Laryngeal Abductor Reinnervation With a Phrenic Nerve Transfer After a 9-Month Delay

Julie T. van Lith-Bijl, MD; Robin J. Stolk, MD; Jeroen A. D. M. Tonnaer, PhD; Cees Groenhout, MD; Pierre N. M. Konings, PhD; Hans F. Mahieu, MD, PhD

Background: Successful restoration of laryngeal abductor function, using the phrenic nerve, has been described in the cat model in the acute phase. However, in clinical practice there is usually a considerable delay between injury to the RLN and presentation for treatment. Delayed reinnervation therefore would be more suitable in clinical practice.

Objective: To test the feasibility of delayed selective abductor reinnervation following transection of the recurrent laryngeal nerve (RLN).

Materials and Methods: In 12 cats, the right RLN was severed. Nine months later, the phrenic nerve was anastomosed to the distal RLN stump with all its branches directed toward the posterior cricoarytenoid muscle. For 10 weeks after the reconstruction, electromyography and videolaryngoscopy were performed weekly. Finally, histological analysis of the RLN was performed.

Results: Evaluation was possible in 11 cats. Reinnervation of the right posterior cricoarytenoid muscle with the phrenic nerve occurred in 10 cats following nerve anastomosis, but results of videolaryngoscopy showed adequate to good abduction in only 4 cats. The main limiting factor was reduced mobility of the cricoarytenoid joint. Evidence of spontaneous subclinical reinnervation after the delay was observed in 7 cats but apparently did not impede the surgical reinnervation.

Conclusions: Delayed selective laryngeal abductor reinnervation was feasible, but function recovery was less successful than if performed immediately. Future investigations should concentrate on early determinants of spontaneous restoration of function to allow early selection of patients who are eligible for reinnervation surgery.


THE DIAGNOSIS of recurrent laryngeal nerve (RLN) or vagus nerve paralysis often is made after a considerable delay, particularly in cases of unilateral paralysis, when the symptoms of dysphonia and sometimes aspiration may have been present for weeks or even months before a laryngological consultation is sought. In the less common case of bilateral (RLN or vagus nerve) paralysis, progressive stridor and dyspnea often lead to an earlier diagnosis. Unless respiratory insufficiency warrants urgent airway management, attention is first directed toward the cause, extent, and site of the nerve injury. In addition, before any form of permanent and mostly irreversible surgical treatment is performed, spontaneous recovery or compensation (in case of unilateral paralysis) is awaited for some months. Therefore, a considerable delay between the onset of nerve injury and the timing of surgical intervention generally elapses.

In the case of persistent unilateral paralysis, where dysphonia predominates, vocal fold augmentation or medialization laryngeal framework surgery are the most suitable procedures to correct the incomplete glottis closure. For permanent bilateral paralysis, the present treatment options consist of vocal fold lateralization, arytenoidectomy, posterior transverse cordotomy, or permanent tracheostomy. With the exception of permanent tracheostomy, all of these procedures are aimed at enlarging the airway at the glottic level at the inevitable cost of voice quality and an increased risk of aspiration.

Experimental work has therefore been directed toward reinnervation surgery as well as laryngeal pacing procedures. Both methods aim to improve laryngeal function by restoring laryngeal mobility with opening of the airway during inspiration without sacrificing voice quality or increasing the risk of aspiration. Previous studies have concentrated on reinnervation surgery as a potential technique to restore laryngeal function and have achieved excellent...
MATERIALS AND METHODS

SURGICAL METHOD

Institutional guidelines of the Vrije Universiteit for animal experiments were followed as formulated by Dutch law. Twelve female cats (age, 6 months; weight range, 2200-2800 g) were anesthetized with ketamine chloride (20 mg/kg given intramuscularly) and xylazine hydrochloride (0.5 mg/kg given subcutaneously), allowing spontaneous respiration.

