Comparison of Wedge to Forceps Videothoracoscopic Lung Biopsy

**Gross and Histologic Findings**

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**Background:** The decreased morbidity and rapid recovery after thoracoscopic lung biopsy compared with open lung biopsy by thoracotomy is increasingly recognized, as is the ability to obtain satisfactory diagnostic material thoracoscopically. To our knowledge, however, there has been no systematic comparison of specimen quality using different thoracoscopic biopsy techniques. The purpose of this study was to compare histologic features of lung parenchyma obtained by videothoracoscopic forceps and wedge biopsy techniques.

**Methods:** Five adult swine were anesthetized, intubated, and ventilated. Sequential left and right videothoracoscopies were performed to obtain biopsy specimens of lung parenchyma using 5-mm endoscopic cupped forceps. Specimens were obtained from fully inflated lung and from partially atelectatic (deflated) lung. Electrosurgery was applied during forceps biopsy for airleak closure. Limited wedge biopsy specimens were obtained using an endoscopic stapler. One hundred thirteen forceps biopsy specimens (55 inflated, 58 deflated) and 24 sections from 8 wedge biopsy specimens were examined. Specimens were assessed for overall histologic quality and ease of microscopic interpretation. Specific histologic features were then evaluated including presence of artifact, congestion and hemorrhage, degree of alveolar inflation, and number of bronchioles and vessels per cross-sectional area. Whole lungs from two animals were examined for extent and depth of lung injury at the areas of biopsy.

**Results:** No major differences in overall microscopic specimen quality were detected among the different techniques nor were significant differences noted between lung inflated and lung deflated forceps biopsy technique. Wedge sections contained more vessels per unit area (p<0.001), perhaps reflecting the more peripheral nature of forceps biopsy. Small amounts of thermal or crush artifact were noted on the surface of forceps biopsy specimens, but did not affect overall specimen quality.

**Conclusions:** Multiple 5-mm forceps biopsy specimens were of comparable quality to single wedge biopsy specimens obtained by endoscopic stapling. Although greater numbers of vessels were present in endoscopic stapled wedge biopsy specimens, multiple forceps biopsy specimens in fact, contain amply sufficient vessels for histologic analysis. (Chest 1995; 107:546-50)

- TBB=transbronchial biopsy; VT=videothoracoscopy

**Key words:** histology; lung biopsy; thoracoscopy

Videothoracoscopy (VT) provides direct inspection of the pleural cavity and lung through small intercostal incisions, avoiding the potential adverse effects of thoracotomy. Procedures are performed under local anesthesia or general anesthesia with endotracheal intubation. Because evolving VT techniques and instrumentation have provided new diagnostic and therapeutic options for many pleuropulmonary disorders, it is important to understand why certain thoracoscopic techniques are employed. For example, VT pleural biopsy has greater yield than closed needle pleural biopsy for diagnosis of parietal pleura disorders, probably because of greater specimen size and decreased chance for sampling error.

Videothoracoscopy biopsy of lung parenchyma is substantially less invasive than open surgical biopsy by thoracotomy, yet probably as effective. The ideal VT lung biopsy technique by which specimens are obtained, however, is still undetermined. It is generally recommended that large samples of lung parenchyma are necessary for diagnosis of interstitial or infectious lung disease when analyses of transbronchial biopsy specimens are inconclusive. Lung biopsy by VT wedge resection using an endoscopic stapler provides large specimens that are virtually identical to those obtained by open surgical techniques. However, multiple incisions, costly single-use instruments, and general anesthesia with single lung ventilation are required. Instrument malfunc-
tion may require open thoracotomy for repair. Videothoracoscopic forceps biopsy, however, provides smaller specimens, several lung regions are rapidly sampled, and selective intubation may be unnecessary. Prolonged air leak may occur, however, and specimens are subject to thermal and crush artifact.

Careful selection of specific VT lung biopsy techniques could result in increased cost-effective diagnosis of pleuropulmonary disorders, less parenchymal lung damage, lesser morbidity, and more rapid return to baseline physical activities. The purpose of this experimental study of VT lung biopsy techniques in animal subjects, therefore, was to compare gross and histologic features of lung parenchyma obtained by forceps biopsy of inflated lung, forceps biopsy of partially deflated lung, and limited wedge biopsies using an endoscopic stapler.

