A New Fiducial Marker for Gated Radiotherapy in the Lung – A Feasibility Study of Bronchoscopy Based Insertion and Removal in Göttingen Mini-Pig

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Summary

To develop a new prototype fiducial marker (LS-1) that may be used for gated radiotherapy in the lung.

One LS-1 marker was inserted in the lung of each animal under sedation. Animals were kept under observation for four weeks after insertion. After the observation period the marker was removed. Animals were CT scanned after insertion and before removal of the LS-1 marker. After the last CT scan animals were euthanized and lungs excised for pathology.

The LS-1 marker was successfully inserted in all fourteen animals. Thirteen of fourteen LS-1 marker's were *in situ* after four weeks. Two cases of pneumothorax were seen in connection with insertion. The LS-1 marker could only be successfully removed from eleven of thirteen animals. Damage to the lung was mainly local close to the LS-1 marker insertion site.

The LS-1 marker has the potential to be a fiducial marker suitable for gated external beam radiotherapy in the lung. The method still needs some refinement prior to application in humans.

Introduction

SRT (stereotactic radiotherapy) of localized lung tumors in inoperable patients has demonstrated a 2-year local control rate in excess of 85% for both T1 and T2 tumors after three to eight sessions of high-precision radiotherapy (*Haasbeek et al., 2008*). Clinical studies have demonstrated that higher doses are associated with increased overall survival and that radiation dose may be safely escalated (*Belderbos et al., 2006*;*Bradley, 2005*;*Kong et al., 2005*). Individualized high doses of radiation to patients with NSCLC (non small cell lung cancer) may be achieved using dose escalation based on lung dose–volume toxicity (*Belderbos et al., 2006*;*Kong et al., 2006*). Strategies that compensate for respiratory movement of tumor position may be dynamic beam gating or dynamic beam tracking (*Ozhasoglu et al., 2002*). Such techniques require knowledge of tumor's motion trajectory and the ability to detect and adapt to changes in respiratory motion. External markers cannot correct for day-day shift in mean position and differences in motion described by internal markers (*Korreman et al., 2008*;*Ozhasoglu et al., 2002*). Consequently respiratory gated treatment of lung cancers needs a way to verify tumor position during treatment. One solution is to apply image guidance during treatment. Several studies have reported real time tracking of the movement of lung tumors during radiotherapy using inserted gold markers (*Seppenwoolde et al., 2002*;*Seppenwoolde et al., 2002*;*Shimizu et al., 2001*;*Shimizu et al., 2000*;*Shirato et al., 2000*;*Shirato et al., 2000*;*Keall et al., 2004*). Transcutaneous insertion however car-

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ries a high risk of pneumothorax. The present study describes development of a new removable fiducial lung stent (LS-1) marker for image guided radiotherapy. This new marker is a Nickel Titanium (Ni Ti) stent. The LS-1 marker is tested in a feasibility study based on bronchoscopy based insertion in the lung of Göttingen mini-pigs. The animals are genetically and microbiologically well defined (*Svendsen, 2006*;*Hansen et al., 1997*) and a reliable model for clinical research and fundamental science such as experimental radiotherapy (*Baumann et al., 2000*). The paper reports preliminary results from the study of the first prototype of the marker. This study was approved by The Animal Experimentation Council under the Danish Justice Department (journal no. 2008/561-1473)

Materials and Methods

The marker

Design of the new LS-1 marker is shown in figure 1. This marker is based on the design of the commercially available Memokath™ 051 ureter stent. The lower end (base) expands and anchors the LS-1, when flushed with water hotter than 45ºC. The LS-1 is densely coiled to prevent epithelial tissue growing over the stent. When flushed with water cooler than 10ºC, the LS-1 marker wire becomes soft and easily deformable (no force required). The wire remains soft and easily deformable as long as it is cold. This property makes atraumatic removal possible.

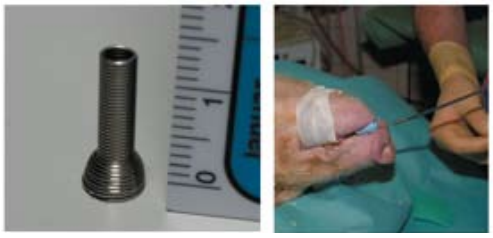

Figure 1. Left panel is a close up photo of the prototype of a new experimental fiducial lung marker (LS-1). The LS-1 marker is shown in the expanded shape. The wire has a diameter of 0.4 mm. Overall length and external diameter and expanded part of the LS-1 marker are respectively 10, 3.5 and 6.5 mm. Right panel demonstrate insertion of the LS-1 marker in the lung mounted on a special insertion catheter (gray plastic tube). The catheter is pushed over the inserted guide wire emerging from the endotracheal tube.

