Bacterial Colonization of Airway Stents

A Promoter of Granulation Tissue Formation Following Laryngotraheal Reconstruction

S. A. Reza Nouraei, MBBChir; Michael A. Petrou, MD; Prem S. Randhawa, MRCS; Arvind Singh, MRCS; David J. Howard, FRCS; Guri S. Sandhu, FRCS

Objective: To investigate whether airway granulation, a common occurrence during laryngotraheal reconstructive surgery and a common cause of delays in definitive treatment and treatment failure, is associated with a microbial etiology.

Design: Prospective case-control study.

Setting: Tertiary referral airway reconstruction unit.

Patients: Patients who had an airway stent as part of their treatment for laryngotraheal stenosis.

Interventions: All airway stents were sent for microbiological analysis. Information about patient demographics, lesion characteristics, and presence of airway granulation tissue at different times during treatment were obtained and correlated against the microbiological findings from airway stents.

Main Outcome Measures: A $\chi^2$ test was used to correlate airway colonization with specific pathogens and occurrence of airway granulation. Logistic regression analysis was used to identify independent microbiological predictors of airway granulation.

Results: Thirty-one airway stents were removed from 26 patients. The mean (SD) age at presentation was 42 (18) years, and postintubation tracheal stenosis was the most common etiology. There were highly significant associations between stent colonization with Staphylococcus aureus and Pseudomonas aeruginosa and the occurrence of airway granulation ($P<.02$), and these microorganisms were independently associated with the risk of developing airway granulation. Furthermore, S. aureus was associated with persistence of airway granulation on average 4 months following removal of the stent.

Conclusions: Airway granulation seems to be associated not with polymicrobial airway colonization but with infection with specific pathogenic microorganisms. All patients undergoing laryngotracheoplasty should receive antibiotic prophylaxis to cover these microorganisms, and the development and use of antibiotic-impregnated airway stents should be explored.


Airway granulation is commonly encountered during laryngotraheal reconstructive surgery. Exophytic granulation tissue narrows the airway lumen, leading to recurrent pulmonary morbidity, difficulties with decannulation, and delays in definitive treatment, which cannot often be undertaken in the presence of active inflammation. In the longer term, an excess of airway granulation tissue promotes circumferential wound contracture, leading to stenosis recurrence. Airway stents are often deployed in these settings as a bridge to definitive treatment, to maintain luminal patency, to prevent wound contracture, and to provide structural support to a reconstructed segment of the airway during the healing phase. Some patients, however, continue to form large amounts of granulation tissue around the prosthesis, and in some others, once the stent has been removed, a recurrence of granulation tissue leads to symptomatic relapse.

Airway granulation is an ongoing inflammatory process, but what drives it is not fully understood. Theories range from mechanical irritation and laryngopharyngeal reflux to response to polymicrobial colonization of the stents by microorganisms such as Streptococci viridans, Neisseria species, Pseudomonas aeruginosa, Staphylococcus aureus, and many others. A better understanding of the etiology of this condition would allow clinicians to institute therapy to prevent or...
effectively treat it and could have major implications for laryngotracheal surgery. We undertook this study to investigate the relationship between airway granulation and microbial colonization of airway stents.

**METHODS**

**PATIENTS AND SURGICAL PROCEDURE**

From a prospective database of patients who had undergone laryngotracheal reconstruction for upper airway stenosis, records were retrieved of those who had had an airway stent as part of their treatment from June 2002 to June 2003. The study was conducted with the approval of the institutional review board. Information was obtained about the etiology of the lesions, patient characteristics, and airway procedures performed.

All patients underwent endoluminal placement and retrieval of Silastic (Boston Medical Products, Boston, Mass) stents with high-frequency supraglottic jet ventilation. The stents had been secured with a single 2-0 polypropylene (Prolene; Ethicon Inc, Somerville, NJ) suture as previously reported. To remove them, the luminal portion of the stent suture was cut with microscissors under endoscopic vision, and the stent was grabbed and removed; care was taken to touch the tracheal and laryngoscope walls as little as possible. All stents were immediately placed in a sterile container with a few drops of sterile isotonic sodium chloride solution, as instructed by microbiological procedures, and sent for analysis. The suture was then removed through a small incision in the neck.