**Materials and Methods**

After approval by the institution’s Animal Subjects Committee, left and right VTs were performed on five adult swine (40 to 60 kg). Following anesthesia with ketamine (40 mg/kg intramuscularly [IM]) and atropine (0.05 mg/kg IM), endotracheal intubation was performed and anesthesia maintained by pento barbital (Nembutal) infusion (25 mg/kg intravenously [IV]). Mechanical ventilation was maintained using a large animal ventilator (Harvard). Exploratory left thoracoscopy was performed through a 1-cm skin incision in the animal’s left chest. After bluntly dissecting the intercostal space, the parietal pleura was penetrated to create a pneumothorax. A 7-mm pleural trocar was inserted to inspect the pleural space and lung with a 7-mm 0° rigid telescope attached to a videocamera (Richard Wolf Co). Under direct VT guidance, a second point of entry was made for insertion of a 5-mm insulated trocar into the pleural space.

**Lung Biopsy Techniques**

With the lung inflated, at least five parenchymal lung biopsy specimens were obtained from upper and lower lobes using an insulated, 5-mm endoscopic cupped-biopsy forceps. Specimens were sheared off on the tip of the pleural trocar as electrosurgery (30 W) was applied to seal the air leak. A second set of specimens was then obtained using identical technique, but with the lung partially atelectatic. The lung was then gently reventilated and additional electrosurgery was applied to the areas from which biopsy specimens were taken. A 3- to 4-cm skin incision was made to allow insertion of a 12-mm diameter endoscopic stapler (Endo GIA 3.0, USCC Autosuture Co). The lung was again partially deflated and grasped with 7-mmatraumaticgrasping forceps. At least one, 3- to 5-cm wedge biopsy specimen was then obtained from the lower or upper lobe under direct VT guidance, taking care to avoid the areas from which biopsy specimens were obtained earlier with forceps. After retrieval of all specimens, the lung was inflated and tested for air leak with 40 cm H2O of positive pressure sustained for 5 s. Trocars were removed and incisions were closed with silk suture. Attention was then turned to the controlateral lung. At the end of intervention, animals were killed by pentobarbital overdose, 120 mg/kg IV.

**Histopathology and Comparative Analysis**

Biopsy specimens were immediately placed into 10% neutral buffered formalin, appropriately labeled, and fixed for at least 12 h. After fixation, specimens were measured and submitted for routine processing in the histology laboratory. Specimens received no special handling and were processed identically to human surgical pathology specimens. Forceps biopsy specimens were submitted for processing intact; wedge biopsy specimens were sectioned and representative sections were submitted. Paraffin sections were stained with hematoxylin-eosin and trichrome/elastic stains. Histologic sections were reviewed by a pathologist blinded as to the method of biopsy, and an overall assessment of specimen quality and interpretability was made (easily interpretable, interpretable with some difficulty, and interpretable with much difficulty). Specimens were designated “easily interpretable” if the pulmonary architecture was intact and well aerated with minimal hemorrhage or biopsy artifact; “interpretable with some difficulty” if there was either partial atelectasis, obscuring blood, or biopsy artifact; and “interpretable with great difficulty” if there was extensive atelectasis, obscuring blood or biopsy artifact. Biopsy specimen size was again measured on the microscopic glass slides. The following specific histologic features were then evaluated: presence, degree, and type of biopsy artifact; number of readily identifiable alveoli, vessels (small arteries and veins) and bronchioles per fragment; extent of alveolar aeration; and presence of hemorrhage and congestion. The number of readily identifiable alveoli was counted in all forceps biopsy specimens and randomly selected wedge biopsy sections. In addition, the heart-lung block was dissected from two animals, and the intact lungs were fixed by inflation with 10% neutral buffered formalin. Biopsy sites were examined grossly and microscopically for extent and depth of lung injury.

**Statistical Analysis**

Analysis focused on determining differences and similarities in distributions of the variables from the three methods of biopsy. Pearson’s χ² test was used to compare frequencies on the nominal classification variables (histologic interpretability, presence of congestion or hemorrhage). Analysis of variance and t test were used to test for differences in the continuous variables (percent inflation, number of bronchioles and vessels per unit area) by biopsy method and by interpretability designation. Vessels and bronchioles per unit area were subjected to a square root transformation before analysis to present a more Gaussian distribution.

