Compliance and Functional Residual Capacity After Staple Versus Combined Staple/Holmium Laser Lung Volume Reduction Surgery in a Rabbit Emphysema Model

Dan L. Serna, MD, Ledford L. Powell, MD, Matthew Brenner, MD, Shannon M. O'Connor, BS, Robert J. McKenna, Jr, MD, Nai-San Wang, MD, and John C. Chen, MD

Divisions of Pulmonary Medicine, Cardiothoracic Surgery, and Beckman Laser Institute, University of California Irvine Medical Center, Orange, California

Background. **There is some evidence to suggest that laser exposure, when added to standard staple reduction techniques, may result in improved physiologic response to lung volume reduction surgery (LVRS). In this study, we compared physiologic responses of staple LVRS with combined staple/laser in a rabbit emphysema model.**

Methods. **Ninety-three New Zealand White rabbits underwent emphysema induction with aerosolized elastase 4 weeks before surgery and were killed 1 week after surgery. Treatment groups were bilateral moderate volume staple LVRS (≤ 3 g, n = 39), combined moderate volume staple (≤ 3 g)/holmium laser LVRS (n = 18), large-volume staple LVRS (≥ 3 g, n = 27), or sham surgery (n = 9).**

Results. **Decrease in postoperative static respiratory system compliance by combined moderate-volume staple/laser treatment (1.22 cc/cm H_2O) was similar to large-volume staple resection (1.40 cc/cm H_2O, $p = 0.39$), and superior to moderate staple resection (0.82 cc/cm H_2O, $p = 0.01$) or sham surgery (0.09 cc/cm H_2O, $p = 0.0001$). Functional residual capacity decrease was greater after combined moderate staple/laser resection (6.46 cc) than large-volume staple resection (4.52 cc, $p = 0.33$), moderate-volume staple resection (4.59 cc, $p = 0.43$), or sham surgery (4.10 cc, $p = 0.29$). Perioperative mortality was highest after laser/staple LVRS (22%, 4/18).**

Conclusions. **In this rabbit model, combined staple/holmium laser reduction for emphysema results in significant improvement in compliance and trends toward improvement in functional residual capacity above staple reduction alone, but with higher mortality.**

(Ann Thorac Surg 1999;68:1003–7)

In human trials, staple lung volume reduction surgical (LVRS) procedures have demonstrated superiority over laser LVRS procedures [1–6]. Although use of lasers has demonstrated effectiveness in improving spirometry and lung hyperinflation for emphysema, most studies also demonstrate increased morbidity and mortality with the use of laser LVRS techniques [1–6]. However, there appears to be a suggestion that laser exposure, when added to standard staple techniques, may result in improved physiologic response to lung volume reduction surgery compared with staple procedures alone [1–6].

Neodymium:yttrium-aluminum-garnet (Nd:YAG) has been the most common laser method used for the laser treatment of emphysema in humans. However, despite benefit Nd:YAG, carbon dioxide (CO_2), and other laser treatments of lung disease produce significant laser-induced lung injury and postoperative morbidity. Previous work in a rabbit bullous emphysema model has demonstrated holmium:yttrium-aluminum-garnet (Ho:YAG) laser treatment to be more effective than Nd:YAG in coagulating bullae without causing more severe lung injury [7]. These results suggest that the Ho:YAG laser technique may accomplish similar benefit to Nd:YAG with potentially less resultant lung injury.

The potential value of lasers as an adjunct treatment to staple LVRS needs investigation. For this reason, we compared the physiologic response of staple LVRS with combined staple and free-beam holmium laser in a controlled treatment trial of LVRS in a rabbit model of heterogeneous emphysema. We hypothesized that bilateral laser exposure in conjunction with bilateral staple procedures would increase pulmonary recoil, and decrease functional residual capacity over that achieved by standard staple techniques alone in this rabbit emphysema model.

Material and Methods

The Institutional Animal Care and Use Committee at the University of California, Irvine approved this protocol.

Presented at the Thirty-fifth Annual Meeting of The Society of Thoracic Surgeons, San Antonio, TX, Jan 25–27, 1999.

Address reprint requests to Dr Brenner, Division of Pulmonary and Critical Care Medicine, University of California Irvine Medical Center, 101 The City Dr, Bldg 53, Rm 119, Orange, CA 92668; e-mail: mbrenner@uci.edu.