For a group of 8 patients with a recent preoperative history of a throat infection or other suspected oral cavity infection, mouthwash specimens were additionally taken and sent for microbiological analysis. This was accomplished by asking the patients to swish and gargle 10 mL of sterile Hartman solution (Baxter Healthcare Ltd, Thetford, England) in the anesthesia room. (Every liter of Hartman solution contains 131 mM of sodium, 5 mM of potassium, 2 mM of calcium, 111 mM of chloride, and 29 mM of lactate.)

**CLINICAL OUTCOME**

All procedures were digitally recorded, and the extent of airway granulation at the time of stent removal was assessed from the videos by 2 experienced otolaryngologists (D.J.H. and G.S.S.) who were blinded to the microbiological outcome. Airway granulation was recorded as a negative, and the patient classified as a control, if there was no or minimal airway granulation and no obstruction of the airway lumen; patients were considered positive if there was an obvious degree of luminal encroachment. These latter patients were classified as cases in the case-control design of the study. Presence of airway granulation was recorded at the time of removal of the stent in all patients and, for those patients who underwent further microlaryngoscopy and tracheoscopy some time after removal of the stent, during the next examination for which the patient received anesthesia.

**MICROBIOLOGICAL ASSESSMENT**

Once received in the laboratory, the stents were examined microscopically for mucus. They were flushed with 2 mL of sterile distilled water or Mucolyxin (Oxoid, Basingstoke, England), a solution containing acetylcysteine that is used to dissolve thick mucoid secretions for microbiological analysis depending on the presence or absence of significant amounts of mucus, as is standard practice. The stents were then vortexed for 1 minute, and a fixed volume of the resultant suspension was inoculated onto blood agar aerobically and anaerobically: MacConkey agar and Neomycin blood agar anaerobically with a metronidazole disk added on the streak 2 cm away from the inoculum; chocolate agar under 10% carbon dioxide; and Sabouraud dextrose agar at 30°C and 37°C. All the inocula were streaked on 4 quarters of the disk (the semiquantitative method). The plates were incubated and were examined for growth after 2, 5, and 7 days of incubation. The suspension was then examined using a fluorescent microscope. A slide was also prepared for gram staining, and this was examined with the aid of a light microscope using oil immersion (Figure 1). Microbial growths were semi-quantified as scanty, moderate, or heavy. For those specimens where fungal growth was seen in the potassium hydroxide preparation, a fully quantitative method was also performed in which serial dilutions were plated out. Both bacteria and fungi were identified to species levels using standard microbiological techniques. A full sensitivity pattern was performed on all S aureus isolates to exclude multiresistant S aureus. No cases of multiresistant S aureus were identified in this series.

**STATISTICAL ANALYSIS**

Variables were presented either as mean (SD) or as binomial percentages when appropriate. The association among different strains of bacteria and occurrence of airway granulation was tested using the Fisher exact test. Stepwise binary logistic regression was performed to identify any microorganisms independently associated with airway granulation. Bivariate correlation, with Kendall τ test, was performed to assess the correlation between bacterial colonization of the oral cavity and microorganisms isolated from the stent in patients who had used a mouthwash (n=8). This test was also used to assess any correlation between airway granulation at the time of stent removal and granulation at the subsequent tracheoscopy. Statistical analysis was performed using SPSS statistical software (release 12.0 for Windows, SPSS Inc, Chicago, Ill).

**RESULTS**

Over the study period, 31 stents were removed from 26 patients, and the mean (SD) in situ time per stent was 5 (3) months. The mean (SD) age at operation was 42 (18) years, and postintubation tracheal injury was the most common etiology. Most patients (88%) had received a tracheostomy during treatment. Table 1 provides further information about patient characteristics.