A midline incision of the neck was performed. Pretracheal muscles were separated in the midline, and the upper part of the trachea was exposed. The right RLN was then identified. At a distance of 2.5 cm from the cricothyroid joint, the RLN was transected. The proximal RLN was then resected as far caudally as possible, usually a length of 4 to 5 cm. The distal stump was then fixed in a small piece of plastic tubing to facilitate reidentification.

After 9 months, the neck was again explored using an extended midline incision. The distal RLN stump was identified and resected slightly more distal to the initial transection, 2 cm from the cricothyroid joint. The larynx was rotated 90° around its longitudinal axis. The inferior pharyngeal constrictor muscle was partly sectioned at its insertion on the lower posterior margin of the right thyroid lamina. A small inferior posterior part of the thyroid lamina was resected to expose the adductor and abductor RLN branches. The abductor branch was preserved, the adductor branch was severed, and the proximal adductor stump was buried in the PCA muscle and fixed with a 10×0 microsuture (Ethilon, Ethicon Inc., Somerville, NJ) and fibrin glue (Tissucol, Austrian Institute for Haemoderivatives, Vienna, Austria). To exclude any influence of the ansa galeni, it too was severed, and its proximal stump was buried in the PCA muscle. The 2 roots of the right PN were identified. In contrast to the situation in humans, the PN in the cat has 2 clearly distinguishable roots originating from C-5 and C-6, respectively. The PN root from C-5 was transected just before it joined the other root. The distal RLN stump was anastomosed to the C-5 PN stump using 3-0 microsuture and fibrin glue. Thus, all reinnervating PN axons were directed toward the PCA muscle (Figure 1), even those that followed the path of the adductor branch or the ansa galeni.

LARYNGEAL ABDUCTOR FUNCTION ASSESSMENT

To assess laryngeal function, videolaryngoscopy and EMG were performed, using the same anesthesia as for the surgical procedure.

Laryngeal abductor function was assessed before and immediately after initial transection of the RLN, before and immediately after retranssection of the RLN, before the PN transfer 9 months later, and weekly during a 10-week follow-up. The time at which a first sign of mobility and EMG reinnervation activity were seen was recorded. After the 10 weeks, a final assessment of laryngeal abductor function (using videolaryngoscopy and EMG) was performed during quiet respiration and respiratory distress. Respiratory distress was achieved by performing a tracheotomy and occluding the tracheotomy tube (type Shiley 00 CFS, Mallinkrodt Medical, Irvine, Calif) for 1 minute. The tracheotomy tube closely fit the trachea in all cases so that on occlusion, there was virtually no passage of air. To eliminate any influence from the cricothyroid muscles or extrinsic laryngeal musculature, these muscles were severed bilaterally before the final assessment.

Electrical stimulation of the right PN was performed proximal to the anastomosis, and mobility and evoked activity were recorded using videolaryngoscopy and EMG.

The right PN-RLN anastomosis was fixed in situ and removed for histological analysis. The animals were killed, and the larynx was excised. The right arytenoid was then palpated for signs of a reduced passive mobility of the cricoarytenoid joint compared with the left cricoarytenoid joint.

VIDEOLARYNGOSCOPIC EVALUATION

The mobility of the reinnervated right hemilarynx was compared with the normal left hemilarynx. Abduction was scored as good if abduction on inspiration occurred almost synchronously with and equal in degree to the abduction of the left side; adequate if abduction on inspiration was more than half the abduction of the left side; limited if abduction on inspiration was half or less the abduction of the left side; or poor if no effective or very slight abduction on inspiration was observed.

EMG EVALUATION

Electromyography was performed using transorally introduced hooked wire electrodes in the left and right PCA muscles and in the left and right vocalis muscles (pars medialis of the thyroarytenoid muscle). An EMG type MS6 (Medelec, Old Walking, England) was used. Respiratory monitoring was performed simultaneously, using a custom-made impedance plethysmograph, registering chest movement.

