Laryngoscope
Investigative Otolaryngology

ORIGINAL RESEARCH OPEN ACCESS

Effects of Inhaled Dexamethasone/Ciprofloxacin on Acute Subglottic Stenosis in a Rabbit Model

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Received: 13 August 2024 | **Revised:** 16 November 2024 | **Accepted:** 20 February 2025

Funding: This study was supported by donations from the Celestial Ball Fundraiser. The authors have no other funding.

Keywords: animal model | dexamethasone/ciprofloxacin | inhalation | subglottic stenosis

ABSTRACT

Objectives: Subglottic stenosis (SGS) is a challenging complication post-airway interventions. Effective preventive strategies lack sufficient evidence. This study investigated the preventive effect of inhalation therapy with dexamethasone/ciprofloxacin on acute SGS in a rabbit model.

Methods: Twenty New Zealand White rabbits underwent subglottic injury via endoscopy and received inhalation therapy twice daily starting on the injury day. Group 1 ($n=4$) received saline for 5 days and was sacrificed on day 5; Group 2 ($n=4$) received dexamethasone/ciprofloxacin for 5 days and was sacrificed on day 5; Group 3 ($n=4$) received the same therapy for 5 days and survived for one week, and was sacrificed on day 15; Group 4 ($n=4$) received the therapy for 10 days and was sacrificed on day 15; Group 5 ($n=4$) received the therapy for 10 days and survived for one week, and was sacrificed on day 22. Rabbits underwent repeat endoscopy and were euthanized at the designated time point. Histological measurements were analyzed statistically.

Results: Histological analysis revealed median cricoid lumen measurements of $20.01 \pm 1.42\,mm^2$ for group 1, $17.94 \pm 3.05\,mm^2$ for group 2, $14.84 \pm 2.55\,mm^2$ for group 3, $17.18 \pm 5.31\,mm^2$ for group 4, and $11.87 \pm 5.68\,mm^2$ for group 5. No significant differences were found between treatment and control groups ($p=0.486$) or between 5-day and 10-day treatments ($p=0.686$). Multivariate statistical analysis indicated that cessation of inhalation therapy ($p<0.05$) and prolonged survival ($p<0.05$) were associated with shorter cricoid lumen measurements.

Conclusion: Short-term dexamethasone/ciprofloxacin inhalation does not prevent acute SGS. No improvements in cricoid lumen diameter were found. Extended survival correlated with shorter cricoid lumen, suggesting SGS progression is time dependent.

Level of Evidence: NA.

1 | Introduction

Acquired subglottic stenosis (SGS) is a prevalent complication of intubation, especially among the pediatric population in pediatric intensive care units (PICUs), where it occurs in up to 9.3% of patients [1]. This condition arises when the pressure exerted by the endotracheal tube (ETT) on the mucosal microcirculation surpasses capillary pressure, leading to tissue ischemia. The resultant tissue edema and hyperemia amplify the ischemic damage, culminating in mucosal ulceration and erosion [2]. Although the wound-healing process initiates after such acute injuries, improper termination of this process can lead to pathological scar formation, thereby causing SGS.

The wound-healing process comprises three overlapping phases: inflammation, proliferation, and maturation. The use of pharmacologic management for mitigating disease progression has been widely studied and discussed, with conflicting results. A systemic literature review [3] suggested the use of antibiotics, steroids, and antireflux treatments for preventing and treating subglottic stenosis.

Steroids, which have been widely studied for their role in modulating inflammation and cell proliferation, are utilized to manage wound healing through their anti-inflammatory and immunosuppressive properties and their ability to inhibit fibroblast proliferation [4–7]. The use of antibiotics complements steroid treatment, particularly when airway granulation tissues, which can obstruct the airway lumen, test positive for bacterial cultures [8]. These infections not only exacerbate stenosis but are also implicated in the development of complications such as periodontitis and chondritis, further increasing the risk of subglottic stenosis [9].

Previous research has focused primarily on the systemic administration of treatments, which raises significant concerns about systemic impacts and side effects. Studies have noted that the use of systemic dexamethasone in preterm infants is associated with neurodevelopmental impairments and the need to manage hyperglycemia [10, 11]. Consequently, intralesional therapies have been introduced as effective scar-modifying therapies for SGS [12, 13]. However, even these methods are not without issues, as they still result in systemic absorption of steroids, leading to acute hypothalamic–pituitary–adrenal (HPA) axis suppression. There have also been reports of the development of Cushing syndrome [14]. In light of these challenges, we explored the feasibility and efficacy of nebulized steroid treatments, which offer a noninvasive and nonsystemic alternative.

