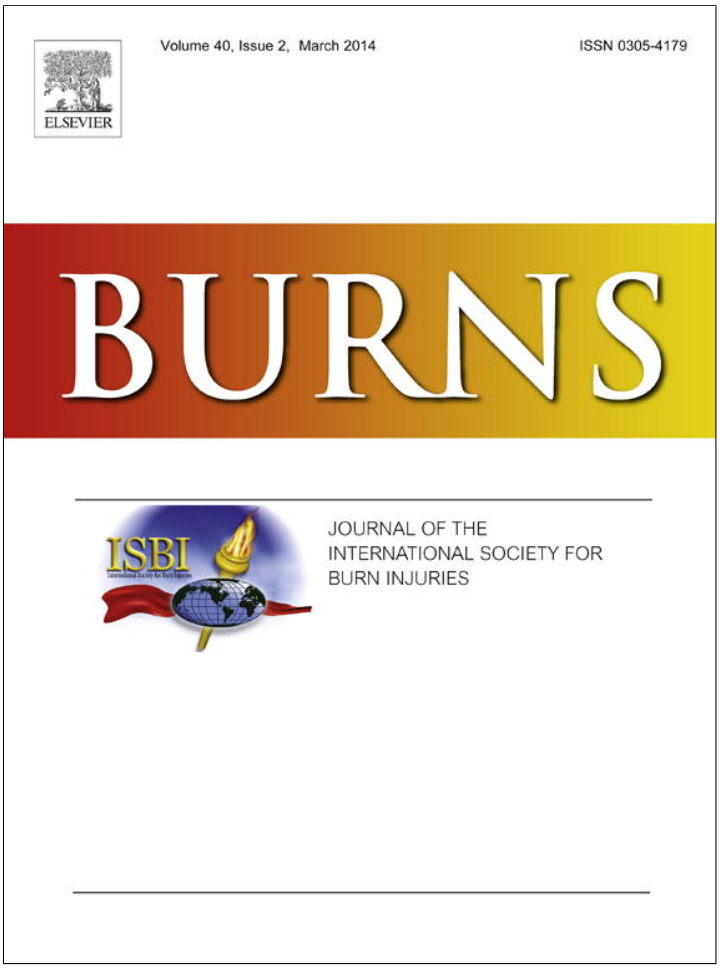

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Pathological changes of the three clinical types of laryngeal burns based on a canine model

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ABSTRACT

Objective: The study was designed to examine pathological changes of inhalational laryngeal burns of three clinical types: congestive, oedematous and obstructive.

Methods: A total of 18 healthy, male, adult Beagle dogs were randomly assigned to inhale hot dry air at room temperature (group C), 80 °C (Group 1), 160 °C (Group 2) or 320 °C (Group 3) for 20 min to induce inhalation injury. Each larynx was evaluated and scored based on the 'clinical scoring and typing system of laryngeal burns at early stage'. Tissue samples of the epiglottis, laryngeal vestibule, vocal folds and infraglottic cavity of the larynx were observed microscopically and evaluated based on a 'pathological scoring system'.

Results: Pathological changes of the larynxes of groups 1 and 2 were primarily characterised by slight atrophy of the mucosa and mild oedema of the submucosal tissues. Group 3 larynxes showed two distinct pathological changes: oedematous and atrophic types. The larynxes of the atrophic type showed lower clinical scores (29.5 ± 0.7 vs. 44.3 ± 2.1) but higher pathological scores (18.6 ± 3.2 vs. 13.7 ± 1.8) than the larynxes of the oedematous type.

Conclusion: Severe laryngeal burns could manifest as severe laryngeal oedema or atrophic change. The laryngeal burns of the atrophic type might suggest an unsatisfactory prognosis, although it had less risk of laryngeal obstruction at an early stage.

1. Introduction

Inhalation injury remains one of the most serious complications of thermal accidents, and it is associated with significant morbidity and mortality. Inhalation injury affects the upper airway, major airway, terminal airway and parenchyma [1]. Due to the special location and structure of the larynx in the upper airway, laryngeal injuries represent a major part of the inhalation injury. For decades, most researchers have placed their emphasis on lower-airway injury related to the inhalation of products from incomplete combustion [1–4]. Much less research has been dedicated to understanding the response to upper-airway burns, especially laryngopharyngeal burns [5].

As an irregular tubular structure, the larynx is the narrowest part of the upper air tract. Stimulated by hot air, the laryngeal tissue might undergo oedematous change of various degrees in a very short time [6]. Mild laryngeal burns cause laryngeal congestion only, whereas moderate-to-severe thermal injury may cause respiratory obstruction that can be

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Table 1 – The clinical scoring system of laryngeal burns at early stage.

Items	Scores			
	0	2	4	6
Symptom	No discomfort	Pharyngalgia	Jam feeling, bucking	Choking
Tone	Normal	Abnormal	Hoarse	Laryngeal stridor
Mucosal colour	Pink	Congestive	Pink-white	Pale
Mucosal appearance	Smooth	Oedema	Small blisters only	Huge blister exceeding glottis
Secretion	Thin	Enhanced	Sticky but clean	Mixed with charcoal powder
Epiglottis	Normal	Move slower	Difficult in lifting	Rigid or ball-like
Angle between vocal cord	>30°	20–30°	10–20°	<10° or hole like

If graded at "<12 h" after injury, an extra "1 score" should be given to each item.

life threatening [7]. The use of fibre-optic bronchoscopy for diagnosing diseases of the respiratory tract is well described and considered a valuable tool for evaluating laryngeal burns [4,8–10]. In 1999, based on the continuous laryngoscopic observations of patients with laryngeal burns in our department, a 'clinical scoring and typing system of laryngeal burns at early stage' was drafted, to classify the laryngeal burns into three types: congestive (slight), oedematous (moderate) and obstructive (severe) [11].

This scoring and typing system (Tables 1 and 2) could make it more efficient and less subjective in evaluating an early-stage patient of laryngeal burns and making medical decisions [12]. However, pathological examinations and analyses about those types of laryngeal burns have never been done hereto fore. Therefore, the current study was designed to investigate the histological morphology and pathological changes of the three clinical types of laryngeal burns, based on a well-established canine model of inhalational thermal injury [13].

2. Materials and methods

2.1. Materials

This study used 18 healthy, male, adult, Beagle dogs weighing approximately 10 kg. The animals were provided by the Animal Lab of Peking University First Hospital (Beijing, China). Dogs with trachyphonia or neck trauma were excluded.

Instruments included a custom-made electrical heating device (Fig. 1), an embedding machine (Sakura Medical, Japan), an ultrathin semiautomatic microtome (R-2, Sakura Medical, Japan), an automatic staining machine (DRS-2000, Sakura Medical Group, Japan), a timer, a divider, a measure gauge and an animal immobilisation device. The electrical heating device consisted of a ceramic tube (4.5 × 3.5 × 30 cm) surrounded with electrical thermal wire (300 W), interfaced to a digital temperature control device (220 V, 20 W, PD90-2, Zhejiang, China).

Table 2 – The clinical typing system of laryngeal burns at early stage.

Total score	Classification	Treatment
<7	Normal	Observe.
8–20	Congestive type	Observe, avoid local irritation.
21–30	Edematous type	Observe closely, prevent laryngeal occlusion.
31–49	Obstructive type	Perform preventive tracheotomy.

2.2. Animal grouping and preparations

All experiments were reviewed and approved by the Animal Ethics Committee of Peking University Health Science Centre and met China's regulations and rules on animal experiments. A total of 18 dogs were randomly divided into four groups: a control group (Group C), with three dogs (Dog A–C) and three experiment groups (groups 1, 2 and 3), with five dogs (Dog A–E) in each group. The dogs were anaesthetised with 3% pentobarbital sodium (25 mg kg^{-1}), at an average intravenous dose of 375 mg at 2 ml min^{-1} via the radial cutaneous vein of the forelimb. A piece of fentanyl transdermal patch (size: 2.60 cm^2; content of fentanyl: 1.0 mg $patch^{-1}$; release rate of fentanyl: 6 μg h^{-1}; and time for analgesia: 72 h) was pasted at the dog's nape for analgesia after skin preparation. The dogs were immobilised in the supine position.

2.3. Experimental procedures

The room temperature was maintained at 26 ± 2 °C; the air humidity was kept at 40 ± 2%. Two rubber stoppers were used to close the nostrils, and the tongue was extended to avoid glossoptosis. A ceramic tube was placed in the mouth between the jaws and parallel with the airway, at a distance of 10 cm from the epiglottis. The inflexible appended thermal probe of the temperature control device was fixed in the oral cavity, 5 cm away from the epiglottis, without tissue contact (Fig. 1). The time for heated air inhalation was 20 min.

The temperature of the heated air was controlled at room temperature (26 °C), 80 °C, 160 °C and 320 °C by the temperature control device for the groups C, 1, 2 and 3, respectively. Heated air was inhaled into the dogs' airway by their spontaneous inspiratory efforts. The temperature of the heated air varied within a range of ± 5 °C.

2.4. Data collection and sample observation

The dogs were allowed to wake up after injury induction, and the recovery times from anaesthesia were recorded. After revival, their basic living conditions (eating and drinking and body movements) were evaluated by two breeders together. Their respiratory rate and breathing sounds were checked at an interval of 4 h. At 24 h after injury, after anaesthesia, all the

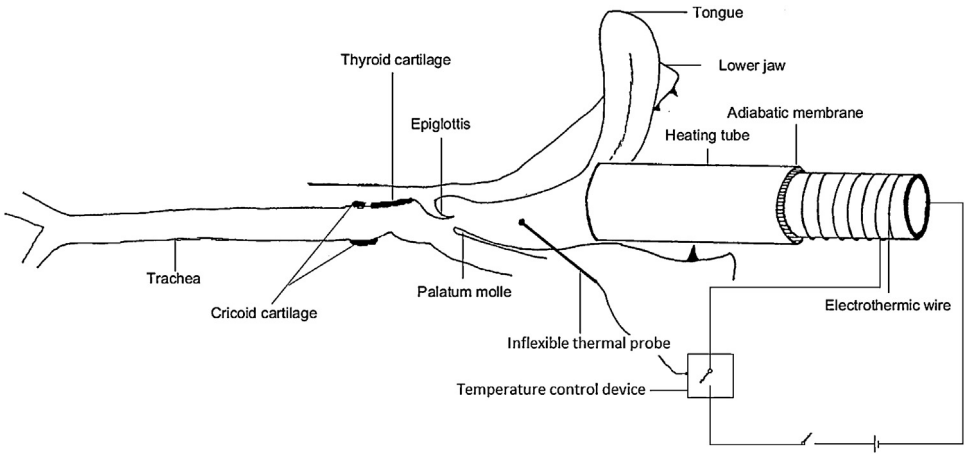

Fig. 1 – The electrical air-heating device.

dogs underwent fibrolaryngoscopy to evaluate and score the severity of their laryngeal burns (Tables 1 and 2), and then they were euthanised by an overdose of 3% pentobarbital sodium (75 mg kg^{-1}).

After cessation of heart beat, the dogs' larynxes were collected and preserved. Parts of the epiglottis, laryngeal vestibule, vocal folds and infraglottic cavity were treated separately and stained with haematoxylin and eosin (HE). The histological changes of all the slices were observed microscopically and scored (Table 3) by two pathologists independently, according to the pathological grading system of inhalational burns developed by the authors, which has been introduced and reported before [13].

2.5. *Statistical analysis*

Data are expressed as mean ± standard deviation (SD). Statistical analysis was performed with SPSS 17.0 statistics software. The differences between groups were analysed by one-factor analysis of variance (ANOVA). Probability values < 0.05 were considered significant.

3. Results

3.1. *Monitoring of vital signs (Table 3)*

Three of the 18 dogs did not survive to 24 h after injury; they expired at 20 min (3-A), 8 h (3-B) and 12 h (3-C) after injury, respectively, and the laryngoscopic examinations and sample collections were performed immediately after their deaths.

For all the dogs, their recovery time from anaesthesia ranged from 2.9 to 5.5 h, with one dog (3-A) dying before recovery. After revival, animals of group C (3/3, A–C), group 1 (4/5, A, C–E) and group 2 (1/5, C) resumed a normal diet within 24 h, while none of the dogs of group 3 were able to eat or drink normally. As to the body movement, compared to the control animals (+++++), dogs of group 1 showed full ability of body

Table 3 – Pathological grading system of airway tissue after inhalational thermal injury.

Pathological changes	Scores			
	0	1	2	3
Mucosa				
Cilia exfoliation[a]	None	≤1/3	1/3–2/3	≥2/3
Epithelial cells	Normal	Swelling	Polarity disappeared	Disorganised
Nuclear apoptosis[b]	None	≤1	2–4	≥5
Nuclear necrosis[b]	None	≤1	2–4	≥5
Submucosa				
Neutrophils infiltration[b]	None	≤20	21–49	≥50
Gland cells	Normal	Swelling	Atrophy	Necrosis
Erythrocyte exudation[b]	None	≤20	21–49	≥50
Cartilage or muscle				
Chondrocyte degeneration[b]	None	≤1	2–4	≥5
Muscular cell	Normal	Swelling	Nuclear apoptosis	Fibre degeneration

Total score (TS) range: 0–27. TS < 9, slight injury; 9 < TS < 18, moderate injury; TS > 18, severe injury.
[a] Length proportion of exfoliated cilia, in 200× field.
[b] Number of cells per high-power field (400×).

Table 4 – Observational data of canines after injury.

Group	Death time (h)	Recovery time (h)	Eating and drinking[a]	Movement[b]	Respiratory rate (/min)	Clinical scores
C						
A–C	24	3.0 ± 0.2	3/3	+++++	18 ± 5	2.3 ± 0.6
1						
A–E	24	3.2 ± 0.5	4/5	++++	17 ± 6	15.2 ± 3.5[c]
2						
A–E	24	5.2 ± 0.4	1/5	++	39 ± 12[c]	27.2 ± 4.2[c]
3			0/5		42 ± 19[c]	38.4 ± 8.3[c]
A	0.3	–	–	–	52	45
B	24	4.5	–	+	36 ± 7	30
C	8	4.3	–	+	49 ± 10	46
D	12	4.0	–	–	22 ± 8	42
E	24	4.6	–	+	41 ± 6	29

[a] The number of "A/B" indicates the proportions of the dogs in each group which resumed normal diet within 24 h after injury. A: the number of the dogs which resumed normal diet within 24 h after injury in each group; B: the total number of the dogs in each group.
[b] The number of "+" is proportional to the amount of activities that animal did before death. The "+++++" means animal did the same amount of movements as usual, and the "–" means animal had no movements at all.
[c] Indicates the significant difference between the labelled item and the control group ($p < 0.05$).

movement but less active exercises (++++). However, group 2 animals were only capable of standing up and walking around slowly (++), whereas the animals of group 3 could only lie still and had difficulty standing (+/–). After injury, recorded at an interval of 4 h, the respiratory rates of animals in groups 2 and 3 were significantly higher than that of group C ($p_2 = 0.012$, $p_3 = 0.007$), while no significant difference between group C and group 1 was found ($p_1 = 0.423$).

The post-injury breathing sounds of group 1 dogs were basically the same as those of group C. Only a slight wheeze could be heard in some animals of group 2; however, inspiratory wheeze, inspiratory laryngeal stridor and sighing breath were noted in dogs of group 3. Laryngoscopic findings also differed between groups. In group 1, no distinct changes but more mucous secretion and congestive mucosa were observed, while in group 2 larynxes, a swollen epiglottis, blisters and oedematous laryngeal ventricle were found. As to the five dogs of group 3, their laryngocopic appearances were inconsistent. In the three early-dead dogs (3-A, C and D), severe swollen epiglottis, huge blister and laryngeal stenosis or occlusion were clearly observed, while in the other two dogs (3-B and E), the laryngeal passage was rather broad, and punctate haemorrhages were scattered in paler mucosa. Based on Table 1, the clinical scores of the dogs' laryngeal burns are listed in Table 4, which significantly differed between groups ($F = 35.535$, $df = 3$, $p = 0.000$). Except for the groups 2 and 3 ($p_{2-3} = 0.198$), differences between any other two groups were significant ($p_{0-1} = 0.001$, $p_{0-2} = 0.001$, $p_{0-3} = 0.003$, $p_{1-2} = 0.008$, $p_{1-3} = 0.013$).

3.2. *Pathological observations*

Compared to the control group, tissues of the experimental groups mainly showed oedematous changes, companied by atrophic degenerations in epithelial cells. However, in two dogs of group 3 (3-B and 3-E), histologic appearances were characterised by charring in the epithelium and atrophy in the submucosa, which were typically different from the severe oedematous manifestations of the other three dogs (3-A, 3-C and 3-D). Therefore, they were distinguished as the oedematous type and atrophic type, respectively.

3.2.1. *Epiglottis (Fig. 2)*

Compared to the control group, the epithelium of the epiglottis of groups 1 and 2 and the oedematous type larynxes of group 3 appeared slightly to substantially atrophic, while the lamina propria, structures of the submucosa and deeper cartilage showed mild to severe oedema and some mucoid degenerations were observed. Meanwhile, the atrophic type larynxes of group 3 showed epithelial disarrangement and atrophic changes in the submucosa and cartilage.

3.2.2. *Laryngeal vestibule (Fig. 3)*

Compared to the control group, tissues of groups 1 and 2 and the oedematous type larynx of group 3 demonstrated slightly thinned epithelium, mucosal atrophy and cilia exfoliation. Further, in the submucosa, vascular thrombosis, neutrophil infiltration, erythrocyte exudation and swollen gland cells were observed. However, in the atrophic type of group 3, the mucosa was found to have epithelial degeneration in a large area and basophilic hyaline materials and atrophic glands were seen in the submucosa.

3.2.3. *Vocal folds (Fig. 4)*

Compared to the control group, the group 1 tissue showed normal morphology. In the tissues of group 2 and the oedematous type larynx of group 3, stratified squamous epithelium of varying thickness, pronounced neutrophil infiltration, increased vascular congestion and thrombosis, oedematous gland cells and muscular cells and liquefaction necrosis of fat cells were observed. While in the atrophic type of group 3, nuclear necrosis of epithelial cells, atrophic change of the lamina propria, basophilic hyaline material formation in submucosa, vascular occlusion and erythrocyte leakage, degenerated muscle fibres and mucous glands and atrophic degeneration of cartilage cells adjacent to the mucosa were seen.

3.2.4. *The infraglottic cavity (Fig. 5)*

Compared to the control group, the group 1 tissues showed normal morphology. In the tissues of group 2 and the oedematous type larynx of group 3, cilia exfoliation and epithelial deformation, vascular congestion and distortion,

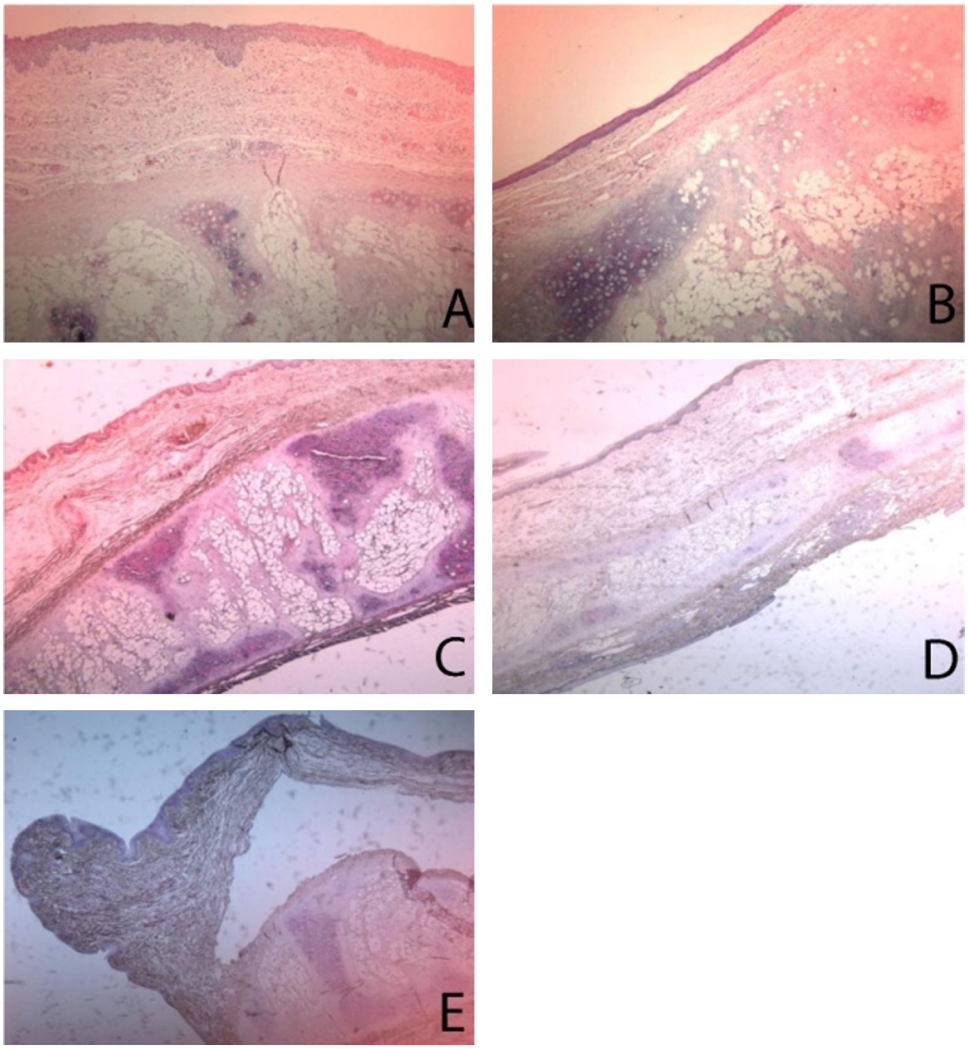

Fig. 2 – Histopathological examination of the lingual surface of epiglottis (HE, ×100). A. Group C, normal tissue. B. Group 1, thinner stratified squamous epithelium, marked atrophy in the upper one third of the epithelial cells, and mild oedema in the lamina propria. C. Group 2, thinned mucosa, vascular congestion and thrombosis, and tissue oedema in perichondrium and submucosa. D. Group 3 (edematous type), apoptosis and necrosis of the epithelial cells, cilia exfoliation, submucosal vascular thrombosis and edematous gland cells. E. Group 3 (atrophic type), distorted arrangements of epithelial cells, poorly arranged elastic fibres, separation of submucosa and perichondrium, and atrophic degeneration of cartilage cells.

erythrocyte leakage and neutrophil infiltration, oedema and degeneration of gland cells were found. While in the atrophic type of group 3, hyaline changes of cells of the mucosa, sporadically distributed atrophic, deeply stained nuclei, indistinguishable structures of the submucosa and basophilic hyaline material formation, atrophic necrosis of gland cells and vacuolar degeneration of cartilage were observed.

3.2.5. *Pathological scores of tissues (Table 5)*

According to the pathological grading system in Table 3, the tissues' pathological scores ranged from 0.0 to 24.0, indicating slight to severe injuries. Generally, for each dog, the thermal injuries at the epiglottis and vocal cords were more severe than at the vestibular folds and infraglottic cavity (Fig. 6). Further, the mean scores of the four parts significantly differed between groups ($F = 47.712$, $df = 3$, $p = 0.000$), and significant differences were found between each two groups ($p_{0-1} = 0.025$, $p_{0-2} = 0.000$, $p_{0-3} = 0.000$, $p_{1-2} = 0.004$, $p_{1-3} = 0.00$, $p_{2-3} = 0.000$).

3.2.6. *Relationship between clinical scores and pathological scores*

The clinical scores and mean pathological scores of the larynxes in groups C, 1 and 2, and each larynx of group 3 are listed in Fig. 7. It was interesting that the two atrophic larynxes (3, B and E) got lower clinical scores but higher pathological scores than the oedematous larynxes (3, A, C and D) in group 3.

4. Discussion

Laryngeal burns are commonly encountered in clinic al situations [14]. Laryngeal obstruction is the most serious

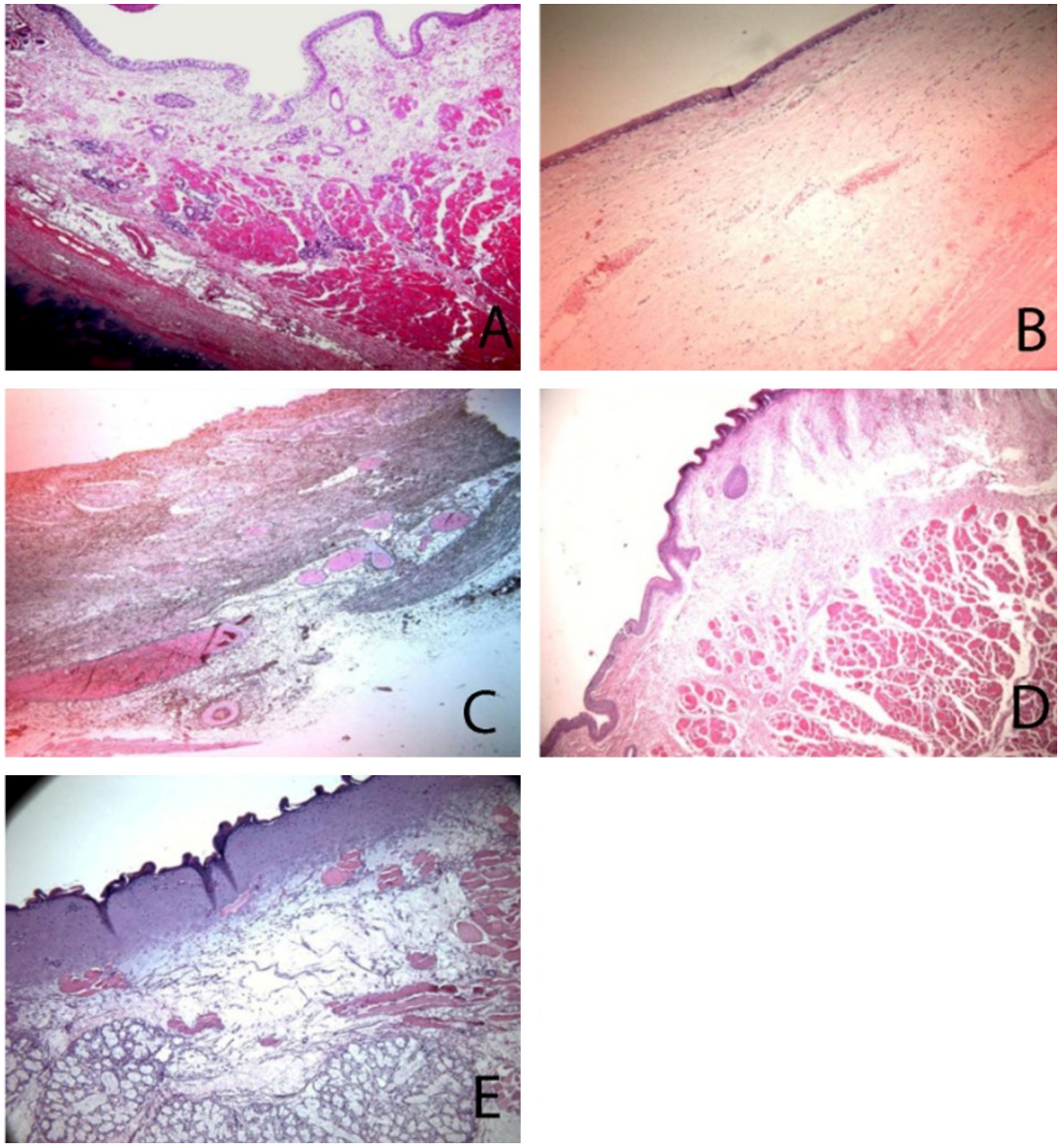

Fig. 3 – Histopathological examination of vestibular folds (HE, ×100). A. Group C, normal tissue. B. Group 1, slightly thinned epithelium, narrowed intercellular space, cilia exfoliation and less goblet cells, and mild vascular congestion. C. Group 2, notable mucosal atrophy, cilia exfoliation, vascular thrombosis, neutrophil infiltration, erythrocyte exudation, atrophy and breakage of collagen fibres, and swollen gland cells. D. Group 3 (edematous type), severe epithelial degeneration and cilia exfoliation, severe submucosal oedema and erythrocyte exudation, and swollen gland cells. E. Group 3 (atrophic type), notable nuclear degeneration and distorted organisation of epithelial cells, patchy basophilic hyaline material at submucosa, vascular leakage, atrophic gland cell and vascular cells.

complication at the early stage (<72 h) of laryngeal burns. Based on clinical experiences, most laryngeal obstructions occurred at 12–36 h after injury [6,12]. Since laryngeal oedema is a dynamic process, it is important for providers to evaluate and predict the risk of laryngeal obstruction in <12 h after injury. In 1999, based on continuous laryngoscopic observation, the authors first presented 'the clinical scoring and typing system of laryngeal burns at early stage' to classify the laryngeal burns into the three types, congestive, oedematous and obstructive, and meanwhile the corresponding suggested clinical strategies were suggested (Tables 1 and 2) [11], which have been well accepted and used by most doctors of burns in China. However, in clinic, some cases of laryngeal burns underwent a longer-than-expected healing time and chronic progressive laryngeal stenosis or occlusion, which were evaluated as the slight type at first. Similar conditions were reported by other physicians [15,16]. Therefore, in the present study, animal models to represent the three clinical types of laryngeal burns were established and their pathological changes were explored. By comparing the pathological scores

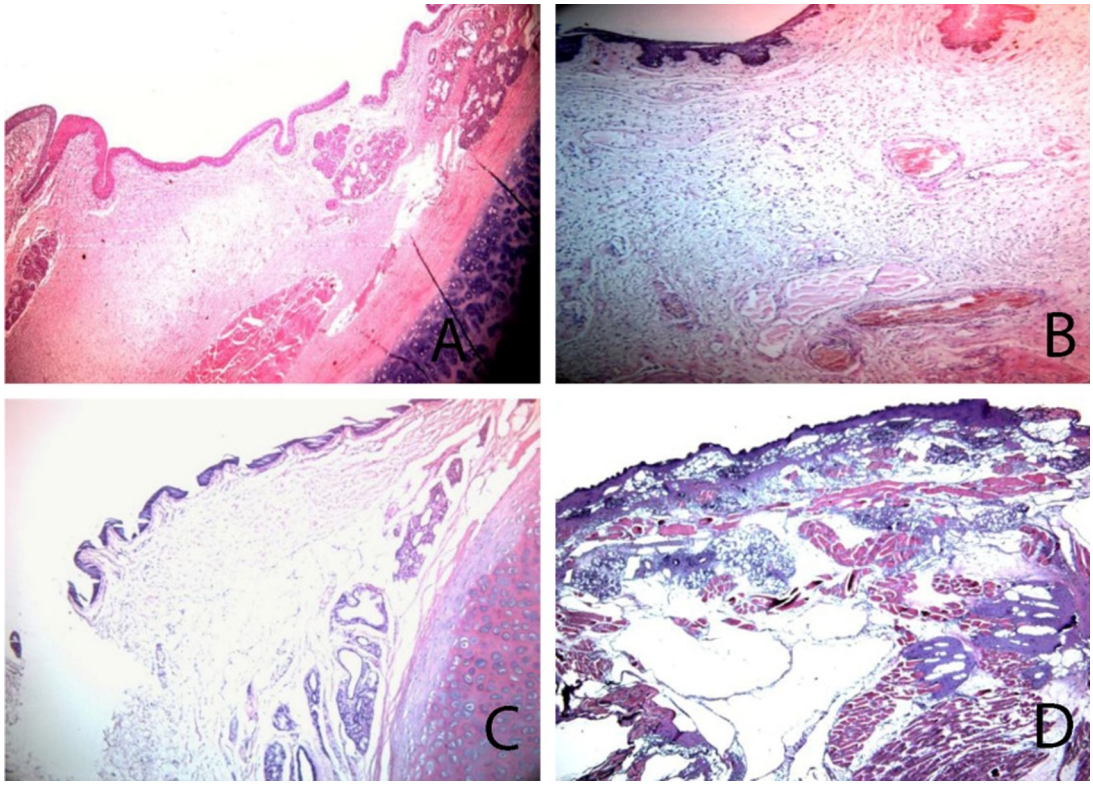

Fig. 4 – Histopathological examination of vocal folds (HE, ×100). A. Group C and group 1, normal tissue. B. Group 2, stratified squamous epithelium of varying thickness, pronounced neutrophil infiltration, increased vascular congestion and thrombosis. C. Group 3 (edematous type), marked thinned stratified squamous epithelium, edematous gland cells and muscular cells, and liquefaction necrosis of fat cells. D. Group 3 (atrophic type), distorted epithelium, nuclear necrosis of epithelial cells, atrophic change of lamina propria, basophilic hyaline material formation in submucosa, vascular occlusion and erythrocyte leakage, degenerated muscle fibres and mucus glands, and atrophic degeneration of cartilage cells adjacent to the mucosa.

and clinical scores of larynxes experimented on, it was realised that a certain part of the severe laryngeal burns might appear as the atrophic type, which has a low risk of laryngeal obstruction in the early stage, but suggests more trouble in the long term.

4.1. *Animal models of different types of laryngeal burns*

To establish the proper animal models of the three clinical types of laryngeal burns, the authors carried out many investigations and improvements [13,17,18] The dog was chosen as our experiment animal because of three reasons: (1) there is much space between the upper and lower jaws of dogs, which is necessary for us to locate the air-heating tube, (2) the dog's larynx has almost the same size and anatomical structures as humans' [19–21], so it is easier to perform laryngoscopy and (3) Beagle dogs are lighter and smaller than goats or swine, which means easier control and less anaesthesia drugs for us.

However, there are some distinct differences between canines and humans in respiratory physiology and functions [22]. First, in stimulation of heated air, dogs' respiratory rate could increase dramatically (as much as 300/min) [23] and meanwhile the tidal volume decreases markedly [24], which could impair our results. Second, the nasal and oral cavity (especially the tongue) of dogs have large potential in heat dissipation, which is absent in human. In the present study, the respiratory rate was controlled at 15–25/min by adjusting the dose rate of the narcotic drug during the experiment, to ensure that enough volume of heated air was inhaled in each breathing cycle. Besides, excluding the influence of heat dissipation from nasal and oral cavity was tried by locating the air-heating tube in front of the larynx directly.

After injury, the dogs were allowed to wake up with a fentanyl transdermal patch for analgesia, and their living conditions, breathing sounds and respiratory symptoms were observed closely and recorded. Generally, in the experimental groups of more severe injury, dogs were less capable of resuming their normal activities and showed more respiratory symptoms and signs, which was in accordance with the conditions of patients with inhalational injuries.

4.2. *Clinical scoring system of laryngeal burns at early stage*

The 'clinical scoring of laryngeal burns at early stage' (Table 1) was determined by four grades (scores 0, 2, 4 and 8) of seven items, based on the respiratory symptoms, signs and

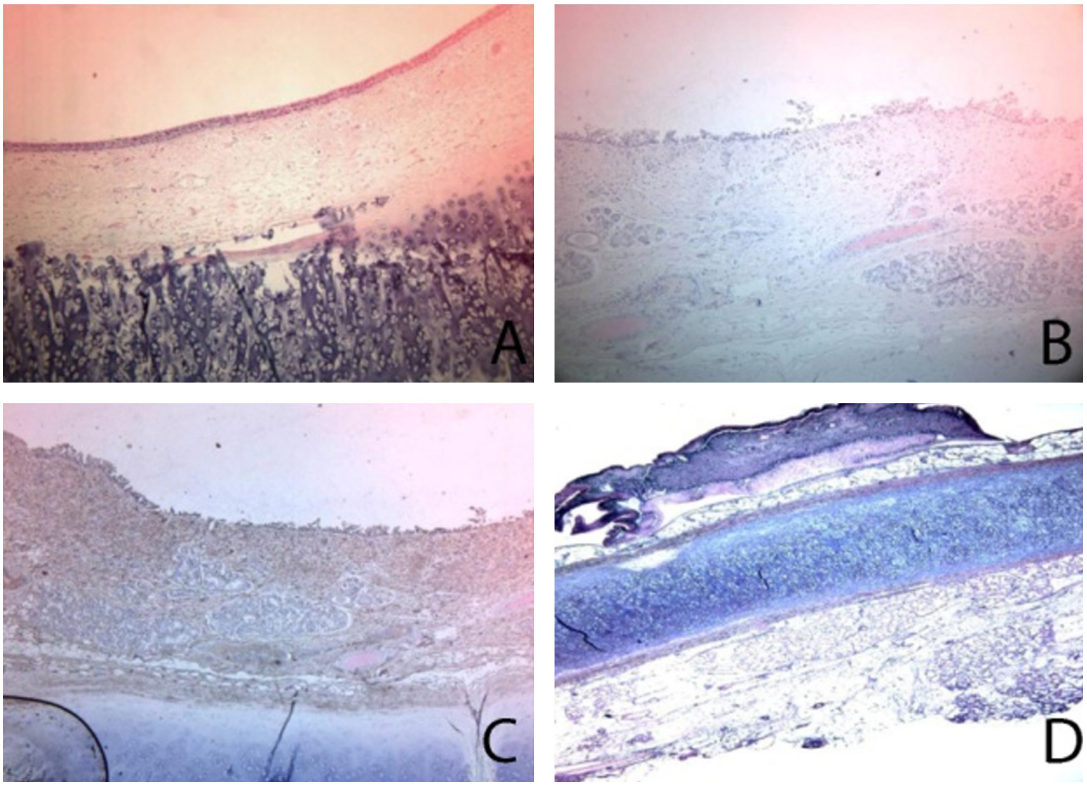

Fig. 5 – Histopathological examination of the infraglottic cavity (HE, ×100). A. Group C and group 1, normal tissue. B. Group 2, cilia exfoliation, nuclear apoptosis of epithelial cells, erythrocyte leakage and neutrophil infiltration in submucosa, enhanced secretion of gland cells. C. Group 3 (edematous type), cilia exfoliation and epithelial deformation, vascular congestion and distortion, erythrocyte leakage and neutrophi infiltration, oedema and degeneration of gland cells. D. Group 3 (atrophic type), hyaline changes of cells of mucosa, sporadic distributed atrophic, deeply stained nuclei, indistinguishable structures of submucosa, atrophic necrosis of gland cells, and vacuolar degeneration of cartilage.

laryngoscopic findings. Since the laryngeal oedema was a progressive process, an extra 1 score should be added for each item if the evaluation was performed within 12 h after injury. The total score range was 0–49, and the demarcation points (Table 2) between the different severity levels were determined by experience and repeated fixations [11]. Until 2012, this clinical scoring and typing method had been used in our institution for more than 15 years, which was efficient and convenient for the clinicians to judge the risk of laryngeal obstructions at early stage after inhalational injury and decide whether preventive tracheotomy was necessary. In this way, the emergency tracheotomy rate could be controlled at the minimum level, which was much more complicated and dangerous than the preventive tracheotomy [25,26], because the tissue oedema in the neck could have been very serious by then. In 2002, after using this scoring and typing system of laryngeal burns for 3 years, the therapeutic information of 164 patients of laryngeal burns was organised and it was found that the early-stage mortality of patients with laryngeal burns reduced from 8.3% (3/36) to 3.7% (6/164), and the complication rate of tracheotomy reduced from 27.3% (3/11) to 7.9% (3/38) [12]. However, during the past 10 years (2002–2012), urgent laryngeal obstruction and emergency tracheotomy were rarely seen in our department.

4.3. *Histological morphology of different types of laryngeal burns*

4.3.1. *Pathological grading system*

The pathological grading system (Table 3) was drafted by the experienced pathologists in our hospital and reported by us in an earlier article [13]. It is a semi-quantitative grading system,

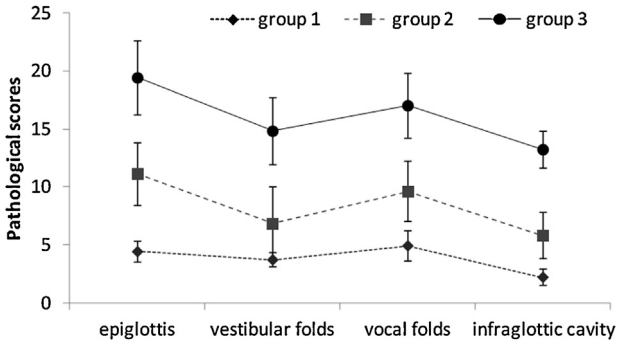

Fig. 6 – Mean pathological scores of the four parts of larynx of the three experimental groups.

Table 5 – Pathological scores of tissues.

Group	Epiglottis	Vestibular folds	Vocal folds	Infraglottic cavity	Mean scores
C					
A–C	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
1					
A–E	4.4 ± 0.9	3.7 ± 0.6	4.9 ± 1.3	2.2 ± 0.7	3.8 ± 1.9[a]
2					
A–E	11.1 ± 2.7	6.8 ± 3.2	9.6 ± 2.6	5.8 ± 2.0	8.3 ± 2.9[a]
3	19.4 ± 3.2	14.8 ± 2.9	17.0 ± 2.8	13.2 ± 1.6	16.1 ± 3.5[a]
A	16	13	14	12	13.8 ± 1.7
B	24	17	20	14	18.8 ± 4.3
C	17	11	15	11	13.5 ± 3.0
D	16	12	14	13	13.7 ± 1.7
E	21	18	20	15	18.5 ± 2.6

[a] Indicates the significant difference between the labelled item and the control group ($p < 0.05$).

in which nine kinds of typical findings at three layers, including cilia exfoliation and epithelial cell degeneration in the mucosal layer, glands and neutrophil infiltration at the submucosa and abnormal cartilage or muscular cells, were graded into four ranks with scores of 0–3 for increasing severity. The total score ranged from 0 to 27, in which score 0 meant normal tissue and scores of 1–9, 10–18 and 19–27 indicated slight, moderate and severe injury, respectively. This grading system was designed especially for the inhalational thermal injury, in order to better report and evaluate the pathological injury severity, instead of clinical diagnosis or classification.

4.3.2. *Difference of the thermal injury severity between groups and places*

In an earlier article [13], we reported the pathological changes of the airway tissues of the epiglottis, cricoid cartilage, trachea bifurcation and terminal bronchioles based on the same animal model. In that study, it was found that the pathological scores of thermal injury decreased along with the increase of the distance from the airway entrance. For example, the epiglottis was injured much more seriously than the other three parts, and the terminal bronchi were basically normal in histology. However, in this study, according to Fig. 6, both the epiglottis and the vocal cords were burnt more severely than the vestibule folds and infraglottic cavity, which seemed different from the trends of our former study. A possible explanation might be the larynx's anatomical internal structure. Being an irregular tubular structure, the most important and narrowest part of the larynx is the glottis, which is the opening between the vocal cords. Therefore, the heated air might encounter more resistance and stay longer at the location of the vocal cords and result in more severe thermal injury.

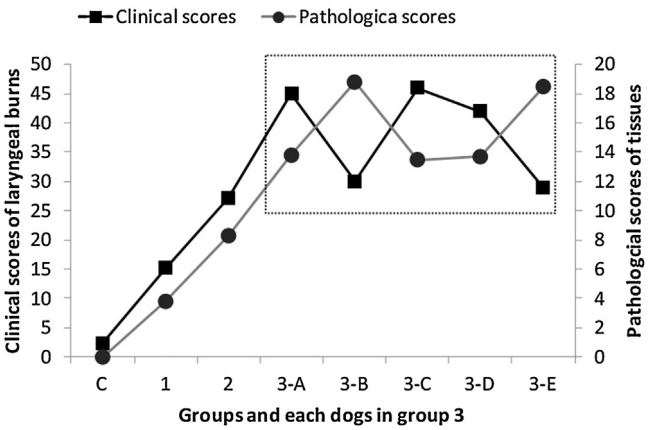

Fig. 7 – Clinical scores and pathological scores of group C, 1 and 2, and each larynxes of group 3. The box indicated the discrepancy between of the clinical scores and pathological scores of larynxes of edematous type and atrophic type of group 3.

4.4. *Discrepancy between the clinical scores and the pathological scores of laryngeal burns*

Through microscopy, the histological appearances of tissues of group 3 gave two distinct impressions: oedematous and atrophic types. According to Fig. 7, the discrepancy between the clinical scores and the pathological scores of the larynxes of group 3 can be observed. It was thought provoking that the two atrophic larynxes (3, B and E) got lower clinical scores but higher pathological scores than the oedematous larynxes (3, A, C and D). As the pathologic change of oedema is reversible, while the atrophic degeneration is irreversible, the pathological scores of the larynxes of the atrophic type (18.6 ± 3.2) were higher than those of the oedematous type (13.7 ± 1.8). By contrast, because the clinical scoring system of laryngeal burns was designed to predict the risk of laryngeal obstruction, which always resulted from tissue oedema, the clinical scores of the larynxes of the atrophic type (29.5 ± 0.7) were lower than those of the oedematous type (44.3 ± 2.1). This finding was meaningful, which suggested a limitation of our clinical scoring system of laryngeal burns. Before this study, what was believed and repeatedly told to patients was that the clinical score indicated the injury severity and the risk of laryngeal obstructions. However, until now, more than 10 cases of laryngeal burns in our department have led to humiliation and frustration, because of the discordance between the promising evaluated scores and the unsatisfactory prognosis. Here in this study, we have found the possible explanation, and later in our future work, we will keep in mind

that some extremely severe laryngeal burns could manifest as the atrophic type, which brings a low risk of early laryngeal obstruction but more problems in the healing process or even delayed laryngeal occlusion [15].

4.5. *Experimental limitations*

In the present study, dogs were used to investigate the pathological changes of laryngeal burns, which could be misleading or biased when extended to humans directly. Therefore, we planned to start a prospective study of patients with laryngeal burns to investigate the relationship between the laryngoscopic appearance and the long-term prognosis of laryngeal burns. Besides, the three dogs of the oedematous type larynx in group 3 died early before 12 h; hence, in fact, their laryngoscopic evaluation and pathological scoring were not matched with others over time. The most possible reason for their early death was the laryngeal obstruction caused by extremely laryngeal oedema; however, the influences from the pentobarbital sodium or fentanyl transdermal patch could not be excluded, overdose of either of which could lead to hypoventilation or respiratory muscle paralysis [27] to cause unexpected death. Lastly, since the key findings of this study were from group 3, the sample size of 5 made it difficult and unconvincing to perform statistical analyses between the oedematous and atrophic types of laryngeal burns.

5. Conclusion

Based on the canine models of the three clinical types of laryngeal burns, the pathological changes of four parts of the larynx were examined. Further, based on 'the clinical scoring and typing system of laryngeal burn at early stage' and 'the pathological scoring system of airway after thermal injury', it was found that some extremely severe laryngeal burns might manifest as the atrophic type, which could be more troublesome in the long-term therapy and prognosis, although they indicate a low risk of early laryngeal obstruction.

Conflict of interest

There are no any ethical/legal conflicts involved in the article.

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