HISTOLOGICAL ANALYSIS

To obtain histological proof of the reinnervation, the nerve anastomoses were fixed in situ after the final assessment in the anesthetized animal, using a cacodylate-glutaraldehyde solution, and then resected for histological examination. After postfixation in the same solution, the tissue was embedded in epoxy resin, and 1-µm-thick transverse sections were cut. These were stained for myelin using 1% paraphenylenediamine. Histological analysis was performed 0.5 cm distal to the anastomosis using computerized screening of a 500-µm-wide band across the widest nerve diameter, and the axon count (axons per square millimeter) and the axon diameters were estimated.

results using surgical reinnervation techniques immediately following nerve injury in an animal model.

In treatment of long-standing paralysis, many factors may adversely influence successful surgical reinnervation and laryngeal function restoration. These include denervation atrophy, spontaneous subclinical reinnervation, inappropriate reinnervation, and fixation of the cricoarytenoid joint. If extensive denervation atrophy has occurred, this may preclude successful reinnervation because of irreversible muscle fibrosis and...
degeneration of muscle endplates.\textsuperscript{13,14} Usually, however, some spontaneous reinnervation takes place due to axonal regeneration across the injury site or neurotization from surrounding musculature. The process of atrophy is then halted and reversed. Such reinnervation generally will not provide laryngeal mobility, a condition known as subclinical (re)innervation.\textsuperscript{15,16} Alternatively, should the reinnervation be sufficient to produce adequate muscular contraction, the regenerated axons may connect to the wrong target muscle, causing synkinesis and thus impairment of function.\textsuperscript{17,18} This condition is referred to as inappropriate reinnervation. In either reinnervated condition, the muscle is no longer susceptible to reinnervation or neurotization from other sources, thus precluding successful reinnervation surgery. These conditions can be diagnosed using laryngeal electromyography (EMG), which typically demonstrates EMG activity in the absence of laryngeal mobility. Subclinical reinnervation and inappropriate reinnervation, rather than true persistent denervation, seem to be the rule some time after serious nerve injury. Furthermore, in long-standing laryngeal immobility, the cricoarytenoid joints may become fixed. This phenomenon appears to be rare in humans,\textsuperscript{19} but extensive fibrous ankylosis has been observed in rabbits after experimental section of the RLN.\textsuperscript{20} This will of course impede restoration of laryngeal mobility despite successful reinnervation.

Considering these factors, time appears to be of paramount importance when contemplating laryngeal reinnervation surgery. Separate selective reinnervation of the adductor and abductor muscle groups can prevent inappropriate laryngeal reinnervation by misdirection of regenerating axons. This reinnervation technique has proved very effective in cats when performed in the acute stage, ie, immediately after denervation by transection of the RLN.\textsuperscript{7,8} Little experimental data are available regarding laryngeal reinnervation surgery after a delay.\textsuperscript{21-23}

We performed this study to test the feasibility of selective abductor reinnervation of the posterior cricoarytenoid (PCA) muscle using a phrenic nerve (PN) transfer after a delay of 9 months after RLN transection.

**RESULTS**

Evaluation was possible in 11 cats. One cat (cat 2) died during the delay.

**LARYNGEAL ABDUCTOR FUNCTION ASSESSMENT**

**Before and After Initial Transection of RLN**

Before transection, normal symmetrical abduction was seen on inspiration in all cats, and normal EMG activity was recorded in the PCA and vocalis muscles. Normally in the PCA muscle, there is a phasic inspiratory activity pattern, whereas the vocalis muscle has only sporadic activity in rest, and a burst of activity is seen during phonation and reflex glottic closure.

Immediately after transection of the right RLN, complete immobility of the right hemilarynx was seen, and there was absence of EMG activity in the right vocalis and right PCA muscles in all cats.