**MICROBIOLOGICAL FINDINGS FROM AIRWAY STENTS**

All airway stents were colonized with mouth commensals including S viridans and Neisseria species, and a number of different microorganisms were grown from stent biofilms (Table 2). A significantly greater percentage of stents removed from patients with airway granulation were colonized with S aureus (P<.02, Fisher exact test; relative risk, 1.73; 95% confidence interval 1.18-2.54) or P aeruginosa (P<.03, Fisher exact test; relative risk, 1.94; 95% confidence interval, 1.17-3.21). One patient had co-colonization with both of these microorganisms. Only 1 patient showed no evidence of granulation in the presence of P aeruginosa, and in this patient the growth of this
A Spearman rank correlation coefficient of 0.73 existed between airway colonization with \textit{S} aureus or \textit{P} aeruginosa and occurrence of airway granulation ($P < 0.001$). Furthermore, a stepwise logistic regression analysis revealed that stent colonization of \textit{S} aureus and \textit{P} aeruginosa were each an independent risk factor for the development of airway granulation. We found no relationship between the length of stent placement and either granulation tissue or airway colonization with \textit{S} aureus or \textit{P} aeruginosa ($P = 0.43$, unpaired \textit{t} test). The relationship between airway stent colonization with different microorganisms and granulation tissue is illustrated in Figure 2.

### PATIENTS WITH MULTIPLE AIRWAY STENTS

Four patients in this series required multiple stent placements as part of their treatment. Three patients had 2 airway stent placements, and a fourth patient required 3 stent placements. Bacterial colonization with \textit{S} aureus or \textit{P} aeruginosa of the first stent was noted in 2 patients. In both instances, considerable airway granulation was present. One of these 2 patients required an additional stent placement, and the other required an additional 2 stents. All 3 stents...
were free of *S aureus* or *P aeruginosa* colonization, and the patients were clinically free of significant granulation at the time of removal of these subsequent stents. In another patient, *S aureus* or *P aeruginosa* was not present when the first stent was removed, but *P aeruginosa* colonized the second stent, removed 4 months later. In this patient, there was minimal airway granulation after the removal of the first stent, but considerable amounts of granulation tissue were present after the second stent was removed. In a fourth patient, despite the presence of significant airway granulation associated with the removal of 2 stents 1 year apart, *S aureus* and *P aeruginosa* were not isolated. This was the only patient in this series for whom this was the case. Findings from granulation tissue biopsy specimens, taken at the time of removal of the second stent, revealed growth of *Aspergillus fumigatus* fungus.

A preoperative mouthwash specimen was taken in the anesthesia room from 8 patients. Separate specimens were taken for culture, and the pattern of microbial growth was correlated with those cultures obtained from the stent using bivariate correlation. There was a perfect correlation between airway colonization with usual mouth commensals and their presence in the mouthwash. There were no other significant associations between microorganisms grown from the stent and the oral cavity.

There were 25 episodes in which the airway was reexamined after removal of the stent (mean [SEM] time after removal, 4 [0.7] months). This was performed with formal microlaryngoscopy and tracheoscopy under general anesthesia. There was a significant correlation between the presence of airway granulation at the time of stent removal and airway granulation at the subsequent tracheoscopy (r = 0.68; *P* < .001, Pearson correlation). The only microorganism whose presence at the time of stent removal was associated with airway granulation at the subsequent microlaryngoscopy was *S aureus* (*P* < .02, Fisher exact test; relative risk, 1.88; 95% confidence interval, 1.17-3.01).