The goal of this study was to investigate the effect of inhaled dexamethasone/ciprofloxacin on acute SGS by using a previously validated rabbit survival model of SGS [15].

2 | Materials and Methods

2.1 | Animals

All animal procedures were reviewed and approved by the Cincinnati Children's Hospital Medical Center Institutional Animal Care and Use Committee (protocol IACUC2015-0083). All animals were acclimated to their environment for at least 72 h before intervention. Twenty adult female New Zealand white rabbits (mean body weight: 3.50 kg, range 3.13–3.82 kg) were randomly divided into five groups depending on the inhalation substance they received and the duration of inhalation after artificially induced airway injury. The experimental groups were as follows: group 1 ($n = 4$), which underwent normal saline inhalation for 5 days and was sacrificed on day 5; group 2 ($n = 4$), which underwent dexamethasone/ciprofloxacin inhalation for 5 days and was sacrificed on day 5; group 3 ($n = 4$), which underwent dexamethasone/ciprofloxacin inhalation for 5 days and then survived for one week and was sacrificed on day 15; group 4 ($n = 4$), which underwent dexamethasone/ciprofloxacin inhalation for 10 days and was sacrificed on day 15; and group 5 ($n = 4$), which underwent dexamethasone/ciprofloxacin inhalation for 10 days and then survived for one week and was sacrificed on day 22.

2.2 | Anesthesia and Sizing of the Airway

Prior to airway exposure via a laryngoscope, the animals were anesthetized by intramuscularly administering a combination of ketamine (35 mg/kg) and xylazine (5 mg/kg) and maintained with 2% isoflurane with spontaneous ventilation. The animals were scoped with a Miller size 1 laryngoscope to expose the larynx after adequate preoxygenation. Atomized 1% lidocaine (0.5 mL) was applied to the larynx to prevent laryngospasm. Photo documentation for airway anatomy was carried out with a 2.7 mm Hopkins Rod telescope (Karl Storz, Tuttlingen, Germany) down to the trachea. Serial endoscopic intubation starting from a small ETT was performed for sizing of the airway, and the size of the subglottis was determined when the largest diameter allowed an air leak at 20 cm H_2O.

2.3 | Induction of Subglottic Stenosis

SGS was induced with cauterization via a previously validated technique [15]. Bugbee monopolar cautery was applied to the posterior 75% of the circumference of the subglottis under endoscopic guidance, followed by 4 h of intubation with a 3.5 mm cuffed Portex ETT (Smiths Medical, Dublin, OH). All animals were closely monitored during the intubation period and sent to a temperature-controlled incubator once extubated until they fully recovered from anesthesia.

2.4 | Nebulization

Inhalation was started the day after the injury and was continued twice daily for 5 or 10 days. The preparation for inhalation was a mixture of 0.5 mL of 0.3% ciprofloxacin ophthalmic solution (Alcon Laboratories, Fort Worth, TX), 0.5 mL of 0.1% dexamethasone sodium phosphate ophthalmic solution (Bausch & Lomb, Bridgewater, NJ) and 2 mL of saline. For experimental group 1, which did not receive the dexamethasone/ciprofloxacin regimen, 3 mL of saline was supplied as a control. A rabbit face mask was connected to a nebulizer containing the preparation and was applied to the rabbit after it had been placed in the rabbit restrainer. Nebulization was carried out for 8–10 min with oxygen provided at a flow rate of 5.0 L per minute.

2.5 | Sacrifice and Histology Measurement

After the designated survival time frame, the animals underwent repeat endoscopic evaluation and sizing of the airway, followed by euthanasia with an intracardiac sodium pentobarbital (100 mg/kg) injection while still under general anesthesia. Induced SGS was documented with the Myer–Cotton grading system [16] (grade I: 0%–50%, grade II: 51%–70%, grade III: 71%–99%, grade IV: 100%). The larynx and trachea were harvested from the hyoid

to 2 cm below the cricoid. The samples were preserved in 10% buffered formalin and embedded in paraffin. The subglottic portion was sectioned at 5 μm per section and prepared with hematoxylin and eosin (H&E) staining. An optical microscope (Carl Zeiss Microscopy, Thornwood, NY) was used to examine the pathology slides and document the images. These images were analyzed via AxioVision software SE64 Rel. 4.9.1 Tool (Carl Zeiss) via delineation of the circumference of the cricoid inner lumen and calculation of its area. The smallest measurement of the cricoid lumen represented the most stenotic part for each subject.