**Before and After Retransection of the RLN**

Before retransection of the RLN, limited abduction mobility of the right hemilarynx was observed in 3 of the cats, using videolaryngoscopy. The EMG recordings of the right PCA muscle showed signs of spontaneous subclinical reinnervation in 7 cats, whereas in the other 4 cats, no EMG activity was found. The EMG activity consisted of an inspiratory pattern (Figure 2, A). After resection of the distal RLN stump, ie, just before performing the delayed PN-RLN anastomosis, the right hemilarynx was immobile in all cats. EMG activity persisted in the right PCA muscle in 3 cats, consisting of a minor inspiratory activity pattern in 1 cat and an uncoordinated pattern in 2 cats (Figure 2, B). The EMG recordings of the right vocalis muscles showed a minor inspiratory activity in 6 cats and an uncoordinated pattern of single motor unit potentials in 5 cats. In the 6 cats with minor inspiratory activity patterns in the vocalis muscle, EMG activity disappeared after retranssection of the RLN, whereas in the 5 cats with uncoordinated single motor unit potentials, the activity persisted. Results of the EMG of the PCA and vocalis muscles are summarized in Table 1.

**Follow-up After PN Transfer**

First signs of PN activity in the EMG recordings, an inspiratory activity pattern starting about 40 to 50 milli-
Respiratory Distress. During respiratory distress, the abductor function was good in 1 cat, adequate in 2 cats, limited in 3 cats, and poor in 5 cats. The 5 cats included the cat with surgical failure. In 10 cats, the inspiratory EMG pattern in the right PCA muscle increased during respiratory distress with recruitment of multiple motor unit potentials. In the cat with surgical failure, no EMG activity was seen during respiratory distress, as in quiet respiration. In the right vocalis muscle, an increase in the inspiratory EMG activity pattern was seen during respiratory distress in 9 of the 11 cats. This occurred simultaneously with the activity in the PCA muscle and indicated synkinesis. The remaining 2 cats showed slight enhancement of the resting activity in the right vocalis muscle, with no coordinated pattern.

Table 1. EMG Recordings in the Right PCA and Right Vocalis Muscles After 9-Month Delay

<table>
<thead>
<tr>
<th>Cat No.</th>
<th>Before Retransection of RLN</th>
<th>After Retransection of RLN</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PCA Vocalis</td>
<td>PCA Vocalis</td>
</tr>
<tr>
<td>1</td>
<td>0 1</td>
<td>0 0</td>
</tr>
<tr>
<td>3</td>
<td>0 S</td>
<td>0 S</td>
</tr>
<tr>
<td>4</td>
<td>1 1</td>
<td>0 0</td>
</tr>
<tr>
<td>5</td>
<td>1 1</td>
<td>0 0</td>
</tr>
<tr>
<td>6</td>
<td>1 1</td>
<td>0 0</td>
</tr>
<tr>
<td>7</td>
<td>1 S</td>
<td>1 S</td>
</tr>
<tr>
<td>8</td>
<td>0 S</td>
<td>0 S</td>
</tr>
<tr>
<td>9</td>
<td>0 1</td>
<td>0 0</td>
</tr>
<tr>
<td>10</td>
<td>1 1</td>
<td>0 0</td>
</tr>
<tr>
<td>11</td>
<td>1 S</td>
<td>0 0</td>
</tr>
<tr>
<td>12</td>
<td>1 S</td>
<td>0 0</td>
</tr>
</tbody>
</table>

*EMG indicates electromyographic; PCA, posterior cricoarytenoid; RLN, recurrent laryngeal nerve; 1, inspiratory EMG activity; S, uncoordinated single motor unit potentials; and 0, no EMG activity.

Electrical Stimulation. Electrical stimulation of the PN proximal to the anastomosis resulted in visible abduction or contraction of the right hemilarynx and evoked EMG activity in 10 of the 11 cats. In the cat with surgical failure, there was no response to electrical stimulation.

Cricoarytenoid Joint Mobility. Palpation of the right arytenoid revealed reduced cricoarytenoid joint mobility in 6 cats. Five of these cats had a poor abductor function, and 1 of them had limited abductor function. The 3 cats with good and the 1 cat with adequate abductor function had a normal passive mobility of the cricoarytenoid joint. In 1 cat (cat 9), palpation of the arytenoid was not conclusive. Spearman rank correlation between restoration of function and normal passive mobility of the cricoarytenoid joint was 0.49.

