell therapy (T0), after one injection (T1) and at the end of the protocol (T2). Significance was set at $p \leq .05$.

3 | RESULTS

3.1 | Safety study

The study involved four healthy mares from the Horse European Centre of Mont-le-Soie. For both doses (2.5 and 5 million cells), none of the horses exhibited blepharospasm, ocular discharge, corneal oedema, cellular infiltration, nor corneal ulcers. Mild to moderate chemosis and conjunctival hyperemia were noted at the injection site in all horses, which resolved within 24 to 48 h. The highest score reached was 5/36, which then dropped to 0 within 48 h.

TABLE 2 Description of breed, gender, age, eye affected, type and duration of IMMK.

Case	Breed	Gender	Age (years)	Eye	IMMK classification	IMMK duration
1	Warmblood	Mare	11	Right	Anterior stromal	4 years
2	Quarter horse	Gelding	24	Right	Anterior stromal	7 years
3	Quarter horse	Gelding	24	Left	Anterior stromal	3 years
4	Quarter horse	Stallion	10	Left	Anterior to mid stromal	1 year
5	Warmblood	Mare	20	Right	Mid stromal	1.5 year
6	Warmblood	Mare	20	Left	Mid stromal	1.5 year

3.2 | Case selection

Patient information is provided in Table 2, with each eye representing one case.

Four horses (two Warmbloods and two Quarter horses) were included in the study. There were two mares, one stallion and one gelding, ranging in age from 11 to 24 years. Two horses had both eyes affected by IMMK. The duration of IMMK ranged from 1 to 7 years, and the classification of the disease was either anterior or mid stromal. None of the cases had achieved medical control of the disease using glucocorticoids and immunomodulators. Case 5 had an history of a perforating ulcer, which was treated with corneal graft and conjunctival pediculated graft. Additionally, a nasal keratectomy was performed to remove calcific degeneration. This surgery was conducted 6 weeks prior to the administration of stem cell therapy.

3.3 | Evolution of ocular lesions

The evolution of the ocular lesion score and the treatment protocol of each case, are presented in Figure 1. A photographic montage of the six eyes is shown in Figure 2.

None of the horses experienced ocular discomfort. Conjunctival hyperemia improved in all cases. Cellular infiltration decreased after the initial injection, but relapses occurred in cases 1, 3, and 4, with only case 3 showing improvement after subsequent injections. In cases 2, 5, and 6, cellular infiltration resolved with the injections. Corneal vascularization remained stable or improved in all cases, particularly in case 6. Cases 1 and 4 showed a mild reduction in the calcific degeneration score while case 6 exhibited excellent improvement after receiving four injections. There was a slight reduction in fibrosis in a few cases, but overall, it remained stable in all cases.

All eyes initially responded positively to therapy, with a reduction in the total lesion score after a single injection. Among the three eyes that experienced recurrence between injections, one eye responded positively to an additional injection. In conclusion, four eyes remained under control with repeated injections throughout the study period.

The mean follow-up time was 9.2 months.

3.4 | Statistical analysis

The total score lesion of T0, T1 and T2 were significantly different ($p = .013$). The total score lesion was significantly different between T0 and T1 ($p = .03$) but not between T0 and T2 ($p = .06$) (Figure 3). Due to low number of eyes treated, further statistical analysis has not been performed.

4 | DISCUSSION

Equine immune-mediated keratitis is characterized by corneal opacities that impair vision and results from local immune system dysregulation.[1,2] Traditional treatment options often require lifelong administration, which can lead to side effects and may become ineffective in some cases.[2] Therefore, the introduction of innovative treatment approaches is crucial. In this study, we investigated the use of subconjunctival autologous mdMSCs in the treatment of six chronically affected eyes that showed poor response to conventional treatment.

The preclinical study and the absence of immediate nor long-term adverse effect in the treated horses demonstrated the safety of this therapeutic approach.

All of the affected eyes initially responded positively to therapy, with a significant reduction in the score lesion after the first injection. Four eyes remained under control with repeated injections throughout the study period. These cases reached better outcomes compared to the previously used conventional medical treatment, and the owners expressed their satisfaction with the progress. Recurrences occurred in three cases between injections, and only one of them responded positively to an increased dose of injected cells, ultimately regaining control. While the total lesion score never reached zero, the inflammatory aspects of the disease, including cellular infiltration and