**Results**

A total of 113 forceps biopsy specimens were taken, 55 from inflated lung and 58 from partially atelectatic lung. Eight wedge biopsy specimens (4 upper lobe and 4 lower lobe) 3 to 5 cm in length were obtained, from which a total of 24 representative sections were analyzed.

**Biopsy Size**

Forceps biopsy specimens were round to ovoid and ranged from 5 to 30 mm² (median, 15 mm²) in cross-sectional area. Individual sections of wedge biopsy specimens ranged from 27 to 146 mm² (median, 61.5 mm²) in cross-sectional area. No statistically significant differences were noted in size of forceps (lung inflated) biopsy specimens compared with forceps (lung deflated) biopsy specimens. Low magnification views of “easily interpretable” forceps and wedge biopsy specimens are shown in Figure 1.

**Artifact**

No significant biopsy-related artifact was seen in
either the forceps or wedge biopsy specimens. Small, focal areas of crush and thermal artifact identified on the perimeter of some forceps biopsy specimens did not affect the overall histologic interpretability. Typical stapler crush artifact was seen grossly but was not evaluated microscopically.

**Alveoli and Alveolar Inflation**

Alveolar space inflation was extremely variable to a similar degree in both forceps and wedge biopsy specimens. Forceps biopsy specimens contained 2,000 to 3,000 readily identifiable alveoli in "easily interpretable" specimens; 300 to 1,700 alveoli in specimens "interpretable with some difficulty," and only 20 to 175 in specimens "interpretable with great difficulty." Alveolar inflation less than or equal to 30% was the most common deficiency in specimens called "interpretable with great difficulty." In total, 10.6% of forceps specimens and two (8.3%) of the stapler section specimens were designated "interpretable with great difficulty." All individual wedge biopsy sections contained at least 8,000 alveoli. Analysis of percent inflation showed highly significant separation by overall interpretability category (p<0.001). The forceps (lung inflated) method showed a higher (t=2.72, 135 df, p<0.01) mean percent inflation (76.6%) than either forceps (lung deflated) or wedge (62.5% and 64.4%, respectively).

**Bronchioles and Vessels**

A minimum of three bronchioles and four vessels were identified in each individual forceps biopsy specimen. No statistically significant differences were noted in number of bronchioles or vessels per square millimeter between forceps (lung inflated) and forceps (lung deflated) specimens. Median composite (combined inflated and deflated techniques) counts were 0.56 bronchioles per square millimeter and 0.62 vessels per square millimeter (p=ns). Median values for wedge biopsy specimens were 0.61 bronchioles per square millimeter and 1.04 vessels per square millimeter. No statistically significant difference in number of bronchioles per square millimeter was noted between forceps and wedge techniques, nor were statistically significant differences found between techniques when numbers of bronchioles or vessels per cross-sectional area were stratified by overall interpretability category. A greater number of vessels per square millimeter, however, was found in wedge biopsy sections compared with those obtained from forceps biopsy specimens (p<0.001, f=14.1;2,128 df).

**Congestion and Hemorrhage**

Mild alveolar wall congestion was noted in 75% of wedge biopsy specimens and in 37% of forceps biopsy specimens (p<0.01, $\chi^2$ 10.0, 1 df). Intra-alveolar or subpleural hemorrhage was noted in 38% of wedge biopsy specimens and 71% of forceps biopsy specimens (p<0.01, $\chi^2$ 8.2, 1 df). Congestion or hemorrhage did not impair specimen evaluation in any of the biopsy specimens.

**Whole Lung Examination**

Forceps biopsy sites on the pleuropulmonary surface were grossly characterized by a central round to oval parenchymal defect surrounded by a concentric area of pallor, surrounded by another concentric area of congestion. The maximum diameter of these changes ranged from 13 to 30 mm. The maximum depth of these lesions ranged from 3.5 to 7 mm (2 to 4 mm of pallor, rimmed by 0.5 to 2 mm of dark red-brown congestion). The gross appearance of biopsy sites was considerably more striking than the microscopic findings. Histologically, there was minor crush and/or cautery artifact at the free edge of the parenchymal defect, and mild to moderate congestion at the periphery. No specific microscopic features were appreciated in the grossly identified areas of pallor.
DISCUSSION