2.6 | Statistical Analysis

Endoscopic findings, Myer–Cotton SGS grades, and descriptive statistics are reported. Because of the small sample size ($n = 20$), a nonparametric test was used, and the median with the interquartile range is reported. The measurements of the cricoid areas were statistically analyzed via the Kruskal–Wallis test and the Mann–Whitney U test. p values < 0.05 were considered significant. All the statistical analyses were performed with MedCalc Statistical Software version 20.114 (MedCalc Software bv, Ostend, Belgium; https://www.medcalc.org; 2020).

3 | Results

3.1 | Endoscopic Assessment of Airway Changes

To evaluate the immediate physical changes in the airway post-cauterization, we utilized endoscopic photo documentation and airway sizing before animal sacrifice. Each rabbit underwent endoscopy, which documented the condition of the mucosa and the presence of debris. In groups 1, 2, and 4, endoscopy revealed sloughing of the mucosa and debris coating the cauterized areas. The debris was mechanically debrided by the ETT during the airway sizing process (Figure 1A,B). However, in groups 3 and 5, which were allowed a survival period of one additional week without nebulization, no similar sloughing or debris accumulation was observed (Figure 2A,B). Inhalation therapy may mitigate the maturation and progression of scar formation in the airway.

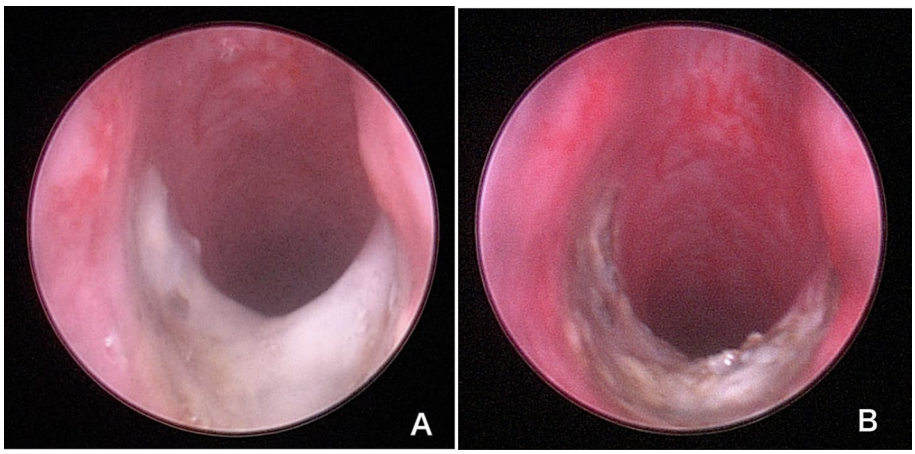

FIGURE 1 | Debridement of Debris from the Cauterized Subglottis after Nebulization. (A) Image of the airway before sizing with the endotracheal tube (ETT), showing the initial condition of the cauterized area. (B) Image of the airway after sizing with the ETT, illustrating the removal of coating debris following 5 days of nebulization with normal saline. The debris is effectively debrided, revealing the underlying mucosal surface.

