Short-term Survival after Radiofrequency Ablation (RFA) of the Lung in Swine Model

BY

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Abstract:

Introduction: Radiofrequency ablation utilizes a high frequency alternating current to create coagulation necrosis. Radiofrequency ablation has been extensively studied in many organs, and in the last few years, investigators have been to understand the effects and safety of RF ablation in lung tissue. Porcine and rabbit models have been used in the past to evaluate the immediate effects of RFA in lung tissue. This is the first series of RFA efficacy in a large animal model to evaluate short-term survival and early complications due to RFA.

Material and Method: Fifteen domestic female swine were divided into two groups. The experimental group underwent radiofrequency ablation of the right lung with the target temperature above 70 C° for at least 5 minutes. In the control group, same method was used, but no RFA energy was delivered. All animals were assessed for vital signs, activity level, respiratory manifestations, general well-being, chest-tube drainage, and surgical wound healing for thirty days. On day 30, animals were sacrificed and their lungs were examined for morphological changes.

Results: The mean values for power, impedance and temperature were 23.63 ± 8.24 W, 88.15 ± 17.87 Ω, and 72.51 ± 8.66°C, respectively. There was no significant difference in vitals signs and general well-being between the two groups. Only the experimental group showed evidence of respiratory distress, which was noticed mainly on the day of procedure. Air leakage and pleural adhesions were major finding in both groups. No evidence of purulent pleural effusion or pneumonia was observed. Gross examination showed a central necrotic area with a surrounding fibrous pseudocapsule and
neovascularization. Important histological features of RF lesions were circumscribed congested necrotic parenchyma, granulation tissue, fibrosis, and vascular thrombosis.

**Conclusion:** Radiofrequency ablation of lung tissue can be achieved in a safe manner with negligible morbidity and no mortality. There was no intra-operative mortality, and the post-operative recovery time was fast and uneventful. Although respiratory distress and pleural adhesions were common findings, they were comparable with any intrathoracic procedure. Pathological examination indicates that RFA causes coagulation necrosis, formation of granulation tissue and tendency for absorption in a predictable fashion.
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I. Introduction:

1.1. Overview of Lung Anatomy:

The respiratory system is divided anatomically into two parts, the upper and lower respiratory tracts, which is separated by pharynx. The lower respiratory tract begins at larynx then continues into the thorax as the trachea. The trachea first divides into left and right main bronchi, which supply each lung. Each primary bronchus gives rise to secondary bronchi supplying the lobes of the lung before dividing again to form tertiary bronchi which supply the segments of each lobe. The right lung has three lobes: upper, middle and lower. The lobar architecture of the left lung is slightly different from the right side, as it has only two lobes: left upper and left lower lobes.

1.2. Lung Blood Supply:

The lung has two blood supplies: the pulmonary system and bronchial system. The pulmonary system is the predominant system, which carries deoxygenated blood from the right side of the heart via pulmonary arteries to each lung. The blood in the pulmonary circulation leaves the right ventricle, and is carried by pulmonary arteries to the right and left lungs. In the pulmonary arteries, the media is about 1/2 as thick as it is in systemic arteries of a corresponding size. The larger vessels consist mainly of elastic tissue, whiles smaller vessels are made of muscular tissue. The pulmonary arteries enter the roots of hila of the lungs with the main bronchi and then divide and course in parallel with the branching airways to supply the pulmonary capillaries surrounding the alveoli. Pulmonary Arterioles differ radically from systemic counterparts. They resemble systemic venules, and are consist of a thin media of elastic tissue covered by endothelium. Pulmonary capillaries form a dense network over the walls of one or more
alveoli. One capillary network passes through more than one alveoli, before reaching
venule. Pulmonary venules and veins are structurally almost identical to the pulmonary
arterioles. The pulmonary arterial system is structurally unusual in two respects. Firstly,
the pulmonary arterial vessels are relatively thin-walled and of large caliber, their
diameter approximating that of the accompanying airway. Secondly, the pulmonary
arteries have the histological characteristics of elastic arteries rather than of muscular
arteries. Elastic expansion and recoil of the vessels maintains the pulmonary arterial
pressure at a relatively constant level throughout cardiac cycle. The bronchial arterial
system constitutes the systemic circulation of the lower respiratory tract. It arises as small
branches of the aorta and supplies oxygenated blood to the tissues of the airway walls and
to the pleura. Part of this system drain into the ride side of the heart via azygos vein, the
rest of it mingles with pulmonary venous blood, constituting a shunt. The lung venous
system returns most of the blood to the left side of the heart via the pulmonary veins,
which are very thin-walled vessels.

There are no lymphatics visible in the alveolar septa, but lymphatics are well developed
around bronchi and pulmonary vessels

1.3. Lung Histology:

Bronchi are lined by pseudostratified columnar epithelium with goblet cells. The
thickness and layering of the epithelium decrease gradually with the decrease in size of
bronchi. A smooth muscle layer encircles a thin connective tissue lamina propria. In
contrast to the trachea, the smooth muscle of the bronchus is arranged in interlacing
spirals around the bronchus. Between the smooth muscle layer and the cartilage is the
submucosa, which may contain seromucous glands. The hyaline cartilage is arranged in
discontinuous plates around the bronchus. Each bronchus continues to divide into smaller
ducts called bronchioles. Bronchioles are airways of less than one millimeter in diameter
and have no cartilaginous support. The bronchiolar epithelium is simple ciliated columnar
and goblet cells are absent. The smooth muscle layer is the most prominent feature.
Terminal bronchioles are the smallest diameter passages of the purely conducting portion
of the respiratory tree. The epithelial cells are no longer ciliated and often take on a dome
shaped appearance. Interspersed are neuroendocrine cells capable of releasing dopamine
and serotonin. These cells concentrate at junctions of the bronchi and bronchioles, and
the bronchiolo-alveolar portals and perhaps are used to regulate airway diameter. The
respiratory bronchiole is a branch of the terminal bronchiole. The epithelium is low
columnar to cuboidal. Cilia are present in the larger bronchioles only. The respiratory
bronchioles have a thin supporting wall of collagenous and elastic fibers and smooth
muscle. The respiratory bronchiole differs from the terminal bronchiole in having alveoli
as outpouchings from its wall. Arterioles and venules are seen in the wall of the
bronchiole, but only capillaries extend beyond the respiratory bronchiole. Alveolar duct
arises by a branching of respiratory bronchioles. Their wall is made up of alveolar sacs
and alveoli. The lining epithelium is reduced to flattened cells with occasional cuboidal
cells. The lumen of the alveolar duct has openings for the alveolar sac. Each alveolar sac
is a cluster of alveoli that is composed of squamous epithelial cells, basement membrane,
and capillaries. Individual alveoli are lined by thin squamous cells (Pneumocyte 1) and
cuboidal cells that bulge into the alveolus (Pneumocyte II). The latter cell is responsible
for the production of surfactant, which maintains the configuration and stability of the
alveolus and plays a role in fluid transport across the alveolocapillary membrane. The
The alveolar sac is the site of respiratory exchange. The alveoli inside the alveolar sac are composed of epithelial cells (Type I or Type II), basement membrane (basal lamina), and endothelial cells. The Type II alveolar cells are cuboidal in shape and appear to have empty vacuoles in their cytoplasm. Type I, squamous alveolar cells constitute 97 per cent of the respiratory epithelium at the alveolar level. The remaining 3 per cent are Type II alveolar cells, which produce the surfactant. Through the alveolar wall, gaseous exchange takes place between blood and air.

1.4. Lung Physiology:

Respiratory physiology can be divided into two broad categories: intrinsic and extrinsic respiration. The function of intrinsic respiration is cell oxygen utilization and handling. Extrinsic respiration is the delivery of oxygen from the external environment to the cells and removal of CO2 from tissue. The lung is the central organ in extrinsic respiration, and has several important functions. One of the main functions of the lung is gas exchange between the alveoli & the blood. It occurs by simple diffusion: O2 diffusing from the alveoli into the blood and CO2 from the blood into the alveoli. Blood enters the lungs via the pulmonary arteries. It then proceeds through arterioles and into the alveolar capillaries. Oxygen and carbon dioxide are exchanged between blood and the air. This blood then flows out of the alveolar capillaries, through vacuoles, and back to the heart via the pulmonary veins. Other functions of the lung include control of airflow for speech, conversion of angiotensin I to angiotensin II by angiotensin converting enzyme, removal of noxious particles by cilia, and defense against infection. In addition to the mucous and cilia of the upper airways, there are macrophages in the distal airways and alveoli. These macrophages contain lysosomes, which digest inspired bacteria and
foreign particles. The lung also has tissue macrophages and polymorphonuclear cells that assist in defense against infection.

1.5. Lung Cancer:

1.5.1. Epidemiology:

Lung cancer is responsible for one-third of all cancer deaths in men and about one-fourth of cancer deaths in women, making it the leading cause of cancer deaths. The mortality rate due to lung cancer surpasses the combined total deaths from colon, breast, and prostate cancer deaths. In 1999, the expected five-year survival for lung cancer patients was 14% compared with 63% for colon cancer, 85% for breast cancer, and 93% for prostate cancer. The current five-year survival rate is only slightly better than the 8% survival rate of the early 1960s. The mortality rates reduced in 1990s in most age and race subgroups, except women over 65 years of age. The incidence of lung cancer has decreased in men and women of less than 65 years of age. The highest lung cancer incidence and mortality rates in all age groups are observed in black men.

1.5.2. Etiology and Risk Factors:

An estimated 87 percent of all cases are directly linked to tobacco smoke. There is a dose response association between the number of cigarettes smoked and the risk of developing lung cancer. Occupational risk factors include exposure to asbestos, arsenic, chromium, nickel, radon, chloromethyl ethers, coal, mustard gas, and vinyl chloride. Other risk factors may include air pollution, genetics, and C.O.P.D.

1.5.3. Pathogenesis:

Carcinogens especially tobacco, result in chromosomal deletion, oncogene activation, and tumor suppressor genes inactivation. Oxygen free radicals from tobacco smoking also
play a role in the pathogenesis of lung cancer by causing cellular damage. The most common genetic abnormality in lung cancer is the loss of the tumor-suppressor gene p53. Once lung malignancy is initiated by these mutations, tumor growth is promoted by epidermal growth factors.

1.5.4. Histological Types:

The four major histological types are squamous cell carcinoma, small cell carcinoma, large cell carcinoma, and adenocarcinoma. For clinical and therapeutic reasons, lung cancers are classified as non-small cell lung cancer (75%) and small cell lung cancer (25%).

<table>
<thead>
<tr>
<th>TUMOR TYPE</th>
<th>GROWTH RATE</th>
<th>METASTASIS</th>
<th>MANIFESTATIONS</th>
<th>DIAGNOSIS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Squamous Cell</td>
<td>Slow</td>
<td>Late</td>
<td>Cough, sputum &amp; airway obstruction</td>
<td>Biopsy, sputum analysis &amp; EM</td>
</tr>
<tr>
<td>Carcinoma</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>Moderate</td>
<td>Early</td>
<td>Pleural effusion</td>
<td>X-ray, EM &amp; bronchoscopy</td>
</tr>
<tr>
<td>Carcinoma</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Large Cell</td>
<td>Rapid</td>
<td>Early/widespread</td>
<td>Cough, sputum, hemoptysis &amp; pain</td>
<td>Sputum analysis &amp; bronchoscopy</td>
</tr>
<tr>
<td>Carcinoma</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Small Cell</td>
<td>Very rapid</td>
<td>Very early</td>
<td>Airway obstruction, hormone secretion</td>
<td>X-ray, IHC, sputum &amp; EM</td>
</tr>
<tr>
<td>Carcinoma</td>
<td></td>
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</tr>
</tbody>
</table>

EM: Electron microscopy, IHC: Immunohistochemistry

Table 1.1. Characteristics of subtypes of lung cancer

1.5.5. Staging:

The summary of 1997 revised international system for staging lung cancer is as follows:

Tis: carcinoma in situ

T1: tumor size ≤ 3 cm

T2: tumor size > 3 cm, with distance from carina of more than 2 cm
T3: tumor of any size within 2 cm of carina in main bronchus or local invasion

T4: tumor of any size with local invasion of mediastinum, or pleural and pericardial effusion

1.6. Standard Treatment Methods:

The optimal treatment regimen for lung cancer depends on many factors, including cell type (small cell lung cancer vs. non-small cell lung cancer), stage of the disease, and patient status. Although curative treatment is the main goal, most lung cancer patients present with advanced-stage disease, where cure is possible only in a few cases. For SCLC, chemotherapy is the front-line therapy. For stage I or II NSCLC, if the patient can tolerate surgery, surgical resection is standard therapy. Treatment of stage III or IV carcinomas may include one or more methods (surgery, chemotherapy, and radiation). The mainstay of treatment for stage I and II NSCLC has been local control of the tumor. So far, no adjuvant or neoadjuvant treatment of any type, including radiation therapy and chemotherapy, has affected the survival of stage I and II patients. Hence, currently aggressive local control of patients with stage I and II NSCLC by surgical resection and/or radiation therapy has been the "best proven method" for treatment.