Published by Elsevier Science Inc

0003-4975/99/$20.00
PII S0003-4975(99)00773-0

All rabbits were cared for in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Animal Preparation

Ninety-three adult male New Zealand White rabbits (2.3–4.8 kg) were anesthetized with a 2:1 mixture of ketamine HCl (100 mg/mL): Xylazine (20 mg/L) at a dose of 0.75 mL/kg i.m. The rabbits were intubated with a 3-mm endotracheal tube and mechanically ventilated (Harvard Apparatus Dual Phase Control Respiratory Pump-Canine; Harvard Co, South Natic, MA) with a tidal volume of 50 mL and a respiratory rate of 30 to 40 breaths per minute. One 20-gauge iv catheter was placed in a marginal ear vein for vascular access. Anesthesia was maintained with 0.3 mL of 1:1 mixture of ketamine HCl (100 mg/mL): xylazine (20 mg/mL) given as iv bolus as needed to maintain apnea throughout all procedures.

Induction of Emphysema

Emphysema was induced in 93 rabbits under general anesthesia by aerosolizing 15,000 U (7.89 mL) of porcine elastase (Worthington Biochemical Corp, Lakewood, NJ) through the endotracheal tube over approximately 1 h. The nebulizer (Respirgard; Marquest Medical Products, Inc, Englewood, CO) was connected to the inspiratory arm of the ventilator circuit, with the ventilator set to a tidal volume of 0 cc and a respiratory rate of 30 breaths/min. The oxygen flow through the nebulizer was adjusted to maintain a peak airway pressure of 20 cm H_2O. An analog manometer placed at the side port of the endotracheal tube monitored the peak airway pressure.

Pulmonary Function Testing

Lung function measurements were obtained at baseline before induction of emphysema, immediately preoperatively at 4 weeks after induction of emphysema, and 1 week postoperatively. Static respiratory system compliance and functional residual capacity were measured at each time interval.

Static Respiratory System Compliance Measurement

To measure static respiratory system pressures, the anesthetized, apneic rabbits were disconnected from the ventilator and placed in the left decubital position. A 60-cc syringe was attached to the end of the endotracheal tubes, and the lungs were inflated to a 60-cc volume above functional residual capacity (FRC) three times in order to establish a constant volume history. The lungs were allowed to passively deflate. Static respiratory system pressures were measured separately at each inflation volume of 60, 50, 40, 30, and 20 cc above FRC. The appropriate volume was injected and the syringe was held in place for 5 seconds while static airway pressure was measured. The syringe was then released and the lungs were allowed to passively deflate. Static respiratory system pressures were measured using a water manometer (10AA25; Meriam Instruments, Cleveland, OH) connected to the side port of the endotracheal tube. The rabbits were returned to mechanical ventilation after the measurements. Compliance was calculated by dividing the sum of the FRC and inflation volume above FRC by the measured static respiratory system pressure at each inflation volume.

Gas Dilution Lung Volumes

The inhalation gasses consisted of 9.30% helium, 60.50% oxygen, 29.05% nitrogen, 0.87% C_2H_2, and 0.28% $C^{18}O$ (Liquid Carbonic Corp, Los Angeles, CA). All gas concentrations were measured by mass spectrometer (MGA 1100; Perkins-Elmer Corp, Pomona, CA). The data were converted to digital information by an AD converter (570; Keithley System, Cleveland, OH) sampling at 20 Hz and stored on an IBM personal computer. The anesthetized rabbits were taken off the ventilator and placed in the left decubital position. The sampling tube of the mass spectrometer was connected to the side port of the endotracheal tube, through which the inspired and expired gas concentrations were continuously measured. A syringe was filled to 60 cc with inhalation gasses and connected to the endotracheal tube. A multibreath helium dilution maneuver was performed by manually pumping the syringe for 10 breaths with a tidal volume of 60 cc over 6 to 10 seconds total time. The initial and final helium concentrations were used to calculate the multibreath helium dilution FRC. Two measurements of FRC were obtained at each trial and averaged. The rabbits were returned to mechanical ventilation after each procedure.

Lung Volume Reduction Surgery

Lung volume reduction surgery (LVRS) was performed 4 weeks after elastase induction of emphysema. The anterior chest wall of the anesthetized and intubated rabbits was shaved and the rabbits were placed in a supine position. Eighty-four rabbits underwent resection of varying quantities of lung tissue. Nine control rabbits underwent median sternotomy and chest tube placement with no excision of lung tissue (sham surgery).