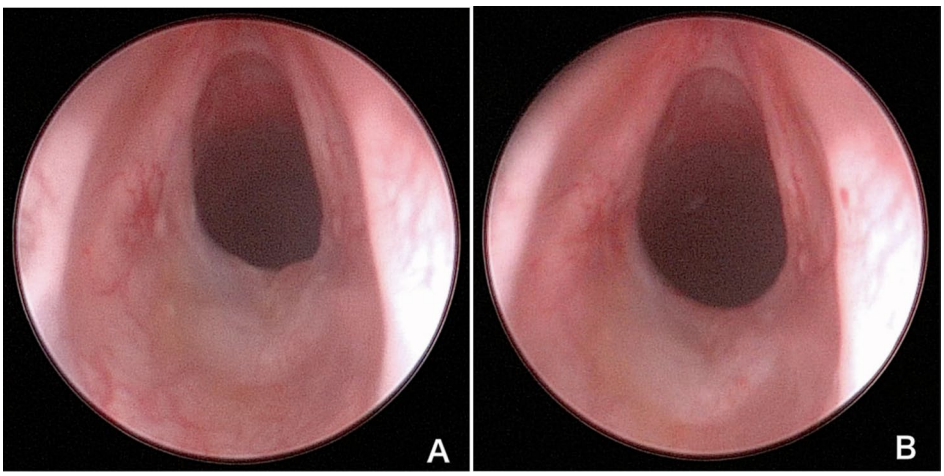

FIGURE 2 | Stability of the Scar Pattern in Group 5 after Treatment. (A) Image showing the scar pattern in the airway of Group 5 before sizing with the endotracheal tube (ETT). (B) Image showing the scar pattern in the airway of Group 5 after sizing with the ETT, illustrating no obvious change in the appearance of the scar.

TABLE 1 | Variation in cricoid lumen measurements across different treatment groups.

Group		Median	IQR	Kruskal–Wallis test $p = 0.018$
1	Normal saline for 5 days	20.01	1.42	Post hoc analysis
2	Dexamethasone/ciprofloxacin for 5 days	17.94	3.05	2 > 3
3	Dexamethasone/ciprofloxacin for 5 days & 1-week survival	14.84	2.55	4 > 5
4	Dexamethasone/ciprofloxacin for 10 days	17.18	5.31	1, 2, 4 > 5
5	Dexamethasone/ciprofloxacin for 10 days & 1-week survival	11.87	5.68	

Note: This table presents the median cricoid lumen measurements and interquartile ranges (IQRs) for each group, along with the results from the Kruskal–Wallis test indicating significant differences between groups ($p = 0.018$). Post hoc comparisons are summarized to highlight specific group differences.

3.2 | Histological Analysis of Changes in the Cricoid Lumen

To assess the effects of different durations of inhaled dexamethasone/ciprofloxacin therapy on the structural integrity of the cricoid lumen, we conducted detailed histological examinations. We measured the median cricoid lumen sizes across five groups with varying durations of therapy and cessation periods. The measurements were as follows: $20.01 \pm 1.42\,mm^2$ in group 1, $17.94 \pm 3.05\,mm^2$ in group 2, $14.84 \pm 2.55\,mm^2$ in group 3, $17.18 \pm 5.31\,mm^2$ in group 4, and $11.87 \pm 5.68\,mm^2$ in group 5 (Table 1). The Kruskal-Wallis test indicated a significant difference across the five groups ($p = 0.018$). However, post hoc analysis showed no significant differences in lumen diameter between the saline control group and the 5-day steroid/antibiotic treatment group (group 1 and 2, $p = 0.486$) or between the 5-day and 10-day steroid/antibiotic treatment groups (groups 2 and 4, $p = 0.686$) (Figure 3). Compared with saline, short-term inhalation therapy did not significantly affect cricoid lumen size, suggesting limited efficacy in preventing stenosis within the studied duration.

3.3 | Statistical Analysis of Therapy Outcomes

To assess the effects of cessation of inhalation therapy on cricoid lumen size and to explore the impact of the duration since the initial injury on the development of SGS, multivariate statistical analysis was used. The study compared cricoid lumen sizes among