Pneumonectomy/lobectomy provides 40-65% five-year survival rate in patients with stage I and stage II of lung cancer. Although sublobar or wedge resection and/or segmentectomy has been tried in the past, lobectomy is preferred if the patient can tolerate the procedure because of higher long-term survival rate. Advances in closed transthoracic radiation, thermal ablative therapy techniques, and gene therapy, may eventually replace surgical resection of tumors to achieve local control.2
1.7. Complimentary Therapies:

Several alternative methods have been suggested for local control of lung tumors including laser, cryotherapy, photodynamic therapy (PDT), electrocautery, endobronchial brachytherapy (EB), and radiofrequency ablation (RFA).

1.7.1. Photodynamic therapy (PDT):

Photodynamic therapy involves the preferential retention of a photosensitizer by malignant or premalignant tissue. This is followed by the use of light to excite the sensitizer such that it interacts with oxygen to produce cytotoxins, which injures the abnormal tissue.

1.7.2. Endobronchial brachytherapy (EB):

Endobronchial brachytherapy is another alternative method that can be used in patients with lung cancer. The objectives of endobronchial brachytherapy are to provide therapeutic irradiation to the tumor and to curtail normal airway damage.

1.7.3. Laser bronchoscopy:

Laser bronchoscopy with (Nd:YAG) laser is preferred for endobronchial disorders because it is compatible with both rigid and flexible bronchoscopy, offers deeper tissue penetration, and preserves good coagulation, cutting, and carbonization properties. The predominant tissue effects of Nd:YAG lasers are thermal necrosis and photocoagulation.

1.7.4. Electrocautery:

In electrocautery, electrical energy is used by one of several introducer devices to produce heat and to cut and/or destroy tumor cells. As the tissue temperature rises, cellular water evaporates, and cellular elements break down chemically. This may result in coagulation, cutting, or vaporization of underlying tissue.
1.7.5. Cryotherapy:

As opposed to electrocautery, cryotherapy utilizes cold to destroy tissues. With the tissue temperature below -15 °C, intracellular dehydration takes place, and intracellular and extracellular ice crystals form. The pH and tonicity of cells change, and the venous and capillary blood flow stagnates. All these factors lead to cellular destruction.

1.7.6. Ethanol Injection:

Ethanol ablation works well only when ethanol can diffuse easily and uniformly, e.g. in hypervascular, well-encapsulated tumors, such as liver tumors. Otherwise, the results with ethanol injection are unsatisfactory, mainly because the distribution of ethanol within the injection site is heterogeneous and tumor necrosis is therefore unpredictable. There are no published reports that ethanol injection has been performed on lung.

1.8. Radiofrequency Ablation:

1.8.1. History of RFA:

Heat has been used clinically for thousands of years dating back to ancient Hindu and Greek healers, who used it for hemostasis. In fact, Hippocrates is reported as saying that “those diseases that medicine cannot cure, the knife cures; those which the knife cannot cure, fire cures.”  

3 The earliest recorded use of heat to treat tumors comes from Egyptian, Greek and Roman descriptions of medical practice when superficial tumors were subjected to cautery.4,5 Rather than applying heat directly, which would have undesirable side effects, medical technicians tried to use the effect of high frequency electric current on tissue. The first high frequency generators were developed in the late 1800 in Leiden/Netherlands and were mostly used for the impressive lighting.
RFA was first introduced for the ablation of the neural tissue to control pain or other neurologic disorders. In 1891, d'Arsonval, showed that the radiofrequency range alternating current when passed through living tissue caused an elevation in tissue temperature without causing neuromuscular excitation. The observation inspired the later development of the surgical Bovie knife.\textsuperscript{6-10}

In the early to mid 1900s, these observations led to the development of electrocautery and medical diathermy. The best known of these developments was the surgical Bovie knife, which was used to cauterize bleeding tissue. The Bovie knife consisted of an alternating electric current generator operated in the range of radiofrequency, a small knife-like electrode, and a large grounding pad. The physical principles of the operation of the device were rather simple. The alternating electric current passes back and forth through the patient between the Bovie knife and the grounding pad. The grounding pad was applied to the patient's thigh, which acted as a large dispersive electrode that allowed the current to pass freely through the patient producing heat only in the Bovie knife. The Bovie knife had a small tip, which was the focal point for the electric current. The current passed between the Bovie knife and the patient, desiccating and charring the underlying tissue.\textsuperscript{11-14}

In 1911, Clark was the first person who used RFA to cut and coagulate small skin and bladder tumors. A few years later, in 1928, Cushing applied radiofrequency energy to remove intracranial tumors with minimal hemorrhage.\textsuperscript{12,15,16}

Consequently, the Bovie knife underwent modifications, which resulted in its use in destruction of the neuronal pathways in cardiac patients with intractable arrhythmias.\textsuperscript{17-21}
Coley suggested that tumor cells are more sensitive to heat than normal ones.\textsuperscript{22} In the mid-1960s neurosurgeons used RF current as a well-established technique to induce controlled thermal injury in living tissue.\textsuperscript{23}

Organ explained the physical principles of the interaction of the alternating electric current with living tissue. He showed that at low-power settings, the alternating current caused agitation of the ions in the adjacent tissue. The ionic agitation caused frictional heat that expanded into adjacent tissues by conduction. However, at high-power settings the ions were quickly destroyed through desiccation and charring of the superficial tissue and minimal heat was produced.\textsuperscript{24,25}

During the 1970s, the physical dimensions and weight of RF generators was hence reduced and advances were made in the methodology of modulating waveforms, enabling control of the power output and monitoring of the electrode tip temperature. These changes resulted in new applications for usage of radiofrequency current.\textsuperscript{16} In 1985; Huang used RFA for ablation of cardiac conduction pathways.\textsuperscript{26}

In the early 1980s LeVeen and colleagues, explained the role of external radiofrequency in treating tumors located deep in the body\textsuperscript{27}. McGahan et al. initiated the treatment of malignant hepatic tumors with RFA.\textsuperscript{28} Initially, the lesions made with RFA electrodes were no more than 1.6 cm in diameter, but the use of multiprobe arrays\textsuperscript{29} and saline injection prior to RF ablation\textsuperscript{30} solved the size limitation problem. The generation of reliable and predictable large lesions has become possible through the use of monopolar needle electrodes with expandable multiple spring wires and straight tip needle electrode internally cooled with chilled saline.\textsuperscript{31-33} Lesions with up to 5 cm in diameter can be ablated with the new expandable needle electrodes in a single ablation session.\textsuperscript{34}
The first publications on the use of RF ablation for the creation of deep thermal injuries in the liver were published in 1990 by Rossi et al and McGahan et al.\textsuperscript{29,35} In the early 1990s, two independent groups of investigators using modified radiofrequency equipment examined the role of focal thermal injuries using radiofrequency current in the liver.\textsuperscript{28,35} They used equipment similar to that of the Bovie knife. The initial needle design was simple and used standard stock needles insulated to the distal tip. The investigators placed needle electrodes in the liver and created focal thermal injuries around the exposed tip of the needles.\textsuperscript{36} Both investigators suggested that radiofrequency ablation might be an effective technique for destroying small malignant liver tumors.\textsuperscript{28,35} Currently three major companies are designing RFA devices, although there approach is different, but their goal is the same to create larger ablation zones and achieve effective coagulation necrosis in solid organ tumors.

1.8.2. Mechanism of Heat Production in RFA:

RFA, which utilizes a radiofrequency signal of 460 kHz, is the process by which discrete quantities of energy in the form of radiofrequency waves are deposited in specific tissues in an attempt to cause coagulative necrosis in a predetermined area. The RF generator supplies RF power, and establishes a high frequency alternating current, which passes through the exposed uninsulated portion of electrode into the tissue.\textsuperscript{16} It is connected to the shaft of the RF electrode, and the reference electrode. The RF electrode consists of a metal shaft, which is insulated except for an exposed conductive tip that is in direct electrical contact with the targeted tissue volume. The reference electrode or ground pad is usually a wide conductive pad contacting the subject’s skin in an area of relatively good electrical and thermal conductivity (e.g. thigh). The RF generator produces a RF
voltage between the active RF electrode and the ground pad, thus establishing an electric field within the subject's body between the two electrodes. The electric field moves back and forth with the RF frequency causing oscillatory movement of ions in the tissue. The ions oscillates rapidly to follow the direction of alternating current, therefore causing ions, which vibrate at a very fast rate (ionic agitation). The friction or resistance created by ionic agitation produces heat.\textsuperscript{10,38,39}

The heat that is produced during RF ablation depends on the difference between the heat generated by RF current flow and the heat lost from the ablation zone. This balance depends on many factors including RF current intensity, duration of ablation and distance from the tip of the electrodes.

Current intensity plays an important role in heat production. It is proportional to the current (I) delivered by the RF generator, which, for constant resistance (R) of the electrode-tissue is equal to the square root of the RF power (P):

\[ P = I^2 \times R \]

Current flow through a resistive medium causes heating, which is proportional to the square of the current or, locally, to the square of the local current density. When the current is too high, tissue carbonization takes place. When the current is applied too rapidly, scattered areas of boiling and vaporization will result. The deleterious effects of charring and vaporization may be decreased by monitoring temperature and/or impedance during treatment, and adjusting the current accordingly.

A fundamental relationship exists between current, voltage, and resistance. According to Ohm's law, the current varies directly with voltage and inversely with resistance. From this law the following formula is derived:
\[ I = \frac{E}{R} \text{ or } \text{Current} = \frac{\text{Voltage}}{\text{Resistance}} \]

Where \( I \) is current, \( E \) is voltage and \( R \) is resistant.

Resistance is the factor that limits current flow at any particular voltage. Ohm's Law states that the voltage (V) across a resistor is proportional to the current (I) flowing through it:

\[ V = IR \text{ or } \text{Voltage} = \text{Current} \times \text{Resistance} \]

where \( R \) is the resistance.

Power is voltage multiplied by current (Volts \( \times \) Amperes). Power consumed in a resistor depends on the amount of current that passes through the resistor for a given voltage. This is expressed as voltage times current.

\[ P = EI \text{ or } \text{Power} = \text{Voltage} \times \text{Current} \]

Power can also be calculated by substituting other components of Ohm's Law:

\[ P = I^2 R \text{ or } \text{Power} = (\text{Current})^2 \times \text{Resistance} \]

\[ P = E^2 R \text{ or } \text{Power} = (\text{Voltage})^2 \times \text{Resistance} \]

Impedance is a general term used to describe the relationship between the voltage across a component and the current flowing through that device. Unit of measurement is the Ohm. All material impedes the flow of electrical current to some extent. The amount of resistance depends upon composition, length, cross-section and temperature of the resistive material. As a rule of thumb, resistance of a conductor increases with an increase of length or a decrease of cross-section.

Duration of ablation is another important factor in creating heat production. It has a direct effect in protein denaturation and cellular destruction. The distance is the most important factor in limiting the size of ablation, as heat decreases to the fourth power of radius from
the electrode tip. The following formula shows the relationship between heat production and, current intensity, duration of treatment and distance from the electrode:

\[ H = \frac{1}{4r^4} \times I^2 \times T \]

Where “\( H \)” is heat production, “\( r \)” is radius or distance from the tip of the electrode, “\( I \)” is current and “\( T \)” is time or duration of the treatment.\(^{40}\)

A major route of heat loss is from convection via the circulatory system. RFA leads to coagulation of small vessels, but large vessels are usually protected because of their higher levels of blood flow. Although this heat sink effect protects large blood vessels, thereby decreasing the risk of hemorrhage, it can make destruction of tumors immediately adjacent to blood vessels difficult. Heat is also lost by conduction or diffusion, radiation and evaporation.

**1.8.3. Effect of RFA at Cellular Level:**

The cell membrane comprises a phospholipid bilayer that is made of membrane proteins, which regulate the flow of ions, hormones, and drugs into and out of the cell. Hyperthermia is known to have detrimental effects on cell structure and physiology at many levels.\(^{10,41}\) In in-vitro experiments, thermal injuries to cells starts at about 41 °C, and the exposure time necessary for cell death begins to decrease exponentially at temperatures greater than 42.5 degrees C.\(^{42}\) At temperatures between 42 - 45°C, depending upon the tissue type and condition, it takes 3 to 5 hours to achieve cell death.\(^{43}\) At 46°C, it takes only 8 minutes to kill malignant cells, at 51°C it takes 2 minutes to destroy all cells.\(^{44,45}\) However, to produce the same tissue necrosis in vivo, 58°C is necessary because the tissue perfusion by vessels carries away the delivered heat.\(^{46-48}\) When the cellular temperature is increased to more than 105°C, tissue boiling,
vaporization, and carbonization will result, which will eventually decrease ablation because of increased impedance and a secondary reduction in energy transmission. Proteins that make up the cytoskeleton of the cells denature under hyperthermic exposure, and the resultant loss of sub-cellular architecture may be an important factor in cell death. Irreversible cellular damage transpires with changes in fluidity of the cell membrane, which has detrimental effects on cellular electrophysiology due to alterations in transit of ions, including potassium and calcium. Numerous changes also occur in the nucleus, including impaired DNA replication, and RNA transcription. Both extracellular and intracellular water may be driven out of the tissue, resulting in coagulative necrosis. 