Hypothermia was prevented with a surgical warming pad. Ringer's lactate solution was infused through an iv catheter in a marginal ear vein at a rate of 5 to 15 cc/h. The rabbits were mechanically ventilated using the ventilator described above. Oxygen saturation (Ohmeda Biox 3700 Pulse Oximeter; BOC Health Care, Boulder, CO), tidal CO_2 (Ohmeda 5200 CO_2 Monitor; BOC Health Care, Boulder, CO), and electrocardiogram (78353B Continuous EKG Monitor; BioMedical Services, Hewlett-Packard) were monitored continuously.

The shaved chest was prepped with Betadine and draped in sterile fashion. The thorax was entered through a median sternotomy. Bilateral upper and middle lobes were excised using a linear thoracoscopic stapler (Endopath ELC; Ethicon Endo-Surgery, Cincinnati, OH) with 3.5-mm staples. Target quantity of lung tissue removed was 0.4 to 5.8 g. Excised lung tissue was weighed intraoperatively to ensure the achievement of target resection amounts. During sham surgery, no lung tissue was excised. Hemostasis was obtained and a 12 F neonatal chest tube was placed under direct visualization into each pleural space. The two chest tubes were con-

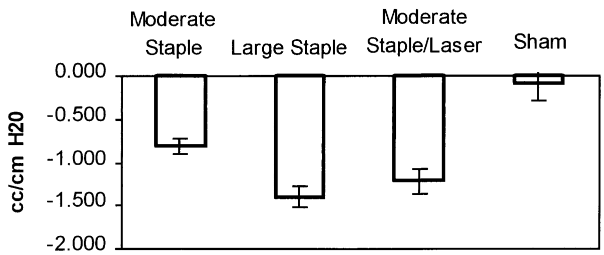

Fig 1. Change in static respiratory system compliance with LVRS. Error bars represent standard error of the mean.

nected to 10 cm of water suction. The sternum was closed with interrupted 0 silk suture, and the chest wound was closed in layers with absorbable monofilament sutures. The rabbits were awakened from anesthesia and extubated. All airleaks, if present, were mild and spontaneously sealed within the first hour of surgery. All chest tubes were removed within 1 hour of surgery.

Laser LVRS

A Trimedyne OmniPulse Holmium laser (Trimedyne Inc, Irvine, CA) with a 1-mm diameter optical fiber and straight-cut end was used in a free-beam mode (10 W delivered, 10 Hz). Fiber energy delivery was calibrated before each use. A 3.5-mm spot size was obtained at approximately 10.3 cm from the tip. The fiber was manually manipulated. Distance and angle were maintained constant with a sterile plastic spacer fixed to the end of the fiber. Surface emphysematous regions were treated as uniformly as possible, covering the lung surfaces with a total 10 second of exposure per side and approximately 60 J per hemithorax. Rabbits were disconnected from the ventilator during laser exposure to prevent lung movement variability.

Statistical Analysis

All compliance and FRC data for each rabbit were tabulated corresponding to preoperative and postoperative measurements. For static respiratory system compliance and FRC, comparisons of the change from the preoperative to the postoperative measurements between the four groups were made using analysis of variance (ANOVA) and two-tailed Student's *t* test (Systat 7.0.1; SPSS, Inc, Chicago, IL), and χ^2 test was performed to test for significance in mortality.

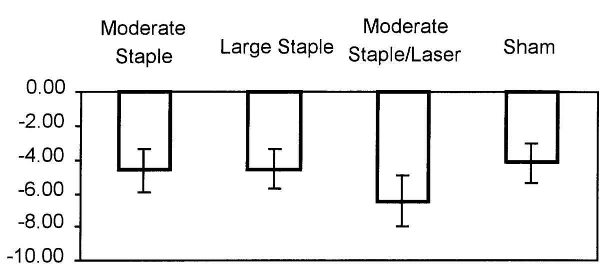

Fig 2. Change in functional residual capacity with LVRS. Error bars represent standard error of the mean.

Table 1. Perioperative Mortality After LVRS

LVRS Technique	Mortality	Chi-square Preop Versus Postop (*p*)	Chi-square Versus Staple/Laser (*p*)
Sham surgery	0% (0/9)	1.00	0.125
Moderate staple LVRS	0% (0/39)	1.00	0.002
Large staple LVRS	6% (1/17)	0.31	0.05
Moderate staple + Laser LVRS	22% (4/18)	0.03	

LVRS = lung volume reduction surgery.

Results

Mean lung volume resection was 2.2 g in the moderate volume staple resection group, 4.2 g in the large volume staple resection group, and 2.4 g in the combined moderate volume staple/laser group. No lung tissue was resected in the sham surgery group.