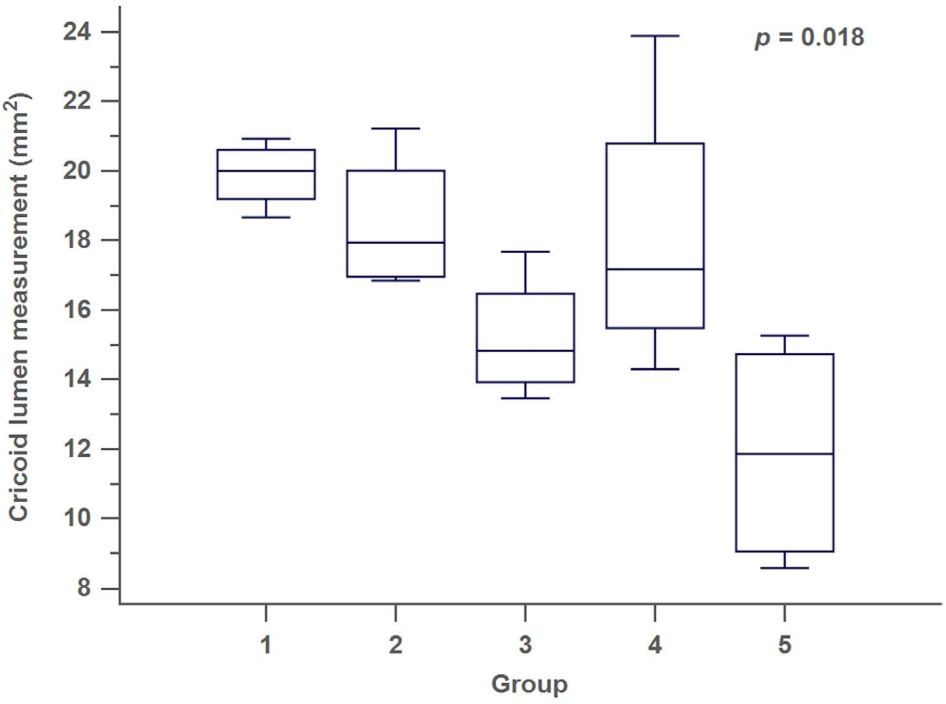

FIGURE 3 | Distribution of Cricoid Lumen Measurements across Groups. Boxplots representing the cricoid lumen measurements for each group. Each boxplot shows the median, quartiles, and range of measurements, facilitating a comparison of lumen sizes across the different treatment groups.

rabbits sacrificed immediately after therapy with those that had therapy ceased but survived longer. The rabbits that ceased inhalation therapy exhibited a significantly smaller lumen size in both immediate cessation groups (Figure 4, $p < 0.05$). Extended survival was associated with progressively shorter cricoid lumen measurements irrespective of the treatment regimen (Figure 5, $p < 0.05$). The therapeutic effects of the inhalation regimen were temporary and did not persist over time. The primary factor influencing the progression of SGS was the duration since the initial injury rather than the specific interventions applied.

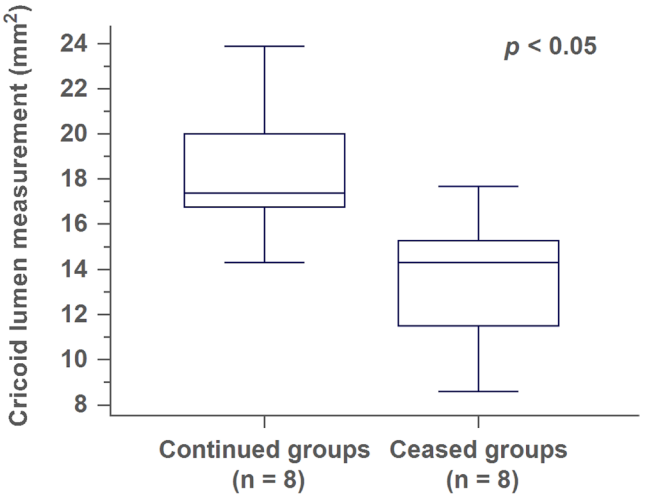

FIGURE 4 | Increased Stenosis in Groups after Therapy Cessation. This figure illustrates the comparison of airway lumen sizes across different groups, specifically highlighting the groups in which therapy was ceased. The statistical significance of the narrower lumens in these groups is indicated ($p < 0.05$).

4 | Discussion

Our study investigated the effectiveness of inhaled dexamethasone/ciprofloxacin in preventing SGS following airway injury in a rabbit model, highlighting several critical aspects of treatment strategies and disease progression. Despite the theoretical benefits of localized steroid and antibiotic application, our findings did not demonstrate a significant advantage over the control in modifying the course of SGS, which aligns with previous research that presented conflicting outcomes [17–19]. This inconsistency likely stems from the complex pathophysiology of the disease, suggesting that the effectiveness of nebulization therapy in clinical settings requires reevaluation. Future studies should consider exploring alternative dosing regimens or combination therapies that might improve drug delivery efficacy to the targeted tissues.