1.8.4. Tissue Response to RFA:

To completely destroy a tumor, an adequate ablating temperature must be reached throughout the whole lesion. Nevertheless, there are multiple and often tissue-specific limitations that preclude heating of the entire tumor volume, most importantly, heterogeneity of heat deposition can occur throughout a given lesion.

The tissue damage and recovery from RFA demonstrates the body's typical pattern wound healing, which include initial tissue injury, followed by scar formation and remodeling. The first stage of wound healing is the acute inflammatory phase, which lasts for 48 – 72 hours and is mainly responsible for cleaning of injured area. In the acute phase of cellular injury, there is an unregulated enzymatic digestion of cell components, loss of cell membrane integrity, and uncontrolled release of cellular products into the extracellular space, initiating an inflammatory response. No microscopic changes are visible hours after cell necrosis. The coagulation necrosis seen in thermal injury is due to
the development of intracellular acidosis, and denaturization of the enzymatic and structural proteins. Under electron microscopy, the first changes in cells undergoing necrosis are mild cytoplasmic swelling, dilatation of smooth endoplasmic reticulum, and loss of ribosomes from the rough endoplasmic reticulum. Blebbing from the plasma membrane also occurs during cellular necrosis. Necrotic cells swell rapidly, and both the plasma and internal membranes begin to rupture. Organelles spill out and found lying in the extra-cellular space. In contrast to apoptosis, nuclear structures remain relatively intact. In addition, Heterochromatin becomes coarser. The early changes are difficult to detect under a light microscope. There is a cellular swelling, with loss of detailed cellular structures, and the cells may have a homogenous, eosinophilic appearance. In addition, chromatin becomes coarser and nuclear staining is lost, which is called karyolysis. The preservation of basic outlines of coagulated cells may span for a few days. There is also edema and congestion within the tissues. A mild to moderate acute inflammatory response can be seen. In acute inflammatory response, leukotrienes play an important role as a mediator of inflammation. They are generated by lipid peroxidation in the membrane of dying cells. Some components of mitochondria are powerful activators of the complement system, which also initiate the acute inflammatory response. Mast cells at the site of initial injury release histamine, which causes vasodilatation of nearby capillaries. Increased capillary permeability is also due to the release of serotonin from platelet and mast cells. This results in accumulation of fluid in the interstitial space and swelling of the tissue. The release of chemotactic factors from mast cells attracts leukocytes. Within hours, neutrophils and macrophages arrive at the site of the injury by the blood stream, and remove the injured cells by the process of phagocytosis. Grossly,
the lesion is well defined, with a rim of hyperemic tissue surrounding the white damaged tissue.

The second stage of wound healing is the reconstructive phase, which peaks for 5 – 7 days. During this phase, fibroblasts release growth factors and collagen. Granulation tissue is highly vascularized, and collagen fibers are an important part of it. At 72 hours, microscopic examination shows advanced cell necrosis with extensive loss of cell nuclei. Ten days after treatment, white tissue surrounds the lesion, indicating fibrosis, with collagen deposition replacing the dead tissue. There is minimal edema, and the chronic inflammation present is associated with fibrosis. At three weeks, the lesion is white and glossy, with well-formed scar tissue. Small blood vessels within the ablation and on its periphery are destroyed during the RFA procedure, but are reformed as the scar develops. The vessels surrounding the lesion remain intact and viable.

The third stage of wound healing is the remodeling stage, which starts 2 – 3 weeks after the ablation. The number of capillaries decrease and collagen synthesis increase. During this period, the necrotic tissue is replaced by scar tissue, which has less volume than healthy cells and thereby creates retraction in the tissue. Over time, this retraction creates a further reduction in overall scar volume.\textsuperscript{24,51,52}

1.8.6. Technical Development of RFA Probes:

1.8.6.1. Monopolar Electrode Techniques:

Initially, the principal use of RF ablation was for neurosurgical and cardiac applications, such as the treatment of benign hyperactive neurologic foci or aberrant intracardiac conductive pathways. The principal advantage of RF techniques for the previously
mentioned applications is the ability to create precise small regions of coagulation necrosis.

Originally, monopolar electrode techniques were used for the ablation of malignant neoplasms in animal liver (1–3) or brain and resulted in the induction of coagulative necrosis with a diameter of less than 2 cm.  

Using a monopolar electrode, Goldberg et al ablated liver and muscle in an ex-vivo animal model. He concluded that the lesion diameter was correlated with the diameter of the electrode and the duration of RF application but that the lesion length (measured parallel to the length of the electrode) was correlated only with the length of the exposed electrode tip.

Further studies confirmed that the electrode surface temperatures are not uniform during RF application, and temperatures are highest at the proximal and distal ends of the electrodes. Additionally, as tip temperature and/or electrode tip length are increased, the variation in temperature along the electrode shaft also increases. Coagulation diameter is well correlated with local mean temperature during the application of RF energy. When surface temperatures are less than 50°C, no necrosis is seen. The size of the lesion is directly related to the electrode surface temperature. However, temperatures higher than 110°C often leads to reduced coagulation necrosis as a result of tissue charring, gas formation, and/or cavitation, all of which result in increased impedance and decreased current flow.

Goldberg et al studied the in-vivo feasibility of performing RF ablation in normal pig muscle and liver and rabbit lung. He found that the length of coagulation necrosis achieved in vivo is comparable to that of ex vivo. However, the diameter of coagulation
necrosis in vivo is considerably smaller than the ex vivo diameter, measuring 1.3 cm for pig muscle, 0.9 cm for pig liver, and 0.8 cm for rabbit lung. The general relationship of coagulation diameter to electrode surface temperature was similar to that observed ex vivo; however, the minimum temperature required to induce coagulation is approximately 8.5°C higher than that for ex vivo specimens, and the variation in temperature along the electrode shaft is much greater than was observed ex vivo. It was proposed that the perfusion-mediated tissue cooling, heat sink effect, is largely responsible for the decrease in coagulation necrosis seen in vivo.\textsuperscript{47,54}

In monopolar probes, the rapid decrease of temperature at increasing distances from the electrode, in conjunction with limitations caused by electrode size, tissue conductivities, and blood-flow convection, created a challenging barrier to enlargement of ablation volume. As a result, maximum coagulation generated in the simple monopolar strategy measured 1.6 cm in diameter, a size much smaller than necessary to adequately treat most tumors.\textsuperscript{37}

1.8.6.2. Multiprobe Arrays

One of the main problems with monopolar electrodes was that the increase in necrosis volume was related to the increase in the length of the probe, but did not produce an increase in ablation diameter. To increase the diameter of coagulation necrosis, Goldberg et al studied the feasibility of simultaneous application of RF energy to arrays of two to five electrodes (with 3 cm tip exposure) in ex vivo calf liver for 6 minutes at 70°–90°C. The volume of coagulation necrosis obtained with simultaneous application of RF energy to all electrodes in such an array was compared with that obtained when RF energy was applied sequentially to each of the individual probes within the array. In all cases, the
volume of coagulation necrosis obtained with simultaneous RF application was greater than that resulting from sequential application.\textsuperscript{29}

Volume of tissue necrosis markedly increased with this approach compared to RF application using single probe, which also reduced the duration of therapy. However, this strategy was also difficult to implement in clinical setting given the technical challenge of precisely positioning multiple needles simultaneously. The development of umbrella RF electrodes with multiple hooked arrays, have overcome many of these problems and have enabled the creation of larger zones of coagulation. Another variation of the multi-probe array system is called the “hooked needle” or “umbrella” system, using an array of multiple, stiff, curved wires in the shape of an umbrella from a single 14 or 16 gauge cannula. Initial devices had 2–4 hooks, and therefore required multiple applications of energy following repositioning of the electrode to achieve complete tumor ablation. However, newer designs with a greater number of active electrodes (up to 12) have been developed and permitted greater contiguous coagulation to 3–5 cm.\textsuperscript{37}

LeVeen, used a 12-hook array in an in-vivo porcine liver, and was able to produce spherical regions of coagulation necrosis measuring up to 3.5 cm in diameter by applying 80W of power for 10–12 minutes.\textsuperscript{55} Similar results were obtained by Siperstein et al, who applied 30–50 W for 15 minutes to a four-pronged umbrella needle system.\textsuperscript{56}

1.8.6.3. Bipolar Arrays:

McGahan et al described a bipolar RFA technique in which they placed a second ground electrode within 4 cm of the active electrode in ex vivo liver. This results in heat being generated not only at the active electrode but also adjacent to the ground needle and between the two electrodes. The diameter of coagulation necrosis is larger in this method
than with the conventional monopolar electrode system, and it is elliptical rather than spherical. The clinical importance of this gain in the lesion volume is difficult to estimate, because most tumors are roughly spherical in shape.\(^{57}\)

1.8.6.4. Saline Solution Injection during RF Ablation:

Miao et al investigated the role of injecting 5% hypertonic saline at a rate of 1 mL/min into freshly excised swine liver before and/or during RF ablation. The lesion diameter increased to larger than 5.5 cm with the saline infusion, while the electrode tip temperature and tissue impedance decreased. They postulated that these effects are due to the increased conductivity of hypertonic saline, as compared with that of blood or other tissues.\(^{58}\)

Livraghi et al described ex vivo and in vivo animal liver experiments, as well as preliminary human studies, in which RF ablation was performed with bolus intraparenchymal saline injection, and continuous saline infusion. Saline pretreatment resulted in larger foci of coagulation necrosis both ex vivo and in vivo. With continuous saline infusion, the ablation lesion diameter is highly dependent on energy deposition, with lesion diameters ranging from 1.8 to 4.1 cm. The authors hypothesized that the increase in the volume of necrosis with saline injection or infusion was due to: (a) enlargement of the effective electrode surface area by means of augmented tissue tonicity; (b) improved tolerance of sustained high generator output due to tissue cooling, decreased tissue impedance, or both; and (c) direct effects of heated saline that subsequently diffuses into tissue. The resulting foci of necrosis were irregular in shape and, the volume of tissue necrosis was often difficult to predict.\(^{59}\) More recently, Polascik et al performed saline-augmented RF ablation of VX2 nodules implanted beneath the renal capsule in rabbit
kidneys. The average size of coagulative necrosis was 1.4–1.8 cm with only 30–45 seconds of RF application.60

1.8.6.5. Internally Cooled Electrodes:

These 14–18-gauge electrodes have an internal lumen through which chilled perfusate is circulated during RF application. The result is to increase generator output and at the same time prevent tissue boiling and cavitation adjacent to the needle tip. Greater tissue necrosis can be achieved by preventing the deleterious increase in circuit impedance that result from cavitation. Lorentzen et al used the internally cooled RF electrodes in ex vivo calf liver, and achieved a greater necrosis volume compared to that of conventional needle electrodes.61 Goldberg et al developed a slightly smaller (18-gauge) internally cooled electrode system and studied its effects in ex vivo calf liver and in vivo pig liver and muscle. Both energy deposition and necrosis were found to be significantly greater with electrode cooling than without it.62 The duration of treatment with internally-cooled electrode is prolonged mainly because the thermal equilibrium inside the tissue takes longer time to achieve.37

1.8.6.6. Clustered Electrodes:

Early experiments with multiprobe arrays were focused on determining the maximum distance that the electrodes could be spaced apart while still ensuring contiguous coagulative necrosis. The use of clustered electrodes offers the potential to substantially increase the volume of necrosis obtained at a single treatment session. By placing electrodes no more than 1 cm apart, large spherical regions of coagulative necrosis can be created. The resulting areas of necrosis is greater than when similar electrodes are used alone or in arrays spaced 1.5–2.0 cm apart. The closely spaced electrodes function
together as if they were a single large electrode, rather than simply heating the same
volume of tissue, as occurs with the multiple probe arrays in which individual electrodes
are spaced further apart.\textsuperscript{63}

Goldberg et al. positioned three electrodes close to one another, and hypothesizing that
closely spaced electrodes might act as a single large electrode and thus may result in an
increased area of necrosis. He found that arrays of three 2-cm, exposed tip, internally
cooled electrodes spaced 0.5–1.0 cm apart could produce a spherical focus of necrosis up
to 4.1 cm in diameter. Simultaneous application of RF to arrays of similar electrodes
positioned 1.5–2.5 cm apart, produced somewhat smaller and/or more irregular zones of
ablation. In another study, three internally cooled electrodes were placed 0.5 cm apart,
and RF energy was applied to ex vivo liver and to in vivo liver and muscle for 5–60
minutes. The largest diameter of necrosis varied from 4.7 to 7.0 cm. In vivo experiments
confirmed 3.1 and 7.3 cm when RF was applied to electrode clusters placed in liver and
muscle, respectively.\textsuperscript{64}

1.8.6.7. Pulsed Application of RF:

To increase the volume of tissue that can be coagulated at a single treatment session,
more energy should be applied, but it should be balanced against the deleterious effects
of tissue charring and cavitation due to too much delivery of energy. Goldberg et al.
investigated the possibility of applying RF in a pulsed, rather than in a continuous
manner to increase RF current density surrounding the electrodes while allowing brief
periods for heat dissipation. RF was applied to ex vivo liver by using internally cooled
electrodes with peak currents of 1,500–2,000 mA. These peak currents were maintained
for approximately 15 seconds, alternating with periods during which RF current was
intentionally reduced to 500 mA. Overall treatment duration was 15 minutes. The diameter of coagulative necrosis achieved was 3.6–4.0 cm when 3–5-cm-long electrodes were used. By comparison, continuous application of the maximum current that could be sustained without increases in impedance using the same electrodes (750–1,350 mA) resulted in foci of coagulation necrosis that are 2.9–3.5 cm in diameter. The in-vivo liver experiments with a 3-cm-long electrode tip for a 12-minute treatment duration, produced coagulative necrosis 2.8 cm in diameter with the pulsed technique but 2.4 cm in diameter with maximal continuous current. More rapid temperature increases and higher overall tissue temperature achieved when the pulsed technique was used.