The animals

Initial procedure development and pre-animal studies were done using fresh cadaver lungs excised from pigs and mounted in an airtight tank that allowed for variation of air pressure. The later part of this study was performed on live barrier-bred Göttingen mini-pigs.

Design

The animal study was designed as a feasibility study and thus number of animals would typically be low. Assuming no high risk (grade 3) endpoints (i.e. resulting in death during the experiment) occur, and that the 95% confidence interval of the true underlying probability of a significant finding must be between 0% and 20%, the number of animals may be estimated to be 14 using the binomial distribution. Endpoints are listed in table I. A comprehensive overview of the workflow is given in figure 2.

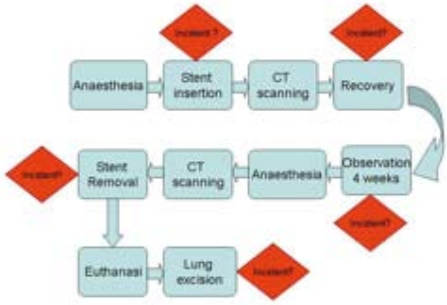

Figure 2. The graph is a comprehensive illustration of workflow in the project. Blue color labels are the individual working procedures. The Red labels represents scoring of adverse events or endpoints predefined in the study.

Table 1. Endpoints defined in risk analysis for the project. A risk score of 3 is considered prohibitive of human application in a feasibility trial. The risk score was calculated in a risk analysis performed prior to the study and not shown.

Endpoint	Consequence	Risk score	Human trials
Bronchospasms	Death	3	Prohibited
Respiratory insufficiency	Death	3	Prohibited
Respiration stop	Death	3	Prohibited
Sepsis	Death	3	Prohibited
LS-1 marker migration	Radiation target miss	2	Possible
Sepsis	Medical intervention	2	Possible
Irritation	Cough	1	Possible
Pneumothorax	Acute respiratory insufficiency	1	Possible
Local burn	Irritation	1	Possible
Local necrosis	Tissue damage	1	Possible
Dyspnoea transient	Troubled respiration	1	Possible
Dyspnoea	Troubled respiration	1	Possible
Stricture	Troubled respiration	1	Possible
Secret	Cough / minor airway obstruction	0	Possible
Local bleeding	Irritation	0	Possible
Major bleeding	Cough / minor airway obstruction	0	Possible
Haemopthysis	Cough / minor airway obstruction	0	Possible
Local infection	Irritation	0	Possible

Insertion procedure

Anaesthesia of the mini-pigs was done preoperatively using a special Zoletil cocktail for pigs Zoletil 50 Vet (6,25 ml Rompun / 1,25 ml Ketaminol (100mg/ml) / 2.50 ml Torbugesic) given as an intramuscular injection 1 ml / 10 kg typically 3-4 ml every 2 hours providing sedation adequate for approaching / handling of the animals. After sedation the animals were weighed and subsequently transferred to the operating theatre. Firstly, endotracheal intubations was performed. Vital signs such as blood pressure, heart rate and oxygen saturation with Pulse Oxymeter were measured during anaesthesia. In case of decline in oxygenation saturation pure oxygen was supplied at the entrance of the tube. Insertion and removal of LS-1 marker were performed using a Storz 11302 BD fiberscope with a working length of 650 mm, an external diameter at the tip of 3,7 mm and work channel diameter of 1.5 mm. All insertion and removal procedures were performed under fluoroscopy. Initially the procedure began with bronchoscope insertion of a guide wire at the intended position. After positioning of the guide wire, the length of the inserted part of the bronchoscope was noted. Next the bronchoscope was retracted leaving the guide wire in place. The insertion catheter with the LS-1 marker was subsequently inserted over the guide wire as demonstrated in figure 1. The LS-1 was moved forward, until the length of inserted catheter was equal to the above recorded length

from the bronchoscope. Finally the correct position of the LS-1 marker was checked with fluoroscopy as shown in figure 3. With the LS-1 in correct position 5 ml 58 – 60 degree Celsius hot water was flushed through the insertion catheter. The hot water emerged at the proximal base of the LS-1 marker. This caused the LS-1 to expand. During the flushing with hot water the temperature in the lung was monitored with a thermistor mounted at the tip of the catheter. After expansion the LS-1 marker was released from the catheter, which was then retracted, leaving the LS-1 marker in the lung. After insertion, the position of the inserted LS-1 marker was inspected with bronchoscopy as shown in figure 3. Also the tissue surrounding the insertion site was inspected for any burning effects from the hot water application.