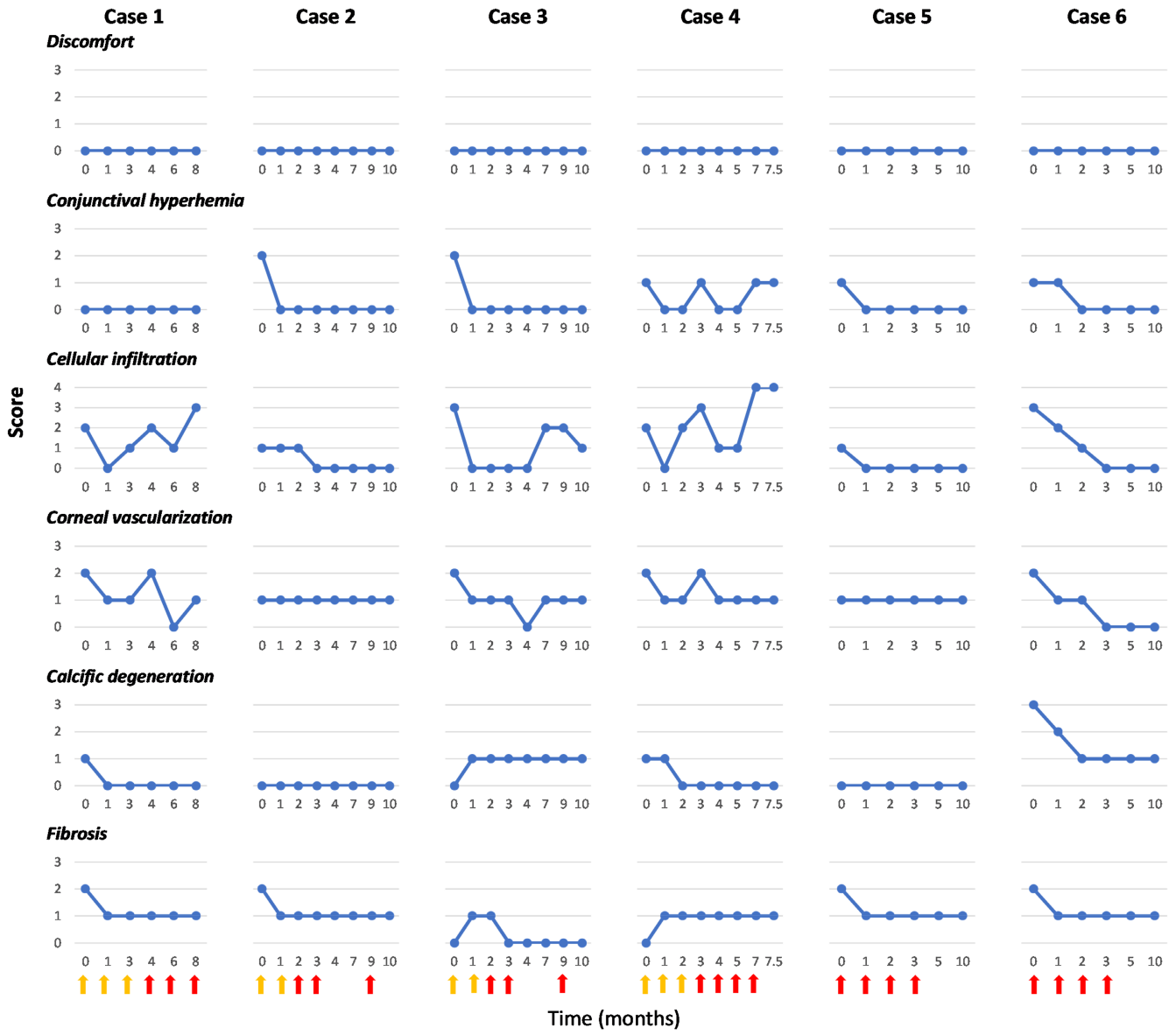

FIGURE 1 Examination results in six eyes based on modified SPOT scoring system: evolution of the ocular lesion score during the follow-up (in months). Yellow arrow = injection of 2.5 million mdMSCS. Red arrow = injection of 5 million mdMSCs.

active vascularization, seemed to be effectively managed in the four responsive cases. It should be noted that complete resolution of fibrosis is rarely achievable in chronic keratitis cases, as observed in our four horses.

Our findings are comparable to those of Davis et al.[14] who investigated the efficacy of subconjunctival bone marrow-derived stem cell (BM-MSC) therapy for equine IMMK. In their study, horses received subconjunctival autologous BM-MSC injections (15 million cells per injection) every 3–4 weeks for a total of 1–5 treatments. Three horses showed a positive response to therapy, while one horse remained unresponsive. The treatment effect was longer in their study (7, 8 and 18 months). However, in their study, horses were maintained on their current medical treatment regimen throughout and sometimes after the BM-MSC treatment period. Although the treated cases were also considered refractory to treatment, a synergistic effect of the two treatments cannot be excluded, which could explain the longer efficacy observed. Further studies are still warranted to achieve long-term control of corneal disease after discontinuation of our treatment protocol.