1.8.7. Strategies for Increasing Necrosis Volume:

A number of approaches have been suggested to enhance tissue–energy interactions and increase the ablation area, which in turn have resulted in the development of new RFA devices and probes. The strategies to increase the volume of tissue necrosis are discussed below.

1.8.7.1. Increasing energy deposition:

Increasing the amount and the rate of energy deposition can be achieved by repeatedly inserting multiple RF probes into the tissue to increase the diameter of induced coagulation. To effectively destroy the entire tumor, multiple overlapping treatments should be carried out in all three dimensions, which is both time-consuming and difficult to use in the clinical settings. However, treatment time can be reduced by simultaneous application of energy using arrays. It is difficult to accurately position multiple probes. However, the newer probes with multiple hooked arrays have enabled the formation of larger areas of necrosis.
Preferentially cooling tissues closest to the probe is another approach to increase overall energy deposition. Two internal lumens inside the probe deliver chilled perfusate to the tip of the electrode and remove warmed effluent out of the ablation zone. This results in a heat-sink effect that takes away heat near the electrode.\textsuperscript{61}

Another method to increase the mean intensity of energy deposition is pulsing of energy. In this approach, periods of high-energy deposition are quickly rotated with periods of low energy deposition. With proper balance between high and low energy deposition, preferential tissue cooling can be achieved close to the applicator. The preferential tissue cooling occurs during periods of minimal energy deposition without significantly decreasing heating deeper in the tissue. Therefore, deeper heat penetration and greater tissue coagulation can be attained by greater energy delivery during periods of high-energy deposition.\textsuperscript{76,77}

Combination of both internal cooling and pulsing has a synergistic effect with greater tissue destruction than with either method alone.\textsuperscript{78}

\textbf{1.8.7.2. Improved Tissue Heat Conduction:}

Injection of saline and other compounds improves heat conduction within the tissues.\textsuperscript{59,79,80} Heat conduction is much faster in heated liquid than in healthy solid tissue. Injection of saline increases tissue ionicity, resulting in better current flow.

Since heat conducts through different tissues at various rates, tissue composition is also important when considering the factors that can change the extent of coagulation necrosis.\textsuperscript{81,82}
1.8.7.3. Mechanical Obstruction of Blood Flow:

Efficacy of RF ablation can be enhanced in human hepatic tumors by mechanical blood flow reduction in patients undergoing intraoperative RF. Some organs such as liver and lung have dual blood supply and angiographic balloon occlusion may not be adequate to decrease blood flow. Embolotherapy prior to ablation may overcome this limitation.\(^37\)

1.8.7.4. Decrease Tumor Tolerance to Heat:

Tumor sensitivity to heat can be enhanced by cellular hypoxia. Antiangiogenesis-factor therapy such as endostatin or prior tumor cell damage from chemotherapy (doxorubicin) or radiation can decrease tumor tolerance to heat. Synergy between chemotherapeutic agents and hyperthermic temperatures (42–45°C) has already been established.\(^{111, 112}\) Concomitant administration of intraarterial or intravenous vasopressin to further reduce splanchnic blood flow, in addition to celiac or hepatic arterial occlusion produced a larger tissue necrosis.\(^37\)

1.8.8. Clinical Applications of RFA:

1.8.8.1. Percutaneous:

The electrode is inserted through the skin to the target location. The physician usually uses CT scan or MRI and/or fluoroscopy to guide the needle to the right location. This is the least invasive way that RF is performed. General anesthesia is usually not necessary, but the patient is sedated. Often the patient is able to go home the same day. If general anesthesia is not used, some discomfort or pain may be felt while the area is being ablated. The potential advantages of such intervention provided via a percutaneous insertion route are reduced trauma, shorter procedure times, and, when provided in a procedure room, shorter recovery times than open or thoracoscopic surgical procedures.
1.10.8.2. Thoracotomy:

A second option is the open approach. The benefit of this approach is direct visualization, and easier access. Potential drawbacks of this method are complications associated with general anesthesia, longer recovery period, increased post-operation morbidity, and increased cost.

1.10.8.3. Video Assisted Thoracic Surgery (VATS):

With this approach, a few small incisions are made in the chest cavity, through which the thoracoscopic instruments are passed. VATS is now being utilized to perform most diagnostic procedures, in addition to therapeutic operations, including major surgical procedures such as pulmonary resection and esophagectomy in specialized centers. Diagnostic and simple therapeutic procedures performed by VATS are now well established and strongly recommended by most surgical centers. The major role of VATS in diagnostic procedures is now well established.\(^{83}\) Staging of lung or esophageal carcinoma may also require thoracoscopy and lymph node biopsy.\(^{84}\) Resection of pulmonary metastasis and mediastinal masses such as thymomas or neurofibromas is usually feasible. VATS is also indicated in recurrent pneumothorax, persistent air leak,\(^{85}\) and resistant pericardial effusions. Its role in major surgery and in extended pulmonary resection is less clear, especially in malignancies, and muscle-sparing thoracotomies.\(^{86}\) Advantages include less invasive access, shorter hospital stay, less postoperative pain and fewer complications such as atelectasis and chest infections.\(^{87}\) Other advantages include lower cost compared with thoracotomy, decrease operative time, better outcome, and a better cosmetic appearance. The risks of thoracoscopy include the following: wound
infection, bleeding, air leak through the lung wall, a longer hospital stay, pain or numbness at the incision site, and pneumonia.

Although thoracoscopic wedge resection for non-small cell carcinoma has received some recognition, it has the same problems as limited resection performed at thoracotomy. The procedure is technically difficult, a full mediastinal lymph node dissection is not consistently possible, careful palpation of the lung for occult extralobar metastatic disease is not always possible, and thoracoscopic lobectomy with an intercostal delivery incision has little postoperative pain benefit over a small muscle-sparing thoracotomy when an epidural and intravenous narcotic analgesia is used. Thoracoscopy and other minimally invasive procedures may manipulate the tumor causing spread of tumor cells into the pleural cavity, chest wall, or within the lung parenchyma. Contraindications include lack of patient cooperation, an uncorrected coagulopathy, cardiac hemodynamic or rhythm instability and unstable angina. Relative contraindications include mechanical ventilation and bullous lung disease. Local chest wall infection must be excluded before passing a needle into the pleural space. It is important to mention that VATS is performed under general anesthesia and therefore carries a substantial degree of risk for patients with severe comorbidities or limited cardiopulmonary function.

1.8.9. Use of RFA in Other Organs:

Radiofrequency tissue volume reduction (RFTVR) has been extensively studied in many medical and surgical specialties including organs such as the heart, bone, liver, pancreas, kidney, uterus, breast, nervous system, prostate, adrenal, and spleen. RFA is the treatment of choice for many symptomatic cardiac arrhythmias. It is
a safe, effective, less expensive, and less traumatic procedure for the treatment of cardiac arrhythmias with fewer complications.\textsuperscript{108}

Tumor ablation using radio-frequency energy has obtained increasing attention as an effective minimally invasive approach for the treatment of patients with a variety of primary and secondary malignant neoplasms. The follow section is a brief review of RFA studies in different organs.

**1.8.9.1. Prostate:**

The technique is well-tolerated\textsuperscript{109}, effective,\textsuperscript{110-113} safe\textsuperscript{111-113} and simple\textsuperscript{112,113} for treatment of benign prostatic hyperplasia, especially in treating elderly patients with a high surgical risk.\textsuperscript{110} Complications are rare, but the cost is difficult to estimate and the long-term outcome is still to be assessed.\textsuperscript{114} In the studies that have been performed so far, the follow-up period were too short to determine whether RF ablation has a place in the management of prostate cancer.\textsuperscript{115,116}

**1.8.9.2. Brain Tumors:**

RFA could be a promising alternative therapy for the treatment of brain tumor as it showed an increased survival rate in rabbits that underwent RF thermal ablation.\textsuperscript{117}

**1.8.9.3. Pancreatic Tumors:**

The role of RFA in the management of small pancreatic neuroendocrine tumors and possibly palliation of unresectable pancreatic adenocarcinoma is promising.\textsuperscript{118}

**1.8.9.4. Renal Tumors:**

RFA offers encouraging anatomic and physiologic results\textsuperscript{119} with minimal morbidity and no mortality,\textsuperscript{120,121} but little is known about RFA’s acute and chronic histologic effects and long-term efficacy as a treatment for renal tumors.\textsuperscript{122}
1.8.9.5. Bone Tumors:

It is a simple, minimally invasive, safe and highly effective technique for the treatment of osteoid osteoma.\textsuperscript{123,124} It provides excellent pain relief and early return to function with minimal morbidity.\textsuperscript{125} Studies show that RFA is equal to operative excision for the treatment of an osteoid osteoma in an extremity.\textsuperscript{90} Although RFA could be useful in metastatic bone disease, more research is required to determine its effectiveness.\textsuperscript{126,127}

1.8.9.6. Spinal Tumors:

The application of RFA energy to spinal tumors resulted in a decrease in pain and back pain-related disability with preservation of neurologic function. Further investigations are required for patients with unresectable spine tumors that do not respond to radiotherapy and chemotherapy.\textsuperscript{128}

1.8.9.7. Liver Tumors:

Although surgical resection remains the gold standard for treatment of liver tumors, but RFA as a safe and effective procedure, has some advantage over surgery, such as minimal invasiveness with a no mortality rate and significantly lower complications, reduced costs and hospital stay.\textsuperscript{129-131}

RFA produces coagulative necrosis in hepatic malignancies, which has been used in patients who do not meet the criteria for resectability of hepatocellular carcinoma and metastatic liver tumors\textsuperscript{132-137} or patients who refuses surgical treatment.\textsuperscript{138,139} This procedure should generally be applied for liver tumors without any evidence of extrahepatic disease, and for tumor histologies with a reasonable probability of disease metastatic only to the liver.\textsuperscript{40} The most common liver tumors treated by RFA are hepatocellular carcinoma and metastases from colon carcinoma.\textsuperscript{138,139} Although the
management of larger HCC is challenging, but one can achieve complete or near complete necrosis with RF ablation in most cases. Currently, survival rates for RF ablation are approaching those reported for surgical removal of metastasis.

1.8.9.8. Breast Tumor:
Although some studies show promising results for breast tumor RF ablation, more studies are required to conclude whether radiofrequency thermal ablation can be used as an alternative method of treatment for breast cancer.

1.8.10. Lung RFA:
In the last few years, there have been a few ex-vivo and in-vivo animal and human studies to understand the effects of radiofrequency ablation on lung tissue. Lung tissue is unique with respect to its structure, function, and blood supply. The effect of RFA in the lung tissue, therefore, can also be unique where it pertains to safety, complications, morbidity, and mortality. Lungs have a light, porous, spongy texture, and are made of highly elastic tissue. The supporting structures of the lung are delicate enough to allow gas exchange, yet strong enough to maintain architectural integrity. Only about 10% of the lung is occupied by solid tissue, while the remainder is filled with air and blood. The high level of air in the lungs creates a rather difficult situation during RFA, because air has high impedance, which prevents delivery of energy and proper heating of the targeted tissue. Another difficulty is the lung dual blood supply. Blood flow causes heat sink effect during in-vivo RF ablation, preventing increase temperature in the tissue and resultant tissue necrosis.