No significant differences were observed between short (5 days) and extended (10 days) durations of nebulized steroid/antibiotic therapy, indicating that prolonging treatment does not necessarily improve outcomes in preventing SGS. However, the cessation of treatment significantly impacted scar maturation, with endoscopic evaluations revealing that the groups that continued treatment exhibited minimal scar formation and presented primarily with easily debrided debris. In contrast, groups that discontinued nebulization for one week presented more mature scars, suggesting that ongoing treatment might be crucial for managing disease progression. Additionally, previous research has demonstrated that nebulization, whether with isotonic or hypertonic saline, can alleviate respiratory symptoms in adults with SGS [20], emphasizing its potential therapeutic benefits. These findings support the notion that optimizing treatment schedules on the basis of the pharmacokinetics and dynamics of drug nebulization for airway tissues could significantly increase

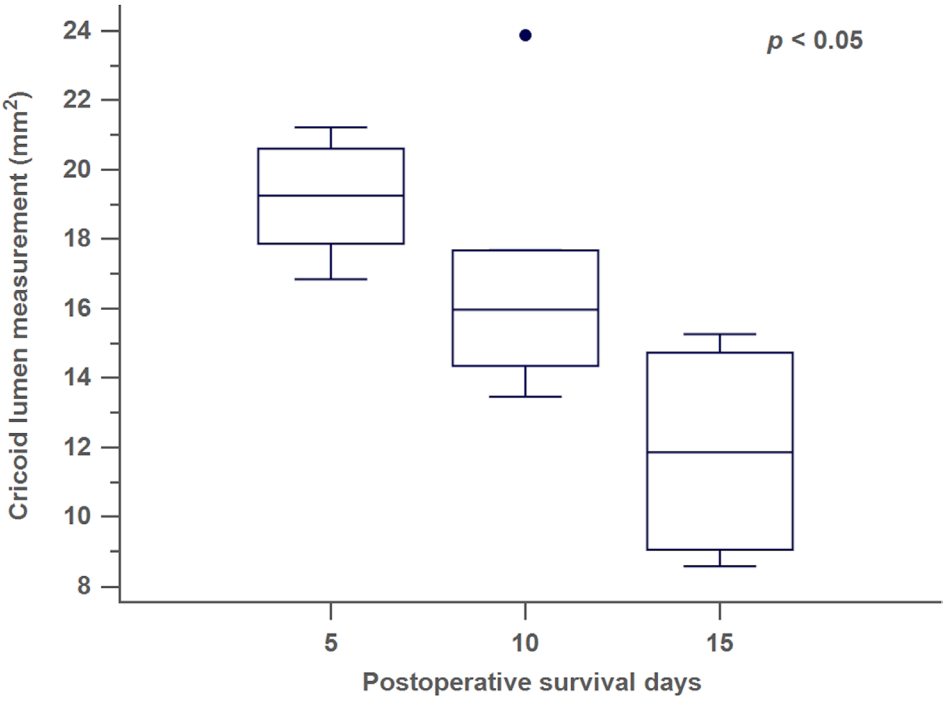

FIGURE 5 | Reduced Cricoid Lumen Size is Associated with Increased Survival Days. This figure displays the cricoid lumen measurements for groups with varying survival days postinjury, highlighting that groups with more survival days present significantly smaller lumen sizes ($p < 0.05$).

the efficacy of treatments, highlighting the need for a tailored therapeutic approach that carefully considers both the timing and duration of nebulization to effectively control the development and maturation of SGS.

A pivotal finding of our study is the critical role of timing since the initial injury in the development of SGS, which appears to be more influential than the type of medical intervention used. Early and consistent postinjury intervention may be crucial in managing the progression of SGS. This highlights the potential benefits of initiating treatment immediately after injury and maintaining it to mitigate disease progression. Defining optimal windows for therapeutic intervention could maximize treatment efficacy, a vital area for future research.

While our study provides initial insights into the potential benefits of inhalation therapies, significant limitations must be acknowledged. The absence of a saline control group with a similar treatment duration and follow-up, particularly a "7-day survival group" after saline treatment, critically undermines our ability to distinguish therapeutic effects from natural healing processes. Furthermore, the follow-up duration was insufficient to observe mature stenosis, which typically develops over a longer period. Addressing these limitations by including longer term studies with appropriately timed control groups is crucial for advancing our understanding of therapeutic interventions for SGS.

5 | Conclusions

In conclusion, while confirming some of the challenges in treating SGS, our study opens avenues for rethinking treatment strategies, particularly in pediatric care, where the risks associated with systemic steroid use are a significant concern. The clinical implications of our findings support the potential development of more effective localized therapies that reduce systemic risk. Future research should explore innovative delivery technologies or new therapeutic agents that target inflammation more effectively without systemic side effects, providing a comprehensive assessment of therapeutic outcomes from acute injury through chronic stenosis.

Acknowledgments

This study was supported by donations from the Celestial Ball Fundraiser.

Conflicts of Interest

The authors declare no conflicts of interest.

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