The precise mechanism through which MSCs exert their effects on keratitis remains to be fully understood. Various potential mechanisms have been proposed.[11,21] MSCs have the ability to secrete various signaling molecules, including neurotrophic factors, growth factors, and cytokines, which can diffuse within the local tissue environment and interact with surrounding cells.[21] This ability enables MSCs to regulate the immune response of the recipient by influencing the maturation and function of

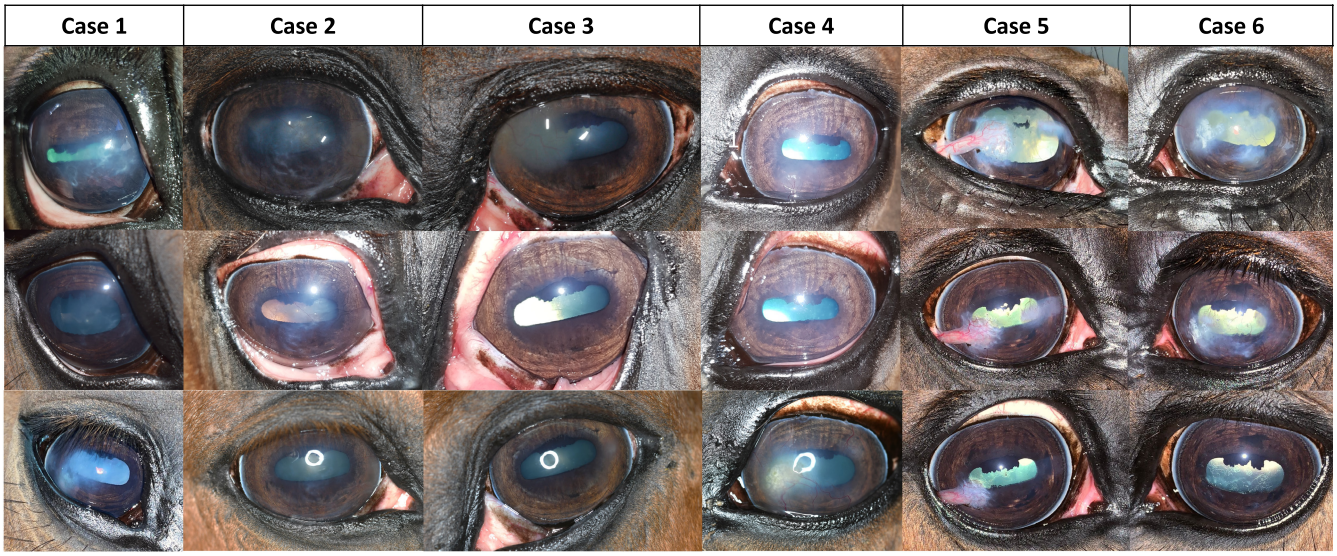

FIGURE 2 Clinical photographs of the six eyes described in this report. The first line shows the eyes prior to mdMSCs subconjunctival injection exhibiting lesion consistent with IMMK. The second line shows the eyes after the first mdMSCS injection, with noticeable improvement in all cases. The third line shows the eyes after mdMSCs therapy. Cases 1 and 4 remained unresponsive at the end of the treatment period.

multiple immune cells. Additionally, MSCs possess antifibrotic properties by secreting human growth factor, promoting matrix remodeling, and enhancing cell survival through the inhibition of oxidative stress.[8,11,22] Studies have also shown that MSCs can inhibit both hemangiogenesis and lymphangiogenesis[23] and have antiapoptotic properties.[24] These immunomodulatory properties, among other mechanisms, likely contribute to the positive effects observed in our clinical cases of IMMK.

One notable advantage of our study is the utilization of muscular-derived stem cells. Collecting this type of stem cells involves a less invasive and less painful procedure compared to bone marrow or fat collection, requiring only local anesthesia and sedation in some cases.[18,19] While mdMSCs may have been studied to a lesser extent compared to other types of stem cells, they demonstrate in vitro immunomodulatory properties,[19,25] especially on lymphocytes, which renders them an attractive therapeutic option.

It is important to note that while MSCs are generally considered to have low immunogenicity, they do express major histocompatibility complex (MHC) class I molecules and, to some extent, MHC class II molecules. This means that MHC-mismatched MSCs have the potential to trigger an immune response both in vitro and in vivo.[26,27] As a precautionary measure, all potential donor MSCs should undergo immunophenotyping and screening for MHC expression. Despite the time required for processing (4–6 weeks), we chose to use only autologous mdMSCs, particularly in light of the need for repeated injections in our treatment protocol.

Various parameters in our treatment protocol can be adjusted to improve its effectiveness. One important factor is the route of MSCs administration, though there is currently no consensus on the optimal route for delivering MSCs to the ocular surface.[28] Subconjunctival injection is a safe, minimally invasive and straightforward technique frequently used in ophthalmology. It enables the local delivery of high cell doses in a small volume. However, subconjunctival injections do have limitations,[28] including the lack of agreement on the ideal cell vehicle solution, the optimal dosage and frequency of injections, and the limited volume that can be administered. Intrastromal injections may offer an alternative administration route.[28] Although more invasive, they could potentially yield better results by directly delivering higher concentrations of MSCs to the lesion. Future studies are required to assess the safety of this MSCs delivery route.