One of the earliest reports of localized interstitial thermal therapy for treating primary lung cancer was described by Lily and colleagues. They noted that if an internal and
external electrode is applied to a solid tumor, significant heat could be produced. Using a
dog model, they treated a 5 cm bronchogenic carcinoma. The tumor was heated easily
and the surrounding tissue was not damaged during RF ablation. Lily suggested that the
procedure could be applied to a variety of operable but unresectable tumors.
Sugaar and LeVeen used two or more pairs of skin electrodes to deliver an RF current in
patients with pulmonary carcinoma. Short bursts of power were sequentially switched to
different electrode pairs to avoid overheating the skin and subcutaneous tissue. Patients
underwent 4-14 sessions of 30 minutes to 1 _ hours of RF ablation. The affected lungs
were removed and histological examination showed different histological changes, the
most advanced being coagulation necrosis.\textsuperscript{142}

Goldberg et al, described higher mean initial lung tissue impedance during ablation than
that of a healthy rabbit liver tissue (509 ± 197 \( \Omega \) versus 180 ± 24 \( \Omega \); \( P < 0.01 \)).
Significantly smaller lesions are created in the lung compared with lesions created in
other solid organs such as liver (0.8 cm versus 1.4 cm; \( P<01 \)). He suggested that
compared with a healthy lung, which had marked variation of impedance, impedance of a
tumor may remain consistent during the procedure.\textsuperscript{47}

Marasso and colleagues examined a series of 98 patients and divided them into two
groups: RF ablation preceded or followed by cryotherapy. RF ablation was completely
successful in 60% and partially successful in 32% of patients. Cryotherapy was also
completely successful in 66% and partially successful in 21% of patients. He concluded
that local control of endobronchial tumors could be easily achieved.\textsuperscript{41}

Goldberg et al. created pulmonary tumors with the injection of a VX2 sarcoma cell
suspension into the right lung of 11 rabbits. Tumors were allowed to grow 14-21 days to
achieve a diameter of 6-12 mm. The experimental group contained seven tumors, which were ablated for 6 minutes at 90°C. The control group consisted of four tumors and was not treated with an RF ablation. Follow-up CT and histopathological analysis was performed on days 0-28. In histological analysis, at least 95% of tumor nodules were necrotic, but residual viable tumors were observed in three rabbits (43%). The control group showed tumor growth without necrosis with a mean survival of 23 days. The author concluded that percutaneous RF thermal ablation could be used to treat small pulmonary parenchymal tumor nodules in an animal model.143

In our lab, we studied the in-vivo effects of temperature, time and an inflated versus deflated lung on ablation size in a swine model. We found out that high impedance of a ventilated normal lung precluded successful ablation of air containing normal lung tissue. Lesion size was directly proportional to temperature but was unaffected by times ranging from 5-10 minutes, which was similar to findings in the liver. It was recommended that treatment time of 5 minutes should be adequate. Lesion size minimally increased in temperature beyond 70°C, and also tissue charring, increase in impedance, and subsequent temperature instability occurred at higher temperatures. It was recommended that a temperature of 70-80°C is reasonable to be used in future applications of pulmonary RFA.

In our lab, we assessed the role of RFA in an ex-vivo human tumor model. The results of our study showed that a solid lung tumor and normal lung tissue behaved differently during RFA, both in the amount of required energy and the level of impedance. Solid neoplasms of the lung responded to the RFA in a predictable manner, however, a partially inflated lung ablated in an inconsistent manner due to high impedance. It was technically
difficult to create an inflated lung ablation in a controlled fashion as tissue impedance usually exceeded the generator capacity. To prevent high tissue impedance, lower power was used, but it was difficult to achieve a continuous temperature of above 70°C in all thermisters. High impedance of a normal inflated lung tissue may have an important role when ablating a lung tumor because it reduces the extent of damage to the surrounding normal inflated tissue.
1.9. Hypothesis:

We hypothesized that radiofrequency ablation of the lung is a safe procedure, and the resolution of lung RFA lesion results in low incidence of post-op complications. In addition, lung tissue presents a unique pathophysiological mix of water-based and air-based tissue resulting in a specific electrophysical RF pattern.

1.10. Objectives of the Study:

The objectives of the study were two folds: To investigate the safety of the procedure by evaluating survival rate, and recognizing complications intra-operatively, post-operatively, and at 30 days, and to observe the incidence of tissue pathology in ablated and non-ablated lung tissue.
2. Materials and Method:

2.1. Study Design:
For the purpose of performing this study, we divided the animals into two groups: study group and control group. The study group included 10 swine, while the control group had five. Initially, there were ten animals assigned to each group, after operating on five control animals, an interim analysis was performed on the control group and it was concluded that due to the similarity within the study group, it was not necessary to use the remaining five animals.

The study group underwent radiofrequency ablation of the lower lobe of the right lung. In the control group, an RF probe was inserted percutaneously into the right chest cavity under direct thoracoscopic visualization in the same way as the study group, but the probe was not deployed inside the lung. The animals in both groups were assessed clinically for thirty days. On day 30, each animal was sacrificed and the lung was examined grossly and microscopically for morphological changes. (Table 1 & 2)

Figure 2.1. Study Design
2.2. Materials:

2.2.1. Animals:

For the purpose of performing this experiment, fifteen four weeks old female domestic swine weighing between 25-38.5 kg were chosen.

2.2.2. Generator:

The generator used in our experiment was model 500 (Rita, Mountain View, California), which had automatic multi-point monitoring of tissue temperatures. Its automatic, internal power adjustment feature insured that target temperatures were maintained with assurance. The generator was small, reliable, and cost-effective, which was simple to setup and operate in 5-10 minutes. Other features included impedance monitoring, manual temperature adjustment and automatic stoppage once impedance reached a cutoff point. It had the capability of delivering 0-50 Watts of power and 460 kHz of frequency. The generator has temperature range of 0-120 degrees C, impedance range of 40-200 Ohms, and timing range of 0-99.9 minutes (in 0.1 minute increments).
2.2.3. Probe:

Model 70 Electrosurgical Device 15 gauge, 25 cm, probe was used in our experiment. The probe had space-filling design (radio-array) with seven uninsulated electrode tips, which provided consistent heat dispersion throughout the tissue. The temperature-sensing thermocouples within the device were designed to monitor temperatures in the ablation zone and provided real-time temperature feedback regarding when and where the target temperatures were reached from the several points at the periphery. The power was automatically adjusted so that target tissue temperatures remained constant, and sufficient heating was produced, while preventing excessive delivery of energy, which could endanger the formation of a complete ablation. The electrodes were made of stainless steel for easy, precise and secure placement into tough tissue. The array size was adjustable, and depending on the size of ablation, different deployment sizes could be
chosen. With the ability to ablate the needle track, the chance of hemorrhage after the ablation process was reduced.

2.2.4. Thoracoscopic Equipment:

The endoscope was a rigid-quartz lens system, with fiber-light bundles incorporated into the shaft using a Xenon light source. Other components of the endoscopic equipment included a camera, a monitor and a video recorder.

2.2.5. Recording Computer:

A laptop computer recorded all the data in real time with a sequence graph produced by ZoMed International Inc. software version 1.9.

2.2.5. Grounding Pad:

Grounding pads functions as dispersive electrodes that concentrate the widely distributed current paths traveling through the animal to complete the electromagnetic circuit. Therefore, the same amount of heating achieved in the tissues surrounding the active RF electrode could occur over the total grounding pad surface area.

2.3. Method:

2.3.1. Pre-operative Assessment:

All animals were thoroughly checked by a certified veterinarian for any sign of illness pre-operatively. The animals were fasted on the night before operation. Vital signs, level of activity, respiratory distress, behavior and appetite of animals were assessed before the procedure.
2.3.2. Operative Method:

2.3.2.1. Anesthesia:

An integral component of veterinary medical care is prevention of infections and alleviation of pain associated with procedural and surgical protocols. Buprenorphine as an analgesic and Penlong as an antibiotic were used for the above purposes.

General Anesthesia was induced with Ketamine 20 mg/kg IM. Ketamine was used for this procedure due to its rapid onset of action that is usually 3-4 minutes, and shorter duration of action of 12-25 minutes. The airway is usually well maintained with Ketamine. In comparison with other intravenous agents, pharyngeal and laryngeal reflexes are somewhat preserved. Ketamine also produces some bronchodilation and increases tracheobronchial secretions. It does not cause cardiac depression; however, mild respiratory depression may be seen.

The anesthesia was maintained with continuous flow of 2% Isoflurane. Isoflurane is a nonflammable halogenated methyl ethyl ether. It is used as an inhalational anesthetic for induction and or maintenance of anesthesia. Time of onset of Isoflurane is dose dependent, and peak effects of it 7-10 minutes. Upon discontinuation of anesthesia, it usually took 15 minutes for the animal to wake up. Isoflurane, depresses ventilation, and relaxes bronchial smooth muscle. Although it can trigger malignant hyperthermia in susceptible swine, no increase in temperature was observed during the procedure. Adequate oxygen was also provided during operation.

Double-lumen endotracheal tube was used to isolate and semi-deflate the lung on the operative side (right lung) and ventilate the opposite lung (left lung). The animal was positioned on the left lateral side. RFA ground pad was attached in the inner thigh of the
animal, and connected to RFA generator through a cable. The right side of the chest was prepped and draped for the procedure. During the operation, heart rate and rhythm using ECG, respiratory rate, mucous membrane color for the level of oxygenation, breathing bag movement for ventilation and temperature were monitored.

2.3.2.2. Procedure:

Before starting the procedure, RFA equipment was set up, and the target temperature of above 70 °C in all thermisters for a period of at least 5 minutes was chosen. Previous studies in our laboratory showed a treatment time of 5 minutes with a target temperature of above 70 °C is sufficient for lung tissue ablation. Other studies indicated that tissue charring, increased impedance and subsequent temperature instability occur at higher temperatures. The author recommended a temperature of 70-80 °C to be used in pulmonary RFA.

The procedure started by making a one cm-incision in the fifth intercostal space in the posterior axillary line. The dissection was performed just above the sixth rib to avoid the neurovascular bundle that runs below each rib. The dissection was carried down to the parietal pleura, and the pleural space was entered using a Kelly clamp. A finger was placed through this opening to access pleural cavity and to prevent lung tissue injury. After the creation of a small intercostal incision, the affected lung collapsed. The lung quickly fell away from the chest wall, allowing clear visualization of virtually all the major structures in the chest cavity. No trocar was used and the thoracoscope was introduced into the chest cavity directly. Once access to the pleural space was achieved, the area of interest, which was the lower lobe of right lung, was identified. A syringe was filled with saline and inserted into the right lower chest cavity at the same level as the
thoracoscope was inserted to find the best angle, location and direction for insertion of RFA probe under direct thoracoscopic visualization. The syringe was then removed and in the same location and direction, the probe was inserted percutaneously into the chest cavity. The probe was flushed with 1 ml of saline before insertion into the lung.

In the experimental group, under direct thoracoscopic visualization, the lower lobe of the right lung, base of the right lung and its posterior margin were identified. A sterilized RFA probe was placed in the lower lobe of the semi-deflated lung 2-3 cm above the posterior margin of the lower lobe of the lung. Once positioned, the probe, which was connected to the generator by a cable, deployed to 3 cm diameter. Upon completion of the ablation, the tract was ablated to decrease bleeding from the injured lung tissue.

In the control group, the same procedure was used except that the RFA probe was inserted in the chest cavity but not into lung tissue, and the generator did not deliver any radiofrequency energy to the lung tissue.

2.3.2.3. Post-procedure:

The probe and the thoracoscopic equipment were removed, hemovac closed drainage system was inserted to observe and record any drainage from the chest cavity. The incision site was sutured.

2.3.3. Clinical Assessment:

Each animal was evaluated based on vital signs, general well-being, respiratory distress, level of activity, chest tube drainage, and surgical wound complications. (Figure 2.5)
2.3.3.1. Vital Signs:

Each animal’s heart rate, respiratory rate and temperature were recorded twice a day for the first three days after the procedure, once a day from day four until day thirty.

2.3.3.2. General Well-being:

General well-being of each animal was assessed by presence of normal appetite, by changes in behavior, and level of alertness.

2.3.3.3. Respiratory Distress:

The respiratory distress graded and assessed semi-quantitively as follows:

- Grade I: respiratory distress at rest – score 1
- Grade II: respiratory distress at activity – score 2
- Grade III: no respiratory distress – score 3

2.3.3.4. Activity level: activity level was also graded and assessed semi-punitively:

- Grade I: no movement – score 1
- Grade II: able to move but unable to stand – score 2
- Grade III: able to stand, but unable to walk – score 3
- Grade IV: able to walk but unable to engage in playing – score 4
• Grade V: playing – score 5

2.3.3.5. Chest Tube Drainage:

The hemovac closed drainage system recorded any drainage from the right chest cavity. The following criteria were used in assessment of chest-tube drainage:

• The number of times the bag was emptied due to air leak graded as:
  - mild (0-3 times – score 1)
  - moderate (4-6 times – score 2)
  - severe air leak (more than 7 times – score 3)

• The type of discharge (bloody or purulent)

• The amount of discharge

2.3.3.6. Surgical Wound:

The wound was observed on a daily basis for evidence of discharge, discoloration, swelling and the condition of sutures.

2.3.4. Post-mortem Assessment:

2.3.4.1 Autopsy:

On the day thirty post-procedure, euthanasia was carried out in a manner to avoid any animal distress. The animal underwent anesthesia with Ketamine for induction, and isoflurane and oxygen for maintenance of anesthesia.

Chest was opened by midsternal thoracotomy, and the right chest cavity was examined for evidence of blood, effusion, infection, fistula and adhesions. The hilum of the right lung was clamped and the hilum was tied by wrapping a suture around it. It was then resected with a surgical scissor and the right lung was removed from the chest cavity.
Following euthanasia with 20ml IV injection of Pentobarbital, death was confirmed by cessation of heart activity.