HISTOLOGICAL ANALYSIS

Histological proof of reinnervation in the RLN was found in all 8 examined specimens. The specimen from the cat with surgical failure was excluded, and specimens from the remaining 2 cats (cats 4 and 5) could not be evaluated due to an unsuccessful fixation procedure. The median axon count was 3771 axons/mm² with a mean axon diameter of 2.17 µm. In a previous study involving cats of the same breed, sex, and weight range as were used in

Final Assessment

Quiet Respiration. Abductor function 10 weeks after reinnervation during quiet respiration was good in 3 cats, adequate in 1 cat, limited in 1 cat, and poor in 6 cats (Table 2). Between the presence of subclinical EMG activity in the right PCA after the 9-month delay, recorded before and after retranssection of the RLN, and the final degree of abductor function recovery, Spearman rank correlations of 0.23 and 0.50, respectively, were found. Evaluation of EMG recordings showed typical PN phasic inspiratory activity in the right PCA muscle in 10 of the 11 cats (Figure 2, C). In the remaining cat (cat 8), the anastomosis was later found to have lost continuity and was therefore considered a surgical failure. In the right vocalis muscle, a weak inspiratory EMG activity pattern was recorded in 9 of the 11 cats (Figure 2, C). In the other 2 cats, uncoordinated series of single motor unit potentials were seen.
this study, we found that the median axon count in the unaffected RLN was 3144 axons/mm², and the mean axon diameter was 4.68 µm. Analysis of variance indicated a significantly higher number of and significantly thinner (regenerating) axons in the reinnervated RLN than in the normal RLN (P = .009).

### Table 2. Results of Final Assessment 10 Weeks After Reinnervation Procedure*

<table>
<thead>
<tr>
<th>Cat No.</th>
<th>Abductor Function</th>
<th>EMG Activity</th>
<th>Electrical Stimulation</th>
<th>Passive Mobility of CA Joint</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Quiet Respiration</td>
<td>Respiratory Distress</td>
<td>PCA</td>
<td>Vocalis</td>
</tr>
<tr>
<td>1</td>
<td>Poor</td>
<td>Limited</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>Good</td>
<td>Adequate</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>Poor</td>
<td>Poor</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>Good</td>
<td>Adequate</td>
<td>1</td>
<td>S</td>
</tr>
<tr>
<td>6</td>
<td>Good</td>
<td>Good</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>7</td>
<td>Limited</td>
<td>Limited</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>8</td>
<td>Poor</td>
<td>Poor</td>
<td>0</td>
<td>S</td>
</tr>
<tr>
<td>9</td>
<td>Poor</td>
<td>Poor</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>11</td>
<td>Adequate</td>
<td>Limited</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>12</td>
<td>Poor</td>
<td>Poor</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>13</td>
<td>Poor</td>
<td>Poor</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

*EMG indicates electromyographic; PCA, posterior cricoarytenoid; CA, cricoarytenoid; 1, inspiratory; 0, no inspiratory; S, single motor unit potentials; R, reduced; and N, normal.

Selective reinnervation of the PCA muscle with a PN transfer has proved feasible even after a delay of 9 months. This was established by EMG PN activity in the PCA muscle in 10 of the 11 cats, PCA muscle contraction on electrical stimulation of the right PN in these 10 cats, and histological proof of reinnervation in examined nerve specimens in 8 cats. Even if subclinical reinnervation persisted following retranssection of the RLN stump, it did not prevent surgical reinnervation in our study. A correlation is even suggested between persistence of subclinical reinnervation after retranssection of the RLN and a good restoration of function (Spearman rank correlation coefficient, 0.50).

No histological examination of the muscular condition was performed, but no apparent denervation atrophy was observed during laryngoscopy or macroscopic postmortem examination. Probably the process of severe denervation atrophy had been prevented or reversed as a result of the subclinical reinnervation.