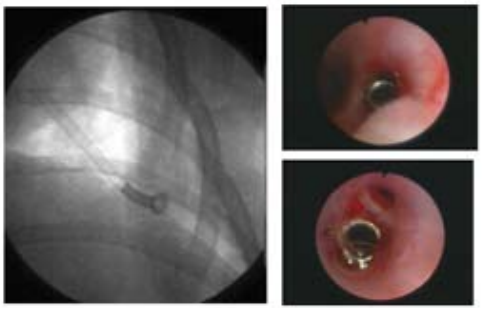

Figure 3. Left panel is X-ray fluoroscopy images of an expanded LS-1 marker inserted in the lung of a Göttingen mini-pig. The insertion catheter has not been removed yet. Right panel shows bronchoscope view of two different examples of inserted LS-1 markers. The images of inserted markers were taken with the bronchoscope immediately after insertion. Notice that the bronchial epithelial looks undamaged, apart from minor abrasive lesions probably due to manipulation with the bronchoscope.

CT scanning

After insertion, the mini-pigs still under sedation were transferred to CT scanning. The thoracic region was scanned using a GE Light speed RT 16 slice scanner. The scanning protocol was a 120kV helical scan with 0.8 sec rotation and a pitch of 0.938 with a slice thickness of 1.25 mm.

Observation

After CT scanning the mini-pigs were transferred back to the animal facilities. The animals were observed for any signs of stress, at 15 min intervals, for the first two hours during recovery from anaesthesia. In the four weeks following the insertion, the animals were kept under observation on a daily basis.

Removal procedure

Four weeks after insertion, removal of the LS-1 from the mini pigs was performed. Initially, the animals were anesthetized and weighed. After CT scanning the mini-pigs were taken to the operating theatre for removal of the LS-1. Removal was performed using either a specially developed removal tool or a grasping rat tooth forceps. Both instruments were inserted during bronchoscopy, using the working channel of the bronchoscope. The special removal

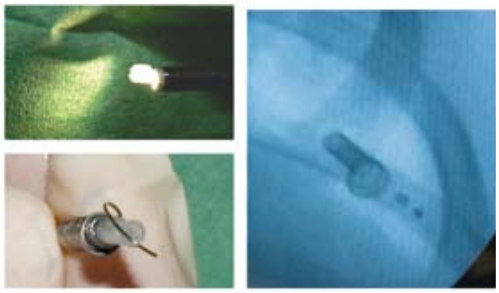

Figure 4. Upper left panel shows the removal tool back loaded in the bronchoscope. The head of the tool is a small plastic sphere lit up by the scope. The image to the right illustrates how passage of the LS-1 marker is checked using fluoroscopy to verify that the small x-ray markers are positioned beyond the LS-1 marker. The lower left panel demonstrate the removed LS-1 marker still on the removal tool. Notice that the collar has been distorted to a slightly twisted wire.

tool consists of a flexible plastic wire. The wire ends in a small sphere at the tip. The sphere has an X-ray marker embedded. The plastic wire was back loaded in the working channel of the bronchoscope. The tip of the wire then travels in front of the bronchoscope as shown in figure 4. The plastic sphere has a diameter of less than 2.7 mm. The diameter was designed for the sphere to be able to pass through the inner lumen of the LS-1 marker as shown in figure 4. The bronchoscope was retracted without changing the position of the plastic sphere. After retraction of the bronchoscope, a removal sheath was inserted over the plastic wire. The distal end of the removal sheath was slit and also contained a small X-ray marker. The slit end was moved through the LS-1 marker and beyond the end of the LS-1 marker. With the sheath in place, the small sphere was then pulled back causing the slit end to open. Correct opening was verified on fluoroscopy by proximity of the two small x-ray markers in the sphere and removal sheath respectively as seen in figure 4. The split end of the removal sheath was retracted until stuck in the expanded end of the marker. Then cold water (0-1 degree Celsius) was flushed through the removal sheath. The cold water emerged at the expanded end of the marker. The expanded part became soft after cooling. The marker was then extracted with a gentle pull. Alternatively the marker was extracted using the bronchoscope in combination with a small rat tooth forceps. After the forceps had grasped the wire of the LS-1 marker, cold water was flushed through the bronchoscope. Subsequently bronchoscope and forceps were pulled back simultaneously. After removal of the inserted LS-1 marker the insertion site was inspected by bronchoscopy for mechanical damage.