2.3.4.2. Gross Examination:

The lung was irrigated with normal saline and photographed. Then the lung was filled through its bronchi with Bouin's fixative using a syringe. Bouin's fixative was also directly injected into areas of the lung, which did not inflated properly. The lung was left in a container full of 10% Formalin for 18 hours. After the completion of fixation period (18 hours), the lung was sectioned a 0.5 cm intervals with a sharp knife from the base to the apex in an axial fashion. The size of each lesion was determined grossly by measuring the longest diameter in the X, Y, Z planes. Following photography of the sections to show the gross changes, blocks of tissue (1x1x0.5 cm each) were removed from the areas of the RFA lesions as well as other abnormal grossly identified areas in the lung. The samples were then imbedded in paraffin for histopathological examination.

2.3.4.3. Microscopic Examination:

The blocks of tissue, which were embedded in and sectioned by a microtome at 3 µm and processed to glass slides. The slides were stained with hematoxylin-eosin for microscopic analysis of tissue changes. Selected areas of interest were photographed under light microscope.

The procedure for H & E staining is as follows:

- Deparaffinize slides in three changes of xylene for 3 minutes each.
- Hydrate slides in 100% alcohol and 95% alcohol, 2 changes for 3 minutes each, and rinse in distilled water until ripples disappear from slides.
- Place in Hematoxylin for 8 - 15 minutes.
- Rinse in tap water until water runs clear.
- Decolorize in 1% acid alcohol, 3 - 6 quick dips.
- Rinse in tap water until ripples disappear from slides.
- Dip in Bluing Agent, 3 - 5 long dips.
- Wash in luke-warm tap water for 5 minutes (37-40°C)
- Stain in Eosin for 30 seconds - 2 minutes.
- Dehydrate in 95% alcohol and 100% alcohol, 3 changes each for 2 minutes.
- Clear in three changes of xylene for 2 minutes each.
- Mount coverglass.

The following criteria apply when interpreting H & E Staining:

- Nuclei: Blue
- Nucleoli: Reddish or reddish-purple appearance
- Cytoplasm and other tissue constituents: Varying shades of red
- Blood: Bright red
- Cartilage and calcium deposits: Dark blue

To confirm tissue necrosis, the NADH staining was applied to the frozen section biopsy of the fresh lung tissue in experimental group specimen. The protocol for performing NADH staining is as follows:

- Thaw NADH solution.
- Add 5 ml NBT solution to the tube with the NADH solution.
- Incubate coverslips in a Columbia staining dish for 30 minutes at 37 °C.
- Wash with three exchanges of tap or deionized H2O.
- Remove unbound NBT from the sections with three exchanges each of the 30, 60 and 90 % acetone solutions in increasing then decreasing concentration.
- Leave the 90 % acetone covering the sections until a faint purplish cloud is seen over the section.
- Rinse several times with deionized H₂O and then mount the coverslips with the aqueous mounting medium onto a labeled glass slide.

NADH staining provides histochemical confirmation of tissue viability, as well as, necrotic areas. Lack of staining with NADH is the current gold standard for determining if the tissue has successfully been destroyed post RFA. The viable cells stain bluish gray from the deposition of blue-black cytoplasmic granules during the NADH oxidation reaction. Dark blue staining of fresh tissue when incubated with NADH and nitroblue tetrazolium for 30 minutes reflects the cell’s enzymes ability to perform the Krebb’s cycle and several other oxidative metabolic pathways and thus implies viability of the tissue. These pathways persist only up to one hour after removal of the tissue from the subject.

The blocks of tissue from both groups underwent microscopic assessment with H & E staining for the presence original RFA probe hole, tissue necrosis, congestion, hemorrhage, inflammation, granulation tissue, fibrosis and scar formation, infection, vascular thrombosis, and diffuse alveolar damage. The features were tabulated as being either present or absent on each microscopic slide. Multiple slides of each lesion were assessed and the composite features used to determine the lesional characteristics.
3. Results:

3.1 Electrophysiological Data of RF Ablation:

In-vivo response of normal lung tissue to RFA in the experimental group showed that the mean values for delivered power during ablation was 23.63 ± 8.24 Watts, for the impedance was 88.15 ± 17.87 Ohms, and the temperature recorded by the generator was 72.51 ± 8.66 °C. (Graph 3.1)
3.2. Clinical Assessment:

3.2.1. Vital Signs:

3.2.1.1 Temperature:

The mean rectal temperature for the experimental group was 39.69 ± 0.25 °C, and in the control group it was 39.56 ± 0.26 with a p value of 0.7198. (Graph 3.2)
3.2.1.2. Respiratory Rate:

The mean respiratory rate in the experimental group at rest was 26.01 ± 2.88, and in the control group it was 25.65 ± 3.07 with a p value of 0.8797. (Graph 3.3)
3.2.1.3. Heart Rate:

The mean heart rate was within normal range in both groups, and there was no statistically significant difference in either group (p value 0.0.2228). In the experimental group the mean heart rate was 75.89 ± 10.86, and in the control group the mean heart rate was 78.72 ± 6.76. (Graph 3.4)
3.2.2. General Well-being:

There was no evidence of decreased appetite, change in behavior or alertness of the animals in either group. The animals started eating as soon as they were able to walk, which was within hours of operation.

3.2.3. Respiratory Distress:

Respiratory distress was observed mainly in the experimental group. There was no respiratory distress at rest (grade I) except on the day of procedure (n=2). Respiratory distress at walking (grade II) was more common, and it was noticed in two animals on the day of the procedure, and in two animals on the first post-ablation day. One animal continued to show evidence of shortness of breath for the first five days after ablation. No evidence of shortness of breath was observed in the control group. (Graph 3.5)

Graph 3.5. Recovery from Respiratory Distress
3.2.4. Activity level:

All animals were able to stand and walk within 3 hours of ablation. Every animal was able to walk (grade IV) or play (grade V) on the first day of ablation. No disability in terms of movements or activity was noticed in either group, and they were grade IV or V of activity level for the whole observation period of 30 days. (Graph 3.6)
3.2.5. Chest Tube Drainage:

Although chest tube drainage was a common finding in both groups, the discharge was more frequent in the control group than in the experimental one. Removal of the chest tube was dependent on the severity of discharge, and none of the animals in either group had a chest tube on the third post-procedure day. (Graph 3.7)
3.2.5.1. Air Leak:

In the experimental group, air leakage was a major finding (n=9) especially on the day of procedure, but the level of air leak decreased rapidly in each consecutive post-operative day. Half of the animals had no air leak on the first post-op day, and by the second post-op day, only one animal had an air leak. None of the animals had a chest tube on the third post-procedure day. (Graph 3.8)
In the control group, the air leak was also a prominent finding, and as in the experimental group, the air leakage decreased rapidly. Three animals had air leakage on the first post-operative day and one animal had an air leak on the second post-operative day. None of the animals in the control group had a chest tube on third post-procedure day. (Graph 3.9)
3.2.5.2. Blood Discharge:

Although seven animals in the experimental group had a bloody discharge, the amount of drainage was less than 100 ml (n=6). One animal had a bloody discharge of more than 100 ml. In the control group, four out of five animals had blood discharges. Of these, three had a bloody discharge of less than 100 ml, and one had bloody discharge of more than 100ml. No animal had any type of discharge on the third post-ablation day. (Graph 3.10)

3.2.5.3. Purulent Discharge:

No purulent discharge was observed in any of the animals in either group.
3.2.6. Surgical Wound:

In the experimental group, two animals showed evidence of swelling and erythema of the wound for the first five days. When the sutures were removed, the swelling and the erythema disappeared. There was no evidence of wound discharge or infection.

In the control group, the incision site was erythematous and swollen in one animal for the first three days. Once the suture was removed, the swelling and erythema diminished and it was gone by the fifth day post-operatively. Two of the swine also showed evidence of oozing from the wound. Sterile gauze was placed every day and the wound was cleaned with iodine solution. The oozing continued for four days from the day of procedure.

3.3. Post-mortem Assessment:

3.3.1. Autopsy:

There was no evidence of hemothorax, pyothorax, and fistula in either group. The main finding at autopsy was the presence of adhesions, and it was a prominent finding in both the experimental group (n=8) and the control group (n=3).

<table>
<thead>
<tr>
<th>CRITERIA</th>
<th>EXPERIMENTAL GROUP</th>
<th>CONTROL GROUP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adhesions</td>
<td>90%</td>
<td>80%</td>
</tr>
<tr>
<td>Fistula</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Hydrothorax</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Hemothorax</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Pyothorax</td>
<td>0%</td>
<td>0%</td>
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</tbody>
</table>

Table 3.1 Autopsy Findings
Figure 3.1 Pleural Adhesions at Autopsy

Figure 3.2. No Evidence of Hemothorax or Hydrothorax at Autopsy
3.3.2. Gross:
3.3.2.1. RFA lesion:

- **Shape of RFA Lesion**: No universal RFA lesion shape was observed; often, animals, eight of them showed spherical or ovoid and/or irregular shapes. No RFA lesion was found in the remaining two samples.

- **Features**:
  - **Probe Hole**: Although the probe hole was observed in five animals microscopically, no probe hole was noticed in gross examination.
  - **Central Coagulum**: This is the central dark brown lesion, which corresponds to necrotic lung tissue immediately adjacent to the probe.
  - **Fibrotic Pseudocapsule**: The central necrotic area is surrounded by a thick fibrotic pseudocapsule. It has pale-yellowish color with a thickness of 2-3 mm, and irregular borders.
  - **Neovascularization**: Although neovascularization was seen in the 30-day post-ablation lesion, the vessels had not entered the necrotic area. They are more prominent in areas with thicker pseudocapsules. Although the specimens were 30 day post ablation, the degree of neovascularization varied from sample to sample.

3.3.3.2. Non-RFA Lesions:

Multiple medium brown colored lesions were seen in the lungs of both groups. These lesions had different shapes; most of them were irregular, and were scattered in all three lobes of the lung. The main reason for these lesions could be the remaining blood in the lung after sacrificing the animal.
Figure 3.3. Gross picture of ablation site at 30 days
3.3.4. Microscopic:

3.3.4.1. RFA Lesion:

- Of the ten animals, only eight demonstrated pathology consistent with an RFA lesion. The other two animals showed alveolar hemorrhage, edema and some degree of alveolar capillary congestion. These areas are interpreted as not being produced directly by the effects of RF coagulation but may be due to trauma from the initial placement of the RFA probe.

- **Probe Hole:** In six of eight cases, which showed RFA lesion, residual defects were identified, consistent with the residual cavities made by the deployed probes. In some cases, these cavities were nearly obliterated. They tended to be located at the center of the healing reaction.

![Figure 3.4. Probe Hole](image)
- **Eosinophilic Coagulum**: Seven of eight lesions contained some of the eosinophilic coagulum that forms the tissue reaction directly around the probe defects. It consisted of thermally coagulated lung epithelium, bronchial tissues and extracellular matrix. The alveolar spaces were filled with proteinaceous fluid.

- **Congested Necrotic Parenchyma**: All eight lungs demonstrated remnants of necrotic lung, which appeared as architecturally preserved parenchyma but with hypereosinophilia of the alveolar walls and constituent cells, nuclear pyknosis, capillary congestion and hemorrhage with laked erythrocytes. This necrotic tissue was sequestered at the center of the lesions.

- **Alveolar Hemorrhage**: Intra-alveolar bleeding and congestion was observed in all samples.
• **PMNs:** Polymorphonuclear leukocytes and karyorrhectic debris were identified in all lesions, although the density was variable. They clearly tended to be present at the outer margins of the necrotic parenchyma in a loose laminar distribution, consistent with ingress from the periphery.

Figure 3.6. Tissue necrosis and PMNs
- **Granulation Tissue:** Surrounding the necrotic zone was a rim of granulation tissue characterized by a band of mononuclear inflammatory cells, specifically lymphocytes, plasma cells and macrophages permeating a loose matrix of acid mucopolysaccharide interstitial ground substance as well as some early fibrous tissue. Small, thin walled vessels infiltrate through this loose stroma. Multinucleated histiocytic giant cells were often present at the interface of the granulation tissue with the necrotic lung. In many instances, the connective tissue support was so loose as to cause fragmentation and separation of the infarct.

- **Fibrosis:** Contiguous with and immediately peripheral to the granulation tissue was a thick lamina of formed collagenous fibrous scar tissue. This reaction was present in all lesions and was found to completely circumscribe the necrotic lung in every case. This fibrous reaction was, in most cases limited to the immediate lesion and with little transgression into the adjacent normal parenchyma.
Figure 3.7. Fibroblast production, inflammatory cells immigration and tissue coagulation.

Figure 3.8. Fibrosis, granulation tissue, and necrosis.
- **Circumscription:** As observed on the macroscopic sections, the shapes of the RFA lesions were not perfectly oval or spherical. While the outside fibrous pseudocapsule of the progressing scar demonstrated an even if somewhat lobulated profile in certain areas, fibrous scarring showed some tendency to extend through the interlobular septae that connected the lesion with the periphery of the lung. This imparted a stellate profile to the lesion in such instances. Pleural retraction occurred when the process extended to the periphery. The fibrosed interlobular septae were of variable thickness, with a tendency to demonstrate increased width closer to the lesion. Within these septae, proliferating lymphatic channels were observed. In only two of eight cases was septal lymphatic dilation (from lymph fluid or intralymphatic hemorrhage) observed and such changes were minor and focal in the vicinity of the RFA lesions. The interior of the lesions was more variable, with fibrous septae dividing areas of granulation tissue and residual necrotic lung.