In our study, we initially administered a dosage of 2.5 million cells per injection based on the experience of one of the authors (Didier Serteyn, personal communication). In two cases, disease control was not effectively achieved with an increased dosage of 5 million cells. While a higher dosage, such as the 15 million cells per injection employed by Davis et al.,[14] might have potentially yielded better results, it has not been attempted in our study.

The precise fate of subconjunctivally injected MCSs remains controversial. Different studies have reported the migration of MSCs from the subconjunctival space to inflamed corneal areas[29–31] while others did not observe evidence of MSCs migration.[32–34] However, despite the lack of migration, MSCs still exhibited therapeutic effects

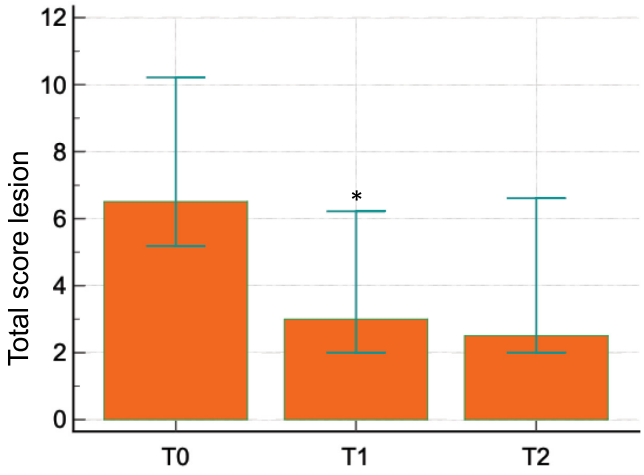

FIGURE 3 Median values of total score lesion before stem cell therapy (T0), after one injection (T1) and at the end of the protocol (T2). Error bars represent the 95% confidence interval. The * represents a statistically different ($p \leq .05$) median value between T0 and T1.

through trophic factors. Discrepancies may be due to timing variations in MSC administration, impacting migratory capacity due to a change in the signals released by damaged tissues. Further evaluation is needed to determine the fate and survival time of subconjunctivally injected mdMSCs in our specific case.

The main limitation of this study is the small number of cases included. We specially chose to treat cases that were nonresponsive to conventional treatment. Further research is necessary to assess the efficacy of our protocol in classical IMMK cases and explore the potential to control these cases through injections, reducing the need for daily topical treatment. Another limitation is the variability in the treatment protocol, including differences in dosages and the number of injections. In the case of Case 5, it is important to consider the potential interaction between the surgery and the progression of corneal lesions. The lamellar keratectomy and the vascular supply of the conjunctival graft may have influenced the local immune response of the cornea. The reasons why Cases 1 and 3 did not respond to repeated injections remain unknown. Performing a corneal biopsy would be relevant to confirm the diagnosis and rule out the presence of corneal lymphoma.

5 | CONCLUSION

In conclusion, our study highlights the potential of subconjunctival autologous mdMSCs as a promising therapeutic option for refractory cases of equine immune-mediated keratitis (IMMK). The initial positive response and subsequent improvement of ocular lesions observed after the first injection suggests the efficacy of this novel treatment approach. Furthermore, the repeated injections were found to be safe, minimally invasive, and beneficial for controlling the disease in our cases. However, further research is needed to fully understand the mechanism of action of MSCs in keratitis and to optimize the therapeutic protocol. This treatment should also be attempted in cases nonrefractory to conventional treatments.

Despite the limitations of our study, such as the small sample size and variable treatment protocols, our findings provide valuable insights into the potential benefits of mdMSC-based therapies for managing IMMK. Future studies should aim to assess the long-term benefits and establish standardized protocols to achieve sustained control of corneal diseases.

AUTHOR CONTRIBUTIONS

Florine Narinx: Conceptualization; data curation; formal analysis; investigation; methodology; project administration; writing – original draft; writing – review and editing. **Aurélie Sauvage:** Data curation; investigation; methodology; project administration; supervision; writing – review and editing. **Justine Ceusters:** Resources. **Sigrid Grulke:** Funding acquisition. **Didier Serteyn:** Conceptualization; resources; software; supervision; writing – review and editing. **Sébastien Monclin:** Investigation; methodology; project administration; supervision; validation; visualization; writing – original draft; writing – review and editing.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

This study complies with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and was approved by the Ethical Committee of the University of Liège. This study was approved by the hospital board of the University of Liège. Animal owners or owners' representatives provided written informed consent for enrolment in the study, procedure(s) and therapy undertaken, and publication of data and images.

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