After the administration of elastase, static respiratory system compliance increased by an average of 0.72 cc/cm H_2O among all rabbits ($p < 0.05$). Similarly, after the administration of elastase, FRC increased among all rabbits by an average of 3.93 cc ($p < 0.05$).

Reduction in static respiratory system compliance by combined moderate-volume staple/laser treatment (1.22 cc/cm H_2O) was similar to that achieved by large-volume staple resection (1.40 cc/cm H_2O, $p = 0.39$), but was superior to reduction in compliance achieved by moderate-volume staple resection alone (0.82 cc/cm H_2O, $p = 0.01$) or by sham surgery alone (0.09 cc/cm H_2O, $p = 0.0001$; Fig 1). ANOVA revealed a significant difference among the four experimental groups ($p < 0.001$).

There was a trend toward greater reduction in FRC after combined moderate-volume staple/laser resection (6.46 cc) than after large-volume staple resection (4.52 cc, $p = 0.33$), moderate-volume staple resection (4.59 cc, $p = 0.43$), or sham surgery (4.10 cc, $p = 0.29$; Fig 2), although these values did not reach statistical significance.

Perioperative mortality (Table 1) was greater with combined moderate-volume staple/laser LVRS (22%, 4 of 18) than with moderate-volume staple resection alone (0%, 0 of 39, $p = 0.002$), sham surgery (0%, 0 of 9, $p = 0.125$), or large-volume staple resection (4%, 1 of 27, $p = 0.05$). Perioperative mortality was significant only in the moderate staple/laser LVRS ($p = 0.03$). Rabbit death after LVRS in this model occurred during the first 5 to 48 postoperative hours.

Comment

In this study, we found that combined moderate-volume staple resection and free-beam holmium laser treatment of emphysematous lung tissue allows some physiologic improvement, comparable with larger-volume staple resections and above what is achieved with similar volume staple resections, but with increased mortality.

A variety of laser techniques have been investigated for the treatment of emphysema by lung volume reduction. Thus far, the most popular laser types for LVRS have been the Nd:YAG and CO_2 lasers [1, 2, 5, 7]. Ho:YAG laser may represent an improvement in laser method for emphysema over other laser techniques. The Ho:YAG laser is 100 times better absorbed in water than the Nd:YAG laser, which is particularly useful in uniformly ablating pulmonary bullae [8]. In a comparison of Ho:YAG with Nd:YAG lasers using a rabbit model of bullous emphysema, the Ho:YAG laser was dramatically more efficient in coagulating bullae and did not result in more acute lung injury than the standard Nd:YAG laser [7]. The Ho:YAG laser also required less exposure at equivalent power and resulted in immediate desiccation of bullae, in sharp contrast to the Nd:YAG laser [7]. The Ho:YAG laser has demonstrated impressive initial results in the ablation of pulmonary bullae in humans [8].

Significantly greater reduction in static respiratory system compliance was achieved by combined laser/moderate-volume staple reduction than by moderate-volume staple reduction alone ($p = 0.01$; Fig 1). The level of improvement in compliance after the combined approach was similar to that achieved by much larger staple reduction alone ($p = 0.39$). Holmium laser treatment produces a band of fibrosis near the visceral surface of the lung that appears to counteract hyperinflation of emphysematous lung, which may be one mechanism of improving dyspnea in emphysema sufferers. Further, this fibrous band may act inwardly to increase recoil or tether collapsible airways, although this hypothesis was not directly tested by the present study.

There was also a trend toward greater reduction in FRC after combined moderate-volume staple/laser resection than after large-volume staple resection ($p = 0.33$), moderate-volume staple resection ($p = 0.43$), or sham surgery ($p = 0.29$, Fig 2), although these values did not reach statistical significance. Reduced lung volumes and decreased thoracic hyperinflation may allow the diaphragm and other muscles of respiration to work more efficiently and may contribute to improvements seen after LVRS [9].