In selected patients with suspected interstitial, infectious, or neoplastic lung disease, conventional practice is to proceed with transbronchial lung biopsy (TBB) after the appropriate imaging studies are obtained. Interestingly, although specimens are usually less than 2 mm² in size, TBB has remarkable sensitivity and specificity, particularly for sarcoidosis, lymphoma, and neoplasm.¹⁰,¹¹ In regards to other parenchymal lung diseases, however, results of TBB have been disappointing. Studies focused on histopathologic or morphometric factors affecting the diagnostic yield of TBB suggest that 5 to 20 alveoli per microscopic field are necessary to determine diagnostic adequacy.¹²,¹³ The question of adequacy, however, cannot be addressed only by noting the number of alveoli per tissue sample because any specimen may be deemed adequate if a definite pathologic diagnosis is made. A well-recognized problem of TBB, however, is lack of representative parenchymal tissue (bronchial wall only, for example) in up to 50% of cases.¹⁴ Indeed, results are very much dependent on operator experience and technique.

Videothoracoscopic lung biopsy, although more invasive than TBB, is an increasingly recognized technique for safely obtaining representative lung parenchyma. Results of VT wedge resection using an endoscopic stapler have been similar to those obtained by thoracotomy.⁴,⁶ Disposable, multifire endoscopic staplers cut between two simultaneously applied rows of staples, providing hemostasis and sealing of air leaks. Unfortunately, the instruments are costly, especially if several lung territories are sampled, and procedures require single lung ventilation and general anesthesia to ensure patient safety. Although large samples may, alternatively, be achieved with lasso sutures followed by resection, most do not recommend this technique because any slippage of the suture could result in substantial air leak or bleeding.

Other methods of VT lung biopsy include simple forceps biopsy, and forceps biopsy accompanied by air leak closure with electrocautery, laser, argon beam coagulation, and cryotherapy.⁶,¹⁵-²⁰ Electrosurgical (electrocautery and electrocoagulation) closure of air leaks caused by removal of parenchymal specimens with biopsy forceps may be incomplete, resulting in prolonged chest tube drainage and hospitalization. Also, if biopsy specimens are inadequate or damaged because of faulty endoscopic technique, diagnostic yield may be unsatisfactory. Cryosurgery may alter the microscopic appearance of biopsy specimens,²¹ and lasers require specific and expensive instrumentation. Studies suggest, however, that lasers produce less extensive parenchymal injury than electrosurgical devices.²²

The diagnostic yield of either VT wedge biopsy or of forceps biopsy is reportedly better than that of TBB and nearly as good as that of limited wedge resection by thoracotomy. Historically, however, VT forceps lung biopsy has been unpopular, probably because of a belief that "big was better" in regards to specimen size. Our hypothesis, that multiple 5-mm forceps biopsy specimens of lung parenchyma provide sufficiently representative tissue for histologic analysis compared with wedge biopsy specimens, is substantiated by the similarities between wedge and forceps biopsies as well as by the few statistically significant differences noted between forceps and wedge samples. Wedge biopsy specimens were larger, however, and contained far more vessels per square millimeter than forceps biopsy specimens. From a standpoint of sample size and overall tissue abundance, therefore, VT wedge biopsy is certainly the more desirable technique. Whether these differences are clinically relevant requires comparative randomized patient studies. Put in perspective, even forceps biopsy specimens designated "interpretable with great difficulty" contained more than 20 alveoli, and most contained more than 2,000.

In regards to forceps biopsy of inflated or partially deflated lung, we found no significant differences in size or in the number of bronchioles and alveoli per unit area. Interpretability was also similar. These findings suggest that atelectasis, such as that obtainable by selective endobronchial intubation, may not be absolutely necessary to improve tissue sampling. From a clinical standpoint, avoidance of selective endobronchial intubation would reduce operating room time and decrease overall procedure cost.

In summary, forceps biopsy provided satisfactory material for histologic study, similar to sections of wedge biopsy specimens obtained by endoscopic stapling in animal subjects. We did not attempt to address technical difficulties or potential complications of either technique. Forceps biopsy of lung parenchyma, even with electrosurgery, exposes patients to the risks of pneumothorax and prolonged air leak. Multiple samples are probably necessary, and must be obtained skillfully to avoid thermal or crush artifact. Although wedge resection provides larger samples, improper handling of an endoscopic stapler can result in significant bleeding and tears of lung parenchyma. Regardless of VT biopsy technique, a chest tube must always be inserted into the pleural space and placed to negative suction. Obviously, VT lung biopsy should be performed only by thoroughly trained thoracoscopists knowledgeable about the indications, potential complications, and available instrumentation.
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REFERENCES