Euthanasia

After marker extraction the mini-pigs were euthanized. Euthanasia was done while the animals still were under full anaesthesia using Pentobarbital 20 % 200 mg/ ml given intravenously. After euthanasia the lung and heart were excised en-block and stored in formaldehyde for later pathological examination.

Pathology

The lungs were cut in 5 mm thick slices for macroscopic and microscopic inspection of changes related to the LS-1.

Results

Data on the fourteen animals are seen in table II. The LS-1 marker was successfully inserted in all fourteen animals. Data on insertion sites in the lung are given in table III. During the insertion procedure variation was seen in blood pressure, heart rate and oxygen saturation (table III). Changes in blood pressure and heart rate were generally small. Decline in Oxygen saturation below 75% was seen in four cases. This was always during insertion of bronchoscope or marker sheath. Oxygen saturation was always rapidly normalised after applying Oxygen at the entrance of the inserted tube. Immediately after LS-1 insertion, bronchoscope inspection of airways and insertion site revealed slight irritation of mucosa or minor abrasive bleeding. No macroscopic burning or necrosis was observed. CT scan

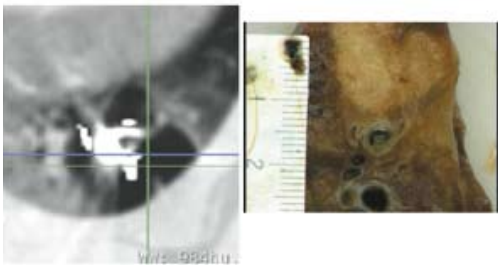

Figure 5. Left hand panel: Axial CT scans of lung tissue surrounding a LS-1 marker insertion site prior to removal. Emphysema is seen as a hypo dense area on the image. Right hand panel: Lung specimen from the pathological examination of the excised lung in the same animal. Notice the LS-1 marker is still visible in the centre of the image, as this was one of the two animals where the LS-1 could not be removed. The specimen is not axial, but more oblique. This explains the different relation between LS-1 and the emphysema change in the lung (light area above the LS-1) compared to the CT scan.

Table 2. Data on the research animals used in the study. The table contains parameters characterizing the insertion of the experimental lung LS-1 marker as well as data on various parameters characterizing animal physiological reactions to insertion procedures.

Parameter	Unit	Median	Min	Max	25% Quartile	75% Quartile
Age	months	16	16	18	16	17
Weight	kg	22.9	18.2	28.6	20.2	26.4
Weight Gain	kg	4.4	0.4	7.0	4	6.4
Insertion						
Guide wire length	cm	46	38	53	42	50
Water temperature	Celsius	59	57	62	59	61
Heart funtion during insertion						
Systolic pressure	mmHg	159	118	193	126	167.5
Drop in systolic pressure	mmHg	12	0	42	0	12.75
Diastolic pressure	mmHg	74	53	98	66	79
Drop in Diastolic pressure	mmHg	0	0	22	0	8.5
Heart rate	Hz	77	45	98	60	85.75
Drop in Heart rate	Hz	6	1	43	4	17.75
Lung function during insertion						
PO2	%	97	90	100	94	98
Drop in PO2	%	10	1	35	6	24

following insertion demonstrated that markers were inserted at branching level 3 or 4. The median values of bronchi diameter proximal and distal of the marker were 6.1 and 3.3 mm respectively. Markers could be inserted close to the lung surface. Inserted low in the lung, markers were located between 3.3 and 12.4 mm above the diaphragm. In two of the fourteen animals CT also demonstrated a pneumothorax. Both cases of pneumothorax were seen to have resolved four weeks later at the CT scanning before removal of marker. Animals recovered rapidly after anaesthesia and were eating within one hour after the procedure. During the four week observation period no adverse reactions (endpoints in table I), were observed in the animals. CT scan before removal of the marker demonstrated hypo dense volumes varying between 5 and 48 ml in 7 of the fourteen animals. One such hypo dense structure is seen in figure 5 together with the corresponding specimen for pathology from the same animal. The hypo dense volumes were typically found laterally or distal to the LS-1 marker in the lung. Finally the CT scan before removal of the LS-1 revealed that in one of fourteen animals the marker had migrated. The marker was removed in eleven of the remaining thirteen animals. In these eleven animals the marker was removed with the experimental tool in seven animals. In six animals the experimental tool

Table 3. Data on LS-1 marker position in the lung of fourteen mini-pigs in the study. Data are determined from CT scan after insertion and before removal of LS-1 marker. Positions in lung are scored H: High M: Mid L: Low. LS-1 marker is removed either using the experimental tool (ET) developed in this project or a Rat Tooth forceps (RTF). Both instruments are applied through the bronchoscope working channel.