**COMMENT**

Our data strongly suggest that a microbial etiology significantly contributes to the occurrence of airway granulation associated with stent placement. To our knowledge, this is one of the largest studies to correlate the microbiological findings of airway stents with the clinical outcome. Several groups have investigated the microbiological findings of airway stents. Noppen et al documented the microbiological findings of airway stents in 14 patients with a mixture and benign and malignant disease, most commonly isolating *S aureus, P aeruginosa, Streptococcus pneumoniae,* and *Klebsiella pneumoniae.* Simoni and Wiatrak found *S viridans,* *P aeruginosa,* *Neisseria* species, and *Haemophilus influenzae* to be the most common microorganisms isolated from airway stents. Schmal et al studied the pattern of microbial colonization of Montgomery T tubes in 10 patients undergoing laryngotracheal reconstruction. They found a significant correlation between airway
granulation and stent colonization with *P aeruginosa* but not *S aureus*.

We found strong associations between the occurrence of airway granulation and both *S aureus* and *P aeruginosa*, which suggests that that airway granulation, rather than being a nonspecific response to polymicrobial stent colonization as has previously been suggested,2,7 is driven to a considerable degree by infection with specific pathogenic microorganisms. We found no correlation between the occurrence of airway granulation and colonization with, for instance, mouth commensals like *S viridans*. On the other hand, *S aureus* and *P aeruginosa* were present in some 90% of patients with significant airway granulation and only in 8% of patients with mild or no granulation tissue (*P<.02*).

Furthermore, we found a strong correlation between the presence of airway granulation at the time of stent removal and the presence of granulation tissue at the subsequent microlaryngoscopy. A significant correlation remained between stent colonization with *S aureus*, but not *P aeruginosa*, and airway granulation at subsequent microlaryngoscopy. Further research is needed to better elucidate the relationship between biofilm microorganisms and airway granulation tissue formation.

We specifically obtained specimens for cultures for anaerobic microorganisms but did not find them present in any significant numbers in the biofilm. Simoni and Wiatrak2 have suggested that anaerobic microorganisms may play a role in the pathogenesis of airway granulation tissue. These authors did, however, use an anaerobic enrichment medium to grow anaerobes. This medium serves to amplify anaerobic microorganisms. In our view, it is highly unlikely that the very small numbers of anaerobic microorganisms that may be present and trapped within a stent biofilm, in one of the most aerobic environments in the body, can make a considerable contribution to the pathogenesis of airway granulation.

The findings of our study are in agreement with animal studies conducted by Sasaki et al,8 which showed that airway granulation is driven by specific pathogenic microorganisms, and have important implications for the perioperative care and antibiotic prophylaxis of patients undergoing laryngotracheoplasty.

Because airway granulation seems to be associated with 2 pathogenic bacteria (*P aeruginosa* and *S aureus*), we recommend antibiotic prophylaxis to cover these 2 organisms for all our patients undergoing laryngotracheal reconstruction for 1 week following surgical treatment. Our antibiotics of choice are flucloxacillin sodium and ciprofloxacin hydrochloride. Antibiotic-impregnated stents would be a different and effective method of reducing bacterial colonization during the crucial acute phases of wound healing in the airway.

In conclusion, our study suggests that airway granulation is strongly associated with infection with *P aeruginosa* and *S aureus*, the latter exerting a more lasting influence on airway granulation. Patients undergoing laryngotracheal reconstruction should receive antibiotic prophylaxis preoperatively and during the active phase of airway wound healing to cover these 2 organisms. When the use of airway stents is necessary, antibiotic-impregnated stents may be of benefit in reducing the incidence and severity of airway granulation.

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**Correspondence:** Michael A. Petrou, MD, Department of Microbiology, Hammersmith Hospital, Du Cane Road, London W12 0HS, England (m.petrou@imperial.ac.uk).

**Author Contributions:** The authors had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. **Study concept and design:** Nouraei, Petrou, Howard, and Sandhu. **Acquisition of data:** Nouraei, Randhawa, and Singh. **Drafting of the manuscript:** Nouraei, Petrou, Howard, and Sandhu. **Critical revision of the manuscript for important intellectual content:** Petrou, Singh, Howard, and Sandhu. **Statistical analysis:** Nouraei. **Administrative, technical, and material support:** Randhawa and Singh.

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**References**