![Figure. 3.9. Circumscription](image)

Figure. 3.9. Circumscription
- **Vascular Thrombosis**: Vascular thrombosis was ubiquitous in these lungs but was confined to the lesion or its immediate periphery. Centrally, vascular remnants, identified by the presence of elastic laminae, showed collapse and obliteration of their lumens by dense fibrous tissue. At the margins of the healing lesions, the process of luminal fibrosis was perforated by small lumens as evidence of recanalization. No fresh thrombi were identified.

- **Airway Damage**: Airway damage was observed in all cases. In the central region of the necrotic zone, necrotic bronchioles with collapsed but occasionally patent lumina were present. Progressing to the healing peripheral reaction, complete fibrosis obliteration of bronchioles and cartilaginous bronchi were seen. Many of the larger bronchi that bordered the lesions demonstrated segmental mucosal thickening, with the expanded portion positioned adjacent to and usually contiguous with the scarred parenchyma enclosing the infarct.

![Infarcted Alveoli & Airways](image)

Figure 3.10. Infarcted alveoli and damaged airways
- **Pneumonia & Diffuse Alveolar Damage**: In the microscopic sections containing the lesions, no pneumonia or diffuse alveolar damage was identified. The only inflammatory/reactive response identified was a focal interstitial process, which was characterized by expansion of the alveolar septae by a combination of fibrous tissue and chronic inflammatory cells in a pattern consistent with lymphoid hyperplasia. Only one animal in experimental group demonstrated an acute inflammatory infiltrate that would be suggestive of early pneumonia.

![Early Bronchopneumonia](image)

Figure 3.11. Evidence of early pneumonia
3.3.4.2. Non-RFA Lesions:

Focal areas of hemorrhage and congestion were evident in areas away from the RFA lesion, and it was thought that these lesions were due to either probe trauma during RF ablation or mechanical trauma of handling the tissue. There was no necrosis and it is likely that these lesions would resolve with time.

Figure 3.12. Non-RFA lesion
4. Discussion:

4.1. Electrophysical Properties of RFA:

The two important factors to consider when ablating lung tissue are airflow and dual blood supply. Aerated lung tissue acts as an efficient thermal resistor, and has high RF impedance, due to high content of air. In addition, the results of ablating a fully inflated lung will be inconsistent because high amount of air increases the impedance at a level that shuts off the generator. The high vascular flow results in a rapid dispersion of heat away from nearby normal tissue, and results in heat sink effect, which will further reduce the amount of heat deposition in the lung tissue. One of the most important determinants of tissue heating, which has direct relation with the extent of coagulation necrosis, is RF current density. The greater the current density surrounding the needle electrode, the more energy is deposited in tissue and the more tissue can be heated to cause cellular destruction. On the other hand, if too much energy is applied too rapidly, the ablated tissues can be heated to temperatures greater than their boiling point, which in turn results in gas production, charring, and cavitation. All of this will lead to increased circuit impedance and reduction in further energy deposition.

There are significant distinctions in tissue characteristics, tumor biology, and tissue impedance of the lung tumor compared with other tissues e.g. liver that will affect the outcome of RF ablation. The air-containing lung tissue has high tissue impedance to electrical conductance. This affects the RF ablation in the lung in two different ways. The positive way is to make an oven effect like the capsule of primary liver tumors, which induces high current density around the needle in the tumor. The negative effect is that
high impedance of the lung tissue precludes creation of a surgical margin around the
tumor, which is related to the high local recurrence rate.  

4.2. Mortality and Mortality of RFA:
Radiofrequency ablation of lung tissue can be achieved in a safe manner with negligible
morbidity and no mortality. There was not any intra-operative mortality in either groups,
and all animals survived for 30 days post-procedure. In both groups, no intra-operative
complications were observed, and the post-operative recovery time was fast and
uneventful. One of the animals in the experimental group had fever in the first 24 hour
after the procedure, which was first thought to be due to pulmonary atelectasis, because
the same animal also had a severe air leak (emptying chest tube over 7 times in 24 hours).
As the fever continued, Penlong was given for two more days (post-op day 1 and 2), and
the temperature decreased by the third post-procedure day. The normal pulse rate in
swine is between 60-100 beats per minute. There was no statistically significant
difference between the 30 days heart rate in the experimental and control groups. The
respiratory rate was higher on the day of the procedure in both groups due to the effect of
the pneumothorax. The rate went down within 24 hour after the procedure as the size of
the pneumothorax was decreased using a hemovac closed suction system. Although the
respiratory rates of both groups were higher than adult swine (12-18/min), the animals in
our experiment were young, which could explain higher respiratory rate in both groups.
Respiratory distress was observed in two animals in the experimental group, but none of
the animals in the control group experienced respiratory distress. There could be many
explanations for this discrepancy including severe lung parenchymal damage and
pulmonary bleeding due to RF ablation, mechanical trauma from the probe, partial lung
collapse due to pneumothorax and infection. Furthermore, respiratory complications may not have been due to RF ablation and could happen due to any open or thoracosscopic procedure.

Although the literature for pulmonary ablation is scarce, reported cases has demonstrated that lung RFA causes little morbidity, no mortality, and promising short-term follow-ups. Lee and his associates assessed the feasibility and safety of CT-guided percutaneous transthoracic radiofrequency ablation (RFA) with saline infusion of pulmonary tissue in rabbits. They chose twenty-eight New Zealand White rabbits and divided them into two groups: an RFA group (n=10) and a saline-enhanced RFA (SRFA) group (n=18). In the RFA group, percutaneous RFA of the lung was performed under CT guidance using an internally cooled electrode. In the SRFA group, 1.5 ml saline was infused slowly prior to and during RF ablation. The rabbits were sacrificed at times from the one day post-procedure to three weeks later. The complications arising in 12 cases were pneumothorax (n=8), thermal injury to the chest wall (n=2), hemothorax (n=1), and lung abscess (n=1). Although procedure-related complications tended to occur more frequently in the SRFA group (55.6%) than in the RFA group (20%), the difference was not statistically significant (p = .11).145

Miao et al. conducted an animal experiment to evaluate the feasibility of RFA in the treatment of pulmonary tumor. He chose 18 rabbits with pulmonary implantation of VX2 tumors were divided into two groups. Group A (n = 12) was treated with RFA by using a cooled-tip electrode technique. Group B (n = 6) received sham operation. The therapeutic efficacy was evaluated by survival rate, magnetic resonance imaging (MRI), postmortem microangiography, and histology. All animals in group B died within 3 months after
tumor implantation. Tumor eradication was achieved in 9 of 12 rabbits (75.0%) in group A, of which 4 rabbits survived longer than 3 months free of disease and another 5 rabbits were found free of viable tumor when sacrificed. One rabbit was subjected to incomplete tumor ablation and two rabbits suffered from local tumor relapse and/or lung metastasis. The 3-month survival rate of RFA-treated rabbits was significantly higher (P < 0.01) than that of control rabbits. He concluded that destruction of pulmonary tumor could be achieved with current RFA technique in rabbits.\textsuperscript{146}

Human lung RFA studies are scattered. The National Institute of Health 2003 RFA Report indicates that over 100 lung cancer patients have been treated with RFA. The outcomes are mixed with short-term local recurrence rates from 33-74 %. There has been one peri-procedural death due to bleeding, and one reported stroke due to cerebral embolism. A common complication is pneumothorax, usually in the range of 10 to 20%. Less common complications are bleeding, fistula, hemoptysis, subcutaneous emphysema, pleural effusion, fever, infection, and pain.\textsuperscript{147}

Toyoshima and associates applied CT-guided RFA in six patients to treat 14 tumors. The median diameter of the tumors was 1.5 cm, ranging 0.8-to 2.4 cm. Good response was confirmed in six tumors on CT images, in one tumor on PET images, and in two tumors by histologic examination. In the other five tumors, curative effect was hard to evaluate due to consolidation shadows encircled the tumors. The complications were pneumothorax (n=5), subcutaneous emphysema (n=3), and pleurisy (n=1). The author concluded that RFA of pulmonary malignancies appears to be a safe and effective if a low power of less than 70 W is applied.\textsuperscript{148}
Dupuy et al. used RFA to treat three patients with lung cancer, one of whom was retreated for residual disease. The second patient received a 2-week course of external beam radiation therapy, which started 3 weeks after the RF ablation. The patient died in a nursing home 1 month after completion of external beam radiation of unknown reasons. The third patient was asymptomatic at 1 and 3 months follow-up.\textsuperscript{149}

Sewell et al. successfully ablated non-small cell cancer lesions in 10 patients with negative PET scans 3 months after RFA.\textsuperscript{150}

Recently, Glenn et al. reported 10 nonsurgical patients with pulmonary metastases from colorectal carcinomas, who have been percutaneously ablated under local anesthetics or sedation/analgesia. RFA was technically successful in 14 of 15 pulmonary metastases; complications requiring intervention occurred in three patients because of pneumothorax and pleural effusion. Follow-up with CT after 3 months showed no evidence of recurrence in any of the lesions treated, and all treated tumors showed a decrease in tumor size.\textsuperscript{151}

Steinke and his colleagues described a case report of a 72-year-old woman that was previously diagnosed with peritoneal leiomyosarcoma, and was in remission since primary surgery. She underwent pulmonary radiofrequency ablation for two metastatic lesions. The patient did well post-operatively apart from subfebrile temperatures (37.4°C) that lasted for 3 days after the procedure, and mild flu-like symptoms. Deep inspiration was painful, but well tolerated with non-opiate analgesics.\textsuperscript{152}
<table>
<thead>
<tr>
<th>CRITERIA</th>
<th>OUR EXPERIMENT</th>
<th>OTHERS EXPERIMENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pneumothorax</td>
<td>Common</td>
<td>Common</td>
</tr>
<tr>
<td>Bronchopleural Fistula</td>
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<td>Infection</td>
<td>No Clinical Evidence</td>
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<tr>
<td>Pulmonary Embolism</td>
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<td>Uncommon</td>
</tr>
<tr>
<td>Death</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Mental Status Change</td>
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<td>Uncommon</td>
</tr>
<tr>
<td>Subcutaneous Emphysema</td>
<td>None</td>
<td>Uncommon</td>
</tr>
<tr>
<td>Pleural Adhesions</td>
<td>Common</td>
<td>Common</td>
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Table 4.1 Complications of lung RFA in our experiment and other experiments.

It is important to understand whether these complications were directly the result of the RFA procedure or whether they can occur in any thoracic procedures including thoracoscopy, VATS and lung biopsy. Generally, the complications of intrathoracic operations include bleeding, air leak, atelectasis, lung collapse, secondary pulmonary infection, and bronchopleural fistula.\(^\text{153}\) Below is a review of morbidity and mortality rate of thoracic interventions including thoracoscopy, open lung biopsy and VATS.

The type and rate of complications for thoracoscopic procedures vary, depending on the type of procedure, the preoperative condition, and the experience of the surgical team. Furthermore, complications can occur intra-operatively, early post-operatively, or late post-operatively. The overall incidence of postoperative complications after thoracoscopy is relatively low, with a 4% incidence reported in 348 procedures in a study by Krasna et al.\(^\text{154}\) Infection is a potential postoperative complication following all surgical procedures. In thoracoscopy, infection ranges from a local wound infection to a pulmonary abscess or an empyema, however, most studies report an overall infection risk of less than 1%. Atelectasis can also develop as after performing thoracoscopic surgery.
The morbidity and mortality associated with open biopsy varies from series to series depending on the underlying pulmonary disease and the general condition of the patient.\textsuperscript{155-159} Mortality rate of open lung biopsy is 0-21%, while that of VATS biopsy varies from 0-10%.\textsuperscript{215-218} In a recent study by Zegdi et al. there were 4.7% deaths after VATS lung biopsy (2 patients were immunodeficient).\textsuperscript{159}

Morbidity has generally been found to occur in 20-50% of patients undergoing open lung biopsy while that of VATS biopsy has ranged from 0-25% in different series.\textsuperscript{215,216,220} Bensard et al (1993) reported that 19% of open lung biopsy patients experienced complications, compared with 9% undergoing a VATS lung biopsy.\textsuperscript{155} Mack and associates, demonstrated the safety and effectiveness of VATS excision of indeterminate solitary pulmonary nodules. There was no mortality or major morbidity in his experiment, and minor complications occurred in 3.6% of patients. The average hospital stay was 2.4 days.\textsuperscript{160}

Transthoracic needle biopsy, performed under CT or fluoroscopic guidance has high rate of pneumothorax (10-30%). A study of the complication rate of percutaneous fine needle lung biopsy in a series of 29 patients was performed. No hemoptysis or mortality was observed, but pneumothorax developed in eight patients (28 percent). Of these eight patients, five were managed without treatment while three required chest tube.\textsuperscript{161}

Overall, radiofrequency ablation of primary and secondary lung malignancies is a safe technique. No death occurred due to lung RF ablation in any of the animal studies. There was a reported case of a human death due to pulmonary embolism and stroke. Generally, the complication rates of the lung RFA are less common than the conventional surgery. Reported adverse effects include pleural effusions (up to 30%), COPD exacerbation,
bronchopleural fistula, pulmonary embolism, reversible mental status changes, hemorrhage, pneumothorax, pain and subcutaneous emphysema. The complication rate in our study was comparable with other lung RFA studies. Pneumothorax was a common complication in our study as well as other animal and human RFA studies, and it was common in non-RFA thoracic interventions (10-30%). The immediate post-procedure hemothorax in this study was short term and easily manageable without any obvious symptomatic manifestations. The rate of bleeding due to lung RFA is similar to percutaneous needle biopsy of lung nodules. While peri-lesional hemorrhage is commonly identified on post-procedural CTs, the rate of symptomatic bleeding is considerably less, and severe hemoptysis is rare. There was no clinical manifestation of pneumonia in our study, and other lung RFA studies show a low lung infection rate. The same low infection rate is seen in other thoracic procedures (less than 1%). Although, pain is a common complication, it is easily managed with the use of analgesic agents. 