Despite slightly greater physiologic improvement after combined staple/laser LVRS, there was greater mortality in this group. This is probably explainable by the damage caused by lasers. Necropsy was performed upon all animals that died after surgery before the time of death. Mortality was caused by combined pneumothoraces/hemothoraces. Increased morbidity, particularly delayed pneumothorax, has been reported in patients treated with laser LVRS compared with staple LVRS [1]. Prior laser LVRS studies in animals have demonstrated that significant lung injury occurs in normal rabbit lungs with both CO_2 and Nd:YAG laser exposure, though the clinical and histologic presentations were distinct [8, 10, 11]. Holmium laser exposure is more efficacious but yields no differences in morbidity compared with Nd:YAG and CO_2 exposures [7]. During Ho:YAG laser treatment of bullae in a rabbit model of bullous emphysema, there was a noticeable acute effect [7]. The visceral surface of bullae was noted to rapidly contract, coagulate, and desiccate [7]. Similarly, in the present model of heterogeneous emphysema, the visceral surface was noted to immediately contract and coagulate within a few seconds of laser application. Immediate bleeding or air leak was not noted in any cases. All chest tubes were removed within 1 h of surgery. It appears air leak and bleeding were either delayed or subacute.

There are several obvious limitations of this study. First, this study is conducted using an animal model of heterogeneous emphysema, and these results may not be fully applicable to human LVRS. Second, this study examines only compliance and lung volumes, whereas it does not address changes that may occur in other pulmonary physiologic variables such as diffusing capacity, pulmonary artery pressures, oxygenation, CO_2 removal, or right ventricular performance after laser/staple LVRS. A better appreciation for the cardiopulmonary physiologic changes and limitations of laser LVRS may be needed to more fully understand the effects of staple versus combined laser/staple LVRS, and ultimately for determination of what if any role there is for lasers in LVRS. We studied only one laser wavelength and exposure setting. Optimization of laser parameters will likely be necessary to determine whether there may be benefit for some patients while minimizing harmful consequences. Third, it is remotely possible that the degree of air leak and bleeding caused by holmium laser treatment of heterogeneous emphysema may not be a significant cause of mortality in humans. Within 1 hour of completion of surgery, there was no significant return of blood or air through the chest tubes and, therefore, all chest tubes were removed from our rabbits at that time. Perhaps, maintaining chest suction in all rabbits for a greater length of time would have produced reduced differences in mortality among the experimental groups, while maintaining benefits in compliance and FRC.

This is an acute mild/moderate emphysema model. More chronic and more severe disease presentations may respond differently to laser/staple versus staple alone exposure. There is a tendency toward subpleural distribution of the emphysema in this model that may favor benefits of these relatively shallow penetrating lasers that may not be as effective in most human presentations. Additionally, animals were followed for only a short time after LVRS and may not reflect longer follow-up results.

In this study, we found that combined staple/laser treatment for heterogeneous emphysema offers significant reduction in compliance and trends toward greater reduction in FRC over staple LVRS alone. However, this is accompanied by greater mortality in this rabbit emphysema model. The future of either combined laser/staple therapy or laser therapy alone for emphysema may require the development of better, safer laser techniques that result in less lung damage. At the present time, data from published patient trials and this animal study suggest that lasers for LVRS should probably be limited to animal models at the present time until safer, more optimal techniques are developed.

This work was supported in part by Department of Energy Grant DE-F603-91ER61227, American Lung Association Grant CI-030-N, California Tobacco-Related Diseases Research Program Grant 6RT-0158, and an American College of Surgeons Faculty Grant.

References

1. McKenna RJ Jr, Brenner M, Gelb AF, et al. A randomized prospective trial of stapled lung reduction versus laser bullectomy for diffuse emphysema. J Thorac Cardiovasc Surg 1996;111:317–21.
2. Wakabayashi A, Brenner M, Kayaleh RA, et al. Thoracoscopic carbon dioxide laser treatment of bullous emphysema. Lancet 1991;337:881–3.
3. Eugene J, Ott RA, Gogia HS, Dos Santos C, Zeit R, Kayaleh RA. Video-thoracic surgery for treatment of end-stage bullous emphysema and chronic obstructive pulmonary disease. Am Surg 1995;61:934–6.
4. Little AG, Swain JA, Nino JJ, Prabhu RD, Schlachter MD, Barcia TC. Reduction pneumonoplasty for emphysema. Early results. Ann Surg 1995;222:365–71.
5. Wakabayashi A, Peters H, Singh N, et al. Thoracoscopic treatment of bullous emphysema using sapphire contact tip neodymium yttrium aluminum garnet laser (contact YAG): preliminary report. Presented at the 73rd Annual Meeting of The American Association of Thoracic Surgery. Chicago, IL, 1993;47:156.
6. Keenan RJ, Landreneau RJ, Sciurba FC, et al. Unilateral thoracoscopic surgical approach for diffuse emphysema. J Thorac Cardiovasc Surg 1996;111:308–15.
7. Brenner M, Wong H, Yoong B, et al. Comparison of Ho:YAG versus Nd:YAG thoracoscopic laser treatment of pulmonary bullae in a rabbit model. J Clin Laser Med Surg 1997;15:103–8.
8. Kaseda S, Aoki T, Hangai N, Omoto T, Yamamoto S, Sugiura H. Treating bullous lung disease with holmium YAG laser in conjunction with fibrin glue and dexon mesh. Lasers Surg Med 1998;22:219–22.
9. Takasugi JE, Wood DE, Godwin JD, Richardson ML, Benditt JO, Albert RK. Lung-volume reduction surgery for diffuse emphysema: radiologic assessment of changes in thoracic dimensions. J Thorac Imag 1998;13:36–41.
10. Brenner M, Milner TE, Wang NS, et al. Doppler grid surface scanning applications for pulmonary subsurface parenchymal perfusion assessment. J Clin Laser Med Surg 1996;14:367–74.
11. Brenner M, Wang NS, Shankel T, et al. Comparison of continuous versus pulsed CO_2 and Nd:YAG laser-induced pulmonary parenchymal lung injury in a rabbit model. Lasers Surg Med 1996;19:416–23.