Ear Tag	Position in Lung	Position in lung	Bronchi Branch	Bronchi proxi-mal	Bronchi distal	Dist lung surface	Pneu-motho-rax inser-tion	Re-moval Method	Re-moved success-ful
				mm	mm	mm			
75585	Left	L	4	6.1	3.1	10.3	N	ET	Y
75584	Right	M	4	6.1	2.8	7	Y	ET	Y
99451	Right	M	3	8.1	4.2	16.8	N	**Dislocated**	
75219	Right	M	3	7.7	3.4	47.1	N	RTP	Y
75218	Left	H	3	5.6	2.6	3.3	Y	ET	Y
75241	Right	L	4	5	2.5	12.7	N	ET	Y
75506	Left	M	4	7	3.7	15.1	N	ET	Y
99516	Right	H	1	6	3.4	12.4	N	RTP	N
99160	Left	L	4	6	3.4	12.1	N	RTP	N
75546	Right	L	4	5	3.3	11.3	N	RTP	Y
75601	Left	M	3	7.7	3.8	20.9	N	ET	Y
98593	Right	L	4	3.4	2	7.4	N	RTP	Y
75600	Right	H	1	4.7	2.5	11	N	ET	Y
75636	Left	M	3	7.9	3.3	18.5	N	RTP	Y

was not usable due to a large angle with the bronchoscope axis, so use of the forceps was attempted. In two of the six animals it was not possible to remove the marker either with the experimental tool or rat tooth forceps. Inspection of the LS-1 insertion site after removal revealed only minor bleeding. When the rat tooth forceps was applied, bleeding was more pronounced. The LS-1 insertion sites in the pathological lung specimens were identified using recorded information from the insertion and 3D projections from the CT scan taken just before removal. The LS-1 insertion site was identified in thirteen of fourteen animals. The site could not be identified in the animal with a dislocated LS-1 marker. There were obviously LS-1 marker related alterations like emphysema, atelectasis and indurations of lung parenchyma in marker related areas. Quantitative measurements of the extent of macroscopic LS-1 related changes in the lung proved to be difficult in some animals, due to patchy indurations in the lung without relation to the LS-1 marker sites. The changes were present in variable degree in all animals, and were seen in both lungs, i.e. also in the lung with no LS-1 marker insertion. The changes were also observed in two control animals, which had not been subjected to the experimental procedure in this study. On the microscopic level the patchy indurations were associated with

interstitial chronic inflammation in the lung parenchyma. Similar changes can also be seen in animals infected with Porcine Circovirus Type 2 (*Timmusk et al., 2008*) or PRRS arterivirus. Results from the pathological examination of the LS-1 marker bronchi and the adjacent tissue, i.e. the tissue close to the LS-1 marker insertion site and in the lung distal to the insertion site are given in table IV.

Discussion

The LS-1 marker was successfully inserted in all fourteen animals. The large decline in oxygen saturation seen in some animals was related to insertion of the bronchoscope itself or the lung LS-1 marker sheath. The decline was believed to be caused by the bronchoscope or insertion sheath critically reducing the area for free ventilation in the endotracheal tube. Bronchoscopy in humans is normally done without anaesthesia, i.e. without insertion an endotracheal tube. Administration of oxygen during this new procedure would seem to be a sensible precautionary measure to take anyhow. Insertion high in the lung proved difficult in this study, particularly when curvature of the bronchi was large. Large curvature gave large resistance because the LS-1 marker was pushed against the bronchial wall,

Table 4. Local tissue changes at the LS-1 marker insertion site from pathological examination of excised lung from the fourteen animals in the study. The column CT represents percentage of marked hypo dense volume around stent determined on CT scan. Visual are macroscopic volume from pathology specimen. Both volumes are in percent of total lung capacity volume determined from CT. Epithelial changes are microscopic local ulceration, granulomatosis and fibrosis at the insertion site. Correspondingly adjacent tissue is lung parenchyma adjacent to insertion site. Obliteration of Bronchi lumen are N: None L: partial or light obliteration O: total obliteration at the insertion site. Distal emphysema, atelectasis and alveolar edema are changes in tissue along the inserted bronchi distally from the insertion site.