4.3. Pathology:

Eight of the ten animals in the experimental group demonstrated lesions compatible with healing radiofrequency ablated lung. In all of the eight cases, the lesions showed consistent finding of a progressive organization and resolution of a necrosis. The central portion of the lesions was characterized by residual changes observed in the acute experimental phase of the study in our laboratory such as the amorphous densely eosinophilic coagulum that is produced by the RF energy in the immediate vicinity of the deployed individual probes. The actual probe holes themselves were not always present, having collapsed or otherwise filled in with other necrotic debris. No large residual cavities beyond several millimeters in diameter were identified in any lung. The various
lamina associated with each individual lesion identified on the gross lung sections correspond to the different stages of healing. Histologically, the lung lesion that was created by RFA displayed a specific pattern that was unique to the lung tissue. There was a central necrotic area surrounded by a thick fibrotic pseudocapsule. Neovascularization, production of fibroblasts and migration of inflammatory cells were evident in a 30-day old RFA lesion. The central necrotic tissue is actively resorbed by a layer of granulation tissue with neovascularization, fibroblast proliferation and some mature fibrous tissue. This appearance confirms the intact ability of the lung to reproduce the sequence of wound healing, specifically: proteolytic degradation of necrotic cells and connective tissue components, inflammatory cell migration into the wound margin, neoangiogenesis and fibroblast proliferation. In our experiment, complete healing did not take place after 30 days of ablation, although the lesion showed evidence of shrinkage. Same results were found in swine lung RFA by Putnam. He described that the 28-day post-RFA lesion completely replaced red damaged zone by light tanned scar tissue, which surrounded the central thermal coagulum.\textsuperscript{163} Goldberg, in a rabbit lung RFA study, observed minimal residual fibrosis and pleural scarring by day 28, and suggested a rapid, and almost complete, recovery of the RFA lesion.\textsuperscript{47} It seems that smaller animals may have faster resolution of the lung injuries. The red blood cells and lung tissue in the central coagulum are destroyed directly by thermal coagulation. The severe occlusive vascular thrombosis prevents blood access to central coagulum. Hence, the central coagulum persists until the neovascularization with its blood flow and inflammatory cells invade the necrotic tissue. The lesions do not heal by one month, but comparable studies of acute and one month old
lesions show that they have a tendency for shrinkage. This tendency is due to contraction of the fibrous scar tissue during normal wound healing.

There were multiple areas of hemorrhage, which were more prominent in the lower lobes. There was no severe damage of vascular structures and the adjacent bronchioles were not injured. Putnam reported resolution of segmental pulmonary hemorrhage and infarction, and formation of pneumonitis and chronic atelectasis by 28 days post ablation. Blood flow had not entered into the coagulum and the organization was present only at the interface of the fibrous scar tissue with the coagulum.\textsuperscript{163}

The concentric thermal damage and healing zones revealed by pathological examination were similar to those demonstrated in RFA thermal coagulation lesions in normal pig lung followed at intervals for up to 3 months. Distinct thermal damage zones with measurable boundaries extend concentrically from the hot center to the cool periphery. The lethal damage in the tissues is due to direct \textit{in situ} thermal coagulation, ischemic necrosis secondary to the thermally induced hemostasis, and thrombosis and sublethal thermal rupture of membranes with release of intrinsic lytic enzymes. Direct thermally coagulated tissues will retain their usual histological architecture when examined at the light microscopic level, resulting in the mistaken interpretation of viability in the center of the thermal lesion. Although the cells appear "normal," electron microscopy reveals granular denaturation of cytoplasmic and nuclear proteins including intrinsic lytic enzymes and massive rupture of cellular membranes. The \textit{in situ} thermal precipitation of these cellular components will retain the pattern of intact cells in stained light microscopic sections.\textsuperscript{152,163}
4.4. The Role of Other Complimentary Interventions:

4.4.1. Photodynamic Therapy (PDT):

Lung dysplasia, CIS, and perhaps microinvasive carcinoma can be treated with photodynamic therapy. In patients who are not a candidate for surgery, PDT is undoubtedly an option. The advantages of PDT include no risk of exposure to ionizing radiation, improvement in bronchial tumor margins if used pre-operatively, and high effectiveness for lesions involving the carina. Side effects are few, the most common being sunburn. Other disadvantages with PDT are shallow depth of penetration, inpatient treatment, delay from time of administration of photosensitizing agent to regression of tumor, and multiple steps of management.

4.4.2. Endobronchial brachtherapy (EB):

Brachytherapy has become an effective tool in the treatment with tracheal bronchial malignancy including primary and recurrent bronchogenic carcinoma and metastatic carcinoma. EB is indicated in patients who either are not candidates for or have failed other therapies, such as surgery or external beam radiation. Intraluminal brachytherapy is effective in palliating complications caused by malignant endobronchial tumors such as dyspnea, hemoptysis, intractable cough, atelectasis, and postobstructive pneumonia. In general, brachytherapy is used only after a full course of external beam radiation. Fatal hemoptysis, fibrinous obstruction, and fistula formation are the serious complications of EB. The role of EB in the treatment of lung dysplasia, CIS, or microinvasive carcinoma is unclear.
4.4.3. Laser:

The safety and efficacy of laser bronchoscopy has been well established, and the reported incidence of complications is low. Laser bronchoscopy is used for palliation of central airway obstruction due to primary or metastatic lung cancer. It is also useful in airway bleeding, and carcinoma in situ. Some of reported complications include retinal damage, endobronchial fire, hemoptysis, fatal bleeding, airway perforation, pneumothorax, and systemic or cerebral air embolism. The contraindications of laser are extrinsic compression, excessively bulky tumor, technical difficulty in aiming the laser because of tumor location, upper lobe lesions due to upward direction of the lumen, and total occlusion of the airway.169,170,171

4.4.4. Electrocautery:

Electrocautery can be used similarly to laser therapy and/or cryotherapy for managing advanced endobronchial lung cancer. Electrocautery could be employed as a palliative measure for endobronchial malignancy and it holds great potential for endobronchial disease management. As with other procedures there are reported complications including endobronchial fires, hemoptysis/hemorrhage, and aspiration pneumonia.10

4.4.5. Cryotherapy:

Cryotherapy is the most extensively researched alternative procedure for cancer treatment, and has been used to treat metastatic disease in the liver and, to a more limited extent, breast cancer. Cryotherapy is less expensive and safer to use than laser therapy, but it has some disadvantages as well including inability to provide immediate effect, and the need for multiple treatment sessions. Some of the reported complications include death rate of 1-5%, respiratory or cardiopulmonary arrest, fatal hemoptysis, and
tracheoesophageal fistula, pneumothorax, bronchospasm, atrial fibrillation, bradycardia, and aggravation of cold-agglutinin anemia. Cryotherapy is time-consuming, because it takes some time for the destroyed tissues to die and become necrotic. This entails removal of necrotic tissue and, in some cases, repeating the procedure.\textsuperscript{10}

4.5. Limitations of the Study:

Our study suffered certain limitations. First, the results obtained in healthy swine lung may differ from those obtained with non-small-cell human lung carcinoma. However, aspects of the process of cell degeneration caused by the effect of heat on tissue lead us to believe that the results might well be the same with either normal lung tissue in swine or human lung cancer cells. Second, animals were less exposed to multiple risk factors than humans, which may affect long-term survival rate. Thirdly, two of the animals did not show evidence of RF ablation, which could have been due to lack of localized RF energy delivery or the heat sink and high impedance of air. The newer generators may have the ability to overcome some of these limitations as they deliver more power and the probes are able to ablate larger tissue size, decreasing the need for multiple or repeated ablations. Air-containing lung tissue has a naturally high tissue impedance, and the creation of a surgical margin around the tumor may thus be difficult, a fact which might have a protective role for areas close to the tumor, but also may result in higher local recurrence rate, because of inability to ablate a fair amount of tumor margin.
5. Conclusion and Future Directions:

The results of our study showed that radiofrequency ablation of lung tissue can be achieved in a safe manner with insignificant morbidity and no mortality. No intraoperative complication was observed, and post-operative morbidity was negligible, short-term, and easily manageable. The main post-operative complication in both groups was pneumothorax, and it can be concluded that it is a common complication of any chest intervention and not just lung RFA. No animal developed clinical evidence of pneumonia or other respiratory and systemic diseases; there was no evidence of fistula, pyothorax and hemothorax at autopsy. Histological exam did not show any sign of widespread parenchymal damage. Adhesion was the main finding in autopsy, which could happen in any thoracic intervention. The 30-day lung RFA lesion has a predictable morphology with a tendency for absorption, and minimal damage to adjacent tissues. The electrophysical properties of lung RFA is unique to the lung tissue. It requires more power to deliver the current necessary to ablate the targeted lung tissue. A better understanding of the effects of tissue perfusion and airflow on ablation, and the development of methods to overcome these problems are necessary to optimize results.

Overall, RFA is a minimally invasive procedure, which is technically simple, repeatable, and takes short period. It is a promising technique and can be used as an alternative method of treatment in patients not eligible for surgical treatments. Although the long-term clinical benefits of RF ablation for the treatment of lung neoplasms remain relatively unproved, the extensive laboratory and animal experience in combination with the results from preliminary clinical series suggest that these techniques may have an
important role in the treatment of primary and secondary lung tumors. Nevertheless, additional research is needed to further optimize RF equipment and ablation techniques. Despite the progress that has been made, a number of challenges remain for the future. These include the development of techniques that can increase the volume of tissue destroyed at a single treatment session, the development of more suitable and accurate imaging tests, and the development of devices that overcome the high tissue impedance. The improvement of techniques to maximize tissue heating and to prevent carbonization and cavitation may permit larger volumes of tissue destruction at a single treatment session. Although multiple commercial devices are now available, it is not easy to predict which method will prove more effective for any given clinical application. These technologies must be able to ablate the desired volume of tissue in a reproducible and predictable fashion. Other factors, including ease of clinical use, the duration of the procedure, and cost, will play a role in determining which of these technologies will receive the greatest attention. It is also important to investigate the effectiveness of RF ablation in the tumors located around large vessels because of potential complications such as thrombosis by coagulation or lung abscess.

Synergistic combination therapies including RF and other adjuvant agents such as chemotherapy or radiotherapy will also likely receive significant attention in the near future. However, further study is necessary to determine under which conditions a particular method will prove superior to others, and to determine whether any of these methods can improve patient outcomes. The answers to these questions will require substantial further research, and it is hoped that this work and well-conducted
randomized, multi-center trials will determine the proper role for this promising technology.

Although there are some studies that have evaluated the in vivo effects of RF ablation in human lung tumors, but the studies are scattered, and mostly concentrated on survival and recurrence rate of lung cancer after applying RFA. The next step is to evaluate the acute in-vivo effects of RFA in respect to human lung cancer, because our study was based on normal lung RFA in swine, and not human lung tumor. It is also important to perform a long-term human survival study to assess mortality and morbidity associated with RF ablation, and to evaluate if RFA improves survival rate and quality of life in patients with lung cancer. A randomized control trial between RFA and standard surgery and/or other complimentary therapies is necessary to make the final decision whether this method of treatment has any value in management of lung cancer.

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All or part of this research has been used for the following presentations and publication.

Presentations:

1. Short-term Survival after RFA of Normal Lung in Swine Model
   Research Rounds, Dept. of Surgery, University of BC
   May 15, 2003

2. Lung Radiofrequency Ablation
   UBC Graduate Students Research Conference
   Oct. 27, 2000, Vancouver, BC

3. Assessment of Complete Tumor and Normal Lung Tissue Necrosis
   Radiofrequency Technique in Lung Tissue Evaluation Meeting
   Oct. 7-8, 2000, San Francisco, CA

4. Ablation of Human Lung Tumor and Normal Lung Tissue
   10th World Congress – World Society of Cardio-thoracic Surgeons
   Aug. 13-16, 2000, Vancouver, Canada

Publications

Short-term Survival after RFA of Normal Lung in Swine Model
Saleem, M., Bucscowski A., Flint J., Scudamore, C.
Manuscript is prepared and will be submitted to Journal of Investigative Surgery
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