DISCUSSION

DR DOUGLAS E. WOOD (Seattle, WA): I congratulate Dr Serna and his colleagues at the University of California at Irvine for their continued leadership and scholarship in the field of emphysema surgery. This group has the largest clinical experience in lung volume reduction surgery and have produced a number of important publications that continue to define the indications and results for emphysema surgery. Although pioneering laser lung reduction, they have critically examined its efficacy in a prospective randomized trial that has largely been responsible for the abandonment of laser lung reduction by most surgical groups.

One may question, therefore, why it is necessary to continue to evaluate laser lung reduction when stapled resection has been shown to be clinically more efficacious with less morbidity and mortality. However, there is a distinct theoretical advantage to reducing lung volumes and improving respiratory mechanics with minimal or no parenchymal resection.

Firstly, an optimization of this technique may allow a minimally invasive procedure that ultimately may evolve to a percutaneous outpatient procedure.

Secondly, an effective and durable method of pleural contracture would allow maximum preservation of pulmonary parenchyma and alveolar gas exchange, even though its function is severely impaired.

Thirdly, a laser procedure that does not require parenchymal resection may be better applied to patients with diffuse or homogenous patterns of emphysema, and may allow intervention in an earlier stage of disease instead of waiting until a patient is debilitated by end-stage emphysema.

The authors have also correctly chosen an animal model to try to determine whether laser techniques may be feasible rather than experimenting on a fragile and desperate patient population. They have pointed out the major weaknesses in this approach, that is, the unclear relevance of an induced acute model of emphysema to the chronic acquired disease in humans.

I have two questions for Dr Serna. You collected baseline pulmonary function data before exposing the animals to elastase and again before surgery. Can you tell us how compliance and FRC changed in your model before surgery to help support its validity in this setting? It is not surprising that the acute effects of a visceral pleural laser injury would result in diminished compliance and FRC. If you have experience in your model following these animals for extended periods of several months and charting the pulmonary function and histologic changes in the chronic phase of injury, this, of course, would be more relevant to the planned clinical application.

Doctor Serna, I enjoyed your paper and I look forward to continuing to see the leadership in emphysema surgery from you and your group. Thank you for the privilege of reviewing your work and to the Society for discussing it at this meeting.

DR SERNA: Thank you, Dr Wood, for your interesting comments. We agree that the examination of laser therapy has been virtually abandoned after that paper in 1996; however, we also agree that the maximal preservation of parenchyma would be of interest to many patients with heterogeneous emphysema if such techniques can be further developed. Similarly, less invasive means of LVRS we anticipate will also become available with maximal employment of laser therapy. We feel that lasers have a lot to offer and that they have not been fully examined as they should be before they are completely abandoned.

Regarding your questions regarding compliance and FRC, we do take data regarding compliance and FRC before the induction or administration of elastase to our rabbits. We have seen from these data, which we have previously published, that compliance significantly increases, or the lungs have less elastic recoil after the administration of elastase. This continues to progress with time after induction. Similarly, with FRC, we have also seen that functional residual capacity will increase after the administration of elastase. It increases at the 4-week point, and if we were to continue these animals out for approximately 8 weeks, it would continue to increase. We have not survived animals out to 8 months.

Once again, thank you very much.