Ear Tag	CT	Visual	Epithelial changes	Adjacent tissue	Bronchi lumen	Distal emphysema	Distal atlektasis	Distal Alveolar edema
75585	0%		Y	N	N	N	Y	N
75584	0%		Y	Y	N	Y	N	N
99451								
75219	10%		Y	Y	L	Y	Y	N
75218	0%		Y	Y	L	Y	Y	Y
75241	2%	1%	Y	Y	O	Y	Y	N
75506	0%		Y	Y	O	Y	Y	Y
99516	4%		Y	N	N	N	Y	N
99160	3%	1%	Y	Y	N	Y	Y	N
75546	0%		Y	Y	O	Y	Y	Y
75601	2%	2%	Y	Y	O	Y	N	N
98593	1%	1%	Y	Y	N	Y	Y	Y
75600	0%		Y	Y	O	N	Y	N
75636	5%	2%	Y	Y	L	Y	N	Y

or caused the LS-1 marker to get stuck at the crest between bronchi branches. Consequently, most insertions were either low or mid in lung. Only three LS-1 markers were inserted high in the lung in an accessory branch, special to the mini-pigs, exiting in trachea above carina. Consequently changes to the current method are necessary, if large curvatures in the bronchial tree are to be overcome. After insertion of bronchoscope, the guide wire was pushed ahead in order to reach bronchi with a diameter of 3 - 4mm. So the guide wire had to be advanced while the bronchoscope was being retracted. This was done using fluoroscopy. Generally, it was difficult to retain position of the guide wire, which was not fixed. Often the guide wire was advanced far beyond the intended LS-1 marker position. This was believed to be the reason for the two observed cases of pneumothorax in the study. This has to be avoided in future applications, and consequently fixation of the guide wire will be necessary. Insertion of the LS-1 marker did not have any apparent adverse effect on the animals during the four week observation in the study, and on average animals gained weight. One LS-1 marker migrated after the observation period of four weeks. In retrospect the migrated marker was inserted in the bronchus with the largest diameter based on the CT scan. Removal of the LS-1 marker after the observation period was only partly successful. The extraction tool method worked in removing 7 of the 13 LS-1 markers *in situ* after the observation period. The reason was difficulties with threading the extraction tool through the LS-1 marker lumen, when the LS-1 marker was positioned at a large angle to the bronchoscope. This was a consequence of limited manoeuvrability of the tip of the bronchoscope in the narrow lumen of the bronchus. Keeping in mind that only few LS-1 markers were placed high in the lung, the problem with the removing tool may have been underestimated. Using the Rat Tooth Forceps the success was also limited as only four out of six markers were removed. It was also clear, that the removal method using the Rat Tooth Forceps produced more damage to the bronchial mucosa as seen by inspection with the bronchoscope after removal. The reason for this was suspected to be insufficient cooling of the LS-1 marker, which can then only be removed by force, or not at all. The long term effect of the LS-1 marker upon the lung tissue seems to be confined to a small distance (<10-20 mm) from the insertion site. It seems that the average affected volume in the inserted lung was 4% (range 0-19%) measured from the CT scan prior to removal of the LS-1 (table IV). The effect on the total lung capacity would then be around 2 %. The pathological examination was hampered because of the diffuse interstitial infiltration in the lung, probably caused by the virus infection in all animals. The pathological examination demonstrated changes in the bronchi mucosa that were most likely inflicted by thermal and or mechanical trauma during insertion or removal. Emphysema and atelectasis in the lung parenchyma adjacent to the LS-1 insertion site are most likely caused by obstruction of free airflow in parts of the lung ventilated through the bronchi with the inserted LS-1. It may be suspected that a shorter version of the LS-1 marker could reduce this type of damage. But as the LS-1 will be inserted close to or inside a lung tumor, this may be less relevant. Measuring dimensions of macroscopic changes in the pathology specimens was subjected to the large uncertainties due the possible viral infection. In the few cases where it was possible to measure the dimensions, the average affected volume was 2% of volume in the LS-1 inserted lung (table IV). The lower values of lesion dimensions in the pathology specimens compared to CT were expected, as the lungs are inflated in the later case. In conclusion it seems that the new prototype LS-1 marker is a promising new fiducial lung marker that may be used for gated radiotherapy of the lung. The damage inflicted on the lung tissue from insertion and removal of this marker seems to be limited and local. The procedures developed in this study needs to be refined before human insertion.

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