Despite evident reinnervation, however, the abductor mobility function following the delay was clearly less successful than after immediate reinnervation with the PN transfer.24 The results of our study are compared with previously reported results following immediate selective abductor reinnervation (Figure 3) during quiet respiration and respiratory distress. The main limiting factor in mobility restoration in our study appears to have been reduced passive mobility of the cricoarytenoid joint. In earlier studies,24,19 in which reinnervation was performed in the acute stage, only 2 of 30 cats showed reduced passive joint mobility. Fixation of the cricoarytenoid joint due to fibrous ankylosis has occurred in rabbits starting at 5 months after RLN section, leading to complete fixation at 12 months.20 In humans, fibrous ankylosis only due to immobility after RLN paralysis appears to be exceptional.19 This difference is explained by lack of passive movement of the arytenoid owing to less use of the vocal folds in animals.19

Inappropriate reinnervation resulting in synkinesis may have been an additional limiting factor in the restoration of mobility in our study. All cats except that with surgical failure showed signs of inappropriate reinnervation of the right vocalis muscle with inspiratory EMG activity. Despite this fact, 3 cats achieved a good abductor function during quiet respiration. However, during respiratory distress, the abductor function decreased in 2 of these cats, although an enhanced inspiratory EMG activity pattern was observed in the PCA muscles. This can be explained only by inappropriate spontaneous reinnervation of the vocalis muscles that were not surgically reinnervated, resulting in synkinesis. Inspiratory EMG activity that was already present in the vocalis muscles during quiet respiration showed a marked increase during respiratory distress. This activity occurred simultaneously with the increased inspiratory activity in the PCA muscles during respiratory distress, impeding abductor function. In a previous study, in which PN transfer was performed immediately following RLN...
transient, no inappropriate reinnervation of the vocalis muscle was observed, and respiratory distress resulted in an increased abdution.

The results obtained in our study cannot be simply translated to humans, but a number of factors are important to take into consideration when contemplating human reinnervation surgery. The best results of reinnervation surgery are obtained if the interval between the injury and reconstruction is short. Future research should be directed toward tests that can predict the chances of spontaneous nerve recovery at an early stage, to select the patients with a poor prognosis of nerve recovery so they can benefit from reinnervation surgery. Although EMG data are believed to be reliable in predicting the spontaneous outcome of laryngeal paralysis, conclusive evidence to support these claims is still lacking. Reinnervation surgery is likely to be beneficial, especially for patients with bilateral paralysis. If there is certainty of loss of continuity of both RLNs, a unilateral reinnervation procedure using the PN may be considered immediately, as no spontaneous recovery can be expected following transection due to the inevitable inappropriate reinnervation and subsequent synkinesis, which has been demonstrated in a previous study.18

For patients with unilateral paralysis and dysphonia, vocal fold augmentation or surgical medialization still appears superior to reinnervation surgery, and therefore early selection is less important in these cases.

In conclusion, reinnervation surgery may be considered immediately after severe bilateral nerve injury or in selected cases of long-standing bilateral RLN or vagus nerve paralysis where there is no hope for spontaneous recovery, provided there is normal cricoarytenoid joint mobility.

Accepted for publication October 21, 1997.

Supported by N. V. Organon, Oss, the Netherlands.

For the histological analysis, we thank Geert J. P. T. Schuijers, MS, N. V. Organon. For the practical performance of the experiments, the help of the staff of the Clinical Animal Laboratory of the Vrije Universiteit, Amsterdam, the Netherlands, especially Joop A. Grimbergen, has been essential. The statistical analysis has been performed with the aid of Hilde Tobi, MS, Department of Epidemiology and Biostatistics, Vrije Universiteit.

Reprints: Hans F. Mahieu, MD, PhD, Department of Otolaryngology—Head and Neck Surgery, University Hospital, Vrije Universiteit, De Boelelaan 1117, 1081HV Amsterdam, the Netherlands.

REFERENCES