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Reproducing severe acute subglottic stenosis in a rabbit model

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ABSTRACT

Objectives: The objectives of this study were to develop an animal model with consistent, severe subglottic stenosis (SGS), and to develop the timeline needed for intervention to limit rabbit mortality.
Methods: Subglottic stenosis was created endoscopically using either a nylon or polypropylene brush in sixteen New Zealand White rabbits. The subglottic cross-sectional area was measured endoscopically using a right-angled probe and an open source image analysis software The cross-sectional areas of the stenotic and native airways were compared to calculate the subglottic stenosis percentage and Myer-Cotton grade classification.
Results: The average diameter of the native subglottis was 4.7 mm (*SD* 0.4). The mean subglottic stenosis percentage was 73% (*SD* 32) for all rabbits. The three rabbits injured with the nylon brush had 30, 52, and 76% stenosis. Nine of the 12 surviving rabbits (75%) injured with the polypropylene brush had a > 86% subglottic stenosis. Four rabbits in the polypropylene brush cohort died from procedural complications.
Conclusion: This study demonstrates a reliable model using a polypropylene brush to create severe acute subglottic stenosis. Cartilage exposure after creating the injury was associated with more severe stenosis. Mortality rate may have been reduced if endoscopic balloon dilation was performed electively seven days after injury, rather than emergently nine days after injury.
Level of evidence: 4.

1. Introduction

Subglottic stenosis (SGS) is a relatively uncommon problem, but the most common airway anomaly requiring a tracheostomy in pediatric patients. The incidence of SGS has been reported to be less than 2%, but the true incidence remains unknown [1]. Subglottic stenosis is defined as a subglottic diameter less than 3 mm in a preterm infant and less than 4 mm in a full-term infant [2]. Subglottic stenosis is most commonly a complication of the effects of prolonged endotracheal intubation for chronic respiratory failure [3–5]. In 1994, Myer et al. [6] described a classification system for grading SGS that is still widely used today (Grade 1 = < 50%, Grade 2 = 51–70%, Grade 3 71–99%, Grade 4 = 100%).

The management of SGS typically involves a combination of endoscopic techniques, adjuvant topical/injectable medications, open airway reconstruction, and/or a tracheostomy. Grade 1 SGS can often be managed without the need for a tracheostomy or major surgery, but most Grade 2–4 SGS usually involves major open airway reconstruction and/or tracheostomy tube placement. The potential impact on preventing a tracheostomy can decrease morbidity, mortality, and health care costs [3,7]. Regardless of the technique used, these patients are often medically complex with prolonged hospitalizations. Studying the treatment effects of a single intervention in these patients is difficult given the variety of confounding variables, including patient age and weight, acuity of injury, severity of stenosis, underlying cardiac or pulmonary comorbidities, bacterial colonization, history of tracheitis, duration of endotracheal intubation, number of reintubations, and the variability in the patient's own innate inflammatory response to an airway injury.

An animal model is designed to study the individual effects of each treatment modality in a controlled setting. The New Zealand White (NZW) rabbit has been the primary animal model to study pediatric SGS. The average subglottic diameter of the rabbits ranges from 5.4 to 5.8 mm, varying only slightly by weight of the animal [8]. This diameter correlates with the outer diameter of a 4.0 endotracheal tube (outer diameter of 5.4 mm), the standard ETT size for a 3–9 month infant [6]. Creating an animal model of SGS has been attempted with many open and endoscopic techniques, including knife incisions, wire

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brushings, electrocautery, otologic burrs, hydrochloric acid, silver nitrate, CO2 laser, and prolonged endotracheal intubation. Regardless of the mechanism of injury, the primary challenge is producing a significant degree of injury safely within a controlled time period.

The objectives of this study were to develop an animal model with consistent, severe SGS, and to develop the timeline needed for intervention to limit rabbit mortality. Two different techniques were used to create SGS in two cohorts of rabbits. This paper describes our experiences in developing a model for concurrent research investigating the rate of restenosis following endoscopic balloon dilation (EBD) using adjuvant topical ciprofloxacin-dexamethasone.

2. Materials and methods

The following procedures were performed under a protocol investigating the effects of endoscopic balloon dilation on SGS that was approved by the Institutional Animal Care and Use Committee at Madigan Army Medical Center. This protocol included a pilot study to refine the anesthesia methods and to develop a technique to create an animal model with severe SGS. Sixteen adult female New Zealand White rabbits involved in this study were maintained in accordance with the 'Guide for the Care and Use of Laboratory Animals' published by the National Research Council/Institute of Laboratory Animal Research (ILAR).

The first cohort (Group A) included three rabbits injured using a cytologic nylon brush (CytoSoft™). Given the inconsistent results using the nylon brush, the lead authors decided to change the method of injury for the second cohort. The second cohort (Group B) of 13 rabbits was injured using a 6.35 mm polypropylene brush (KeySurgical, Inc). General anesthesia was induced using intramuscular (IM) ketamine (10–35 mg/kg) and xylazine (3–5 mg/kg). Dexamethasone SP (2 mg/kg) and buprenorphine (0.01–0.05 mg/kg) were used for airway edema and perioperative pain, respectively. Usually the induction doses were sufficient for the duration of the procedure, but isoflurane was used as needed to maintain a steady state of anesthesia while having the rabbits breathe spontaneously. Yohimbine was given at a dose of 0.2 mg/kg IM postoperatively to reverse xylazine.

A Parsons laryngoscope, Hopkins II 0° telescope, a right angle probe, and a Tele Pack X LED (KARL STORZ, Germany) were used for direct laryngoscopy, bronchoscopy, and image documentation. The larynx was anesthetized with 1 mL of atomized lidocaine (1 mg/ml). The cross-sectional area (CSA) was calculated from an image of the right-angled probe at the level of the subglottis. Our airway measurement technique was similar to a method recently published [9]. Fig. 1 demonstrates the endoscopic measurement method used to calculate the subglottic CSA using an open source image analysis software (ImageJ, NIH, Bethesda, MD, USA). Subglottic stenosis is defined as below:

$$\textit{Subglottic Stenosis}\ (\%) = \left(1 - \left(\frac{\textit{Stenosis CSA}}{\textit{Native CSA}}\right)\right) \times\ 100$$

The nylon or polypropylene brush was rotated in the subglottis until a circumferential mucosal injury was obtained. Cottonoids soaked in oxymetazoline were used for topical hemostasis if needed. The first cohort of rabbits injured with the nylon brush underwent routine surveillance laryngoscopy three weeks after injury. The CSA of the injured subglottis was compared to the native CSA to determine the SGS percentage.

The objective of this study was to develop Grade 3 SGS. Therefore, we utilized a 6.35 mm polypropylene brush for creating the injury in Group B. To limit collateral mucosal injury, the wired polypropylene brush was cut to 5 mm in length. In the first rabbit of this cohort, the cut brush caused polypropylene fragments to shed loose in the airway requiring retrieval with foreign body graspers. This rabbit suffered no postoperative consequences from the foreign body retrieval. Therefore, the remainder of the rabbits in group B were injured using a plastic sheath telescoped over the unmodified polypropylene brush so that only 5 mm of bristles remained unsheathed to cause injury (Fig. 2). The second group of rabbits developed respiratory distress and clinical deterioration eight days after injury requiring emergent endoscopic balloon dilation. For EBD, a 7 mm balloon (Acclarent, Inc) was inflated to 12 atmospheric pressure (atm) for 30 s, and repeated once after the rabbit's oxygen saturation recovered.

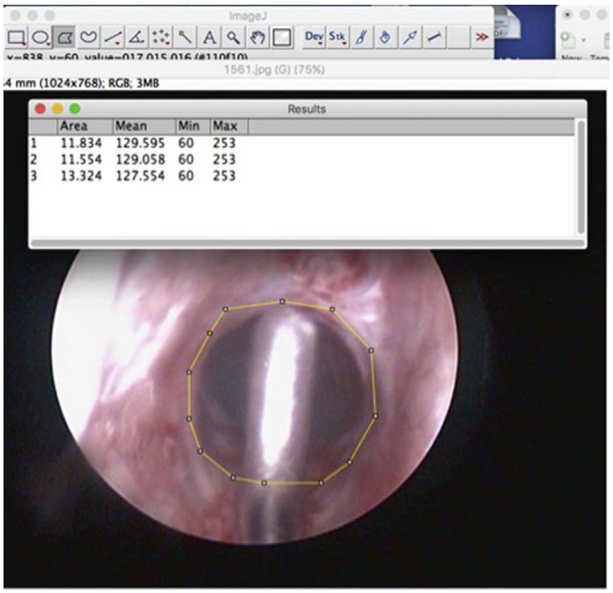

Fig. 1. Endoscopic airway measurement technique using ImageJ software.

3. Results

Table 1 shows the native diameter, native CSA, and the SGS CSA for each rabbit. Fig. 3 shows the distribution of severity of stenosis based on CSA as described by Myer et al., [6] but using our measurement technique. The average diameter of the native subglottis was 4.7 mm (SD 0.4). The mean SGS percentage was 73% (*SD* 32) for rabbits in both cohorts. The three rabbits injured with the nylon brush had 30, 52, and 76% stenosis. The nylon brush required multiple attempts to create a circumferential injury. Nine of the 12 surviving rabbits (75%) injured with the polypropylene brush had a subglottic stenosis CSA > 86%. The polypropylene brush created a more consistent circumferential injury with cartilage exposure. Fig. 4 shows examples of grade 3 SGS in this cohort.

The overall mortality rate for this study was 25% (n = 4). All rabbits in the nylon brush group survived to complete the study. In the polypropylene group, one rabbit died approximately 3 hours after the airway injury procedure due to respiratory failure. Two rabbits died from delayed respiratory failure eight days after injury creation. Both of these rabbits had full thickness mucosal injuries with cartilage exposure, suggesting a more severe injury and inflammatory response. Immediate postmortem necroscopy revealed near complete airway stenosis and severe pulmonary edema. The remaining rabbits were developing significant stridor and respiratory distress. Given the progressive clinical course and the rabbit deaths, six of the remaining 10 rabbits were emergently balloon dilated on the ninth day following injury. One rabbit died immediately upon deflation of the balloon from cardiopulmonary arrest. These rabbits required veterinarian support throughout the night, so the other four rabbits that were stable were dilated two days later given the limits of time and availability of staff support.

Interestingly, the majority of the surviving rabbits in the second cohort had a normal recovery but developed sudden and significant dyspnea, tachypnea, tachyarrhythmias, and hypoxemia approximately