

Cite this article as: Neural Regen Res. 2012;7(9):703-707.

Anatomical feasibility of vagus nerve esophageal branch transfer to the phrenic nerve[☆]

Ce Wang¹, Jun Liu², Wen Yuan¹, Xuhui Zhou¹, Xinwei Wang¹, Peng Xu¹, Jian Chen¹, Guoxin Wu¹, Sheng Shi¹

1Department of Orthopedics, Changzheng Hospital, Second Military Medical University, Shanghai 210000, China

2Department of Orthopedics, General Hospital of Shenyang Military Area Command of Chinese PLA, Shenyang 110016, Liaoning Province, China

Abstract

This study measured the vagus and phrenic nerves from 12 adult cadavers. We found that the width and thickness of the vagus and phrenic nerves were different in the chest. The distance from the point of the vagus nerve and phrenic nerve on the plane of the inferior border of portal pulmonary arteries (T point) was approximately 7 cm to the diaphragm and was approximately 10 cm to the clavicle level. The number of motor fibers in the vagus nerves was $1\,716 \pm 362$, and the number of nerve fibers was $4\,473 \pm 653$. The number of motor fibers in the phrenic nerves ranged from $3\,078 \pm 684$ to $4\,794 \pm 638$, and the number of nerve fibers ranged from $3\,437 \pm 642$ to $5\,071 \pm 723$. No significant difference was found in the total number of nerve fibers. The results suggest that width, thickness, and total number of nerve fibers are similar between the vagus and phrenic nerves, but the number of motor fibers is different between them.

Key Words: phrenic nerve; vagus nerve; esophageal plexus; anatomy; nerve transplantation; nerve fiber

Ce Wang[☆], M.D., Attending physician, Department of Orthopedics, Changzheng Hospital, Second Military Medical University, Shanghai 210000, China

Ce Wang and Jun Liu contributed equally to this article.

Corresponding author: Xuhui Zhou, M.D., Associate professor, Department of Orthopedics, Changzheng Hospital, Second Military Medical University, Shanghai 210000, China; Wen Yuan, Professor, Doctoral supervisor, Department of Orthopedics, Changzheng Hospital, Second Military Medical University, Shanghai 210000, China
shanghaizhouxuhui@163.com;
yuanwenspine@163.com

Received: 2011-12-05
Accepted: 2012-02-03
(N20101015001/WLM)

Wang C, Liu J, Yuan W, Zhou XH, Wang XW, Xu P, Chen J, Wu GX, Shi S. Anatomical feasibility of vagus nerve esophageal branch transfer to the phrenic nerve. Neural Regen Res. 2012;7(9):703-707.

www.crter.cn
www.nrronline.org

doi:10.3969/j.issn.1673-5374.2012.09.011

INTRODUCTION

High cervical spinal cord injury (SCI) patients are associated with quadriplegia and loss of respiratory function. Quality of life suffers as a result of decreased mobility and linguistic function. Moreover, the complications of mechanical ventilation such as mechanical obstruction and pulmonary infection are associated with high mortality^[1-7]. Phrenic nerve pacing can free a quadriplegic patient from ventilatory dependency by simulating a natural negative pressure^[4, 8]. It improves the mobility range of patients and allows normal speech. However, phrenic nerve pacing still has the potential risk of nerve injury, undesirable movement, pacemaker failure, infection, complications from surgery, and does not allow optimal physiological control of respiration^[2, 4, 8-10].

Microsurgery to repair brachial plexus injuries has been able to achieve satisfactory improvement in muscle power, movement of joints, and prevention of deformity by anastomosis^[11-12]. This success has led us to embark on nerve transfer as an alternative to restore respiratory function in patients with high cervical SCI.

The diaphragm plays a significant role in eupnoea. It is also the major muscle of respiratory movement. Ventilatory capacity

induced by the contraction of the diaphragm accounts for 75–80% of eupnoea. Auxiliary respiratory muscles such as the intercostal and abdominal muscles cannot sustain normal respiration alone^[6]. Polentes *et al*^[13] evaluated functional respiratory recovery by recording diaphragm and phrenic nerve activity after transplantation of olfactory ensheathing cells following cervical cord hemisection. The ipsilateral phrenic activity in transplanted rats only achieved 57.5% of that of the control rats after elimination of any contralateral influence *via* contralateral acute C₁ section. Gauthier *et al*^[14] studied unilateral recurrent laryngeal phrenic nerve anastomosis for improving respiratory function of rats after high cervical SCI. The authors found that rats could survive without asphyxiation, even after complete C₂ spinal transection 5 months after nerve transfer. Gauthier *et al*^[14-15] and Vinit *et al*^[16] proposed that neuroplasty and remodeling may have taken place to control respiratory function after nerve transfer. Zhou *et al*^[17-19] studied respiratory function following high cervical cord injury after regeneration of the accessory and phrenic nerves. Anatomical, histological, and electrophysiological analysis on the reconstructed nerve and re-innervated diaphragm confirmed that motor function of the diaphragm was present 6 months after nerve transposition. Although these studies showed good

results, long distance regeneration of the nerve may reduce the survival rate of axons, which may greatly affect the re-innervation of the target organ^[20]. Thus, the current study used the esophageal branch of the vagus nerve as the donor nerve to transfer to the phrenic nerve, which we hypothesized, would minimize the distance of regeneration. We also studied the anatomical relationship and compared the content of nerve fibers between the two nerves.

RESULTS

Vagal track and anatomical characteristics of esophageal plexus

The track of the right and the left vagus nerve in the chest is slightly different. After arriving at the lung root, the left one separates into many branches to form the left lung plexus and the anterior esophageal plexus, and the right one forms the right pulmonary plexus and posterior esophageal plexus. We dissected the vagus nerve carefully and found that the beginning of the esophageal plexus is relatively fixed around the hilum of the lung. Before forming the anterior trunk and posterior trunk of the vagus nerve, the branches of the esophageal plexus show a parallel form or a reticulate form. We concluded that if we selected one of them to transfer to the phrenic nerve, little influence on nerve transfer will be caused.

Positional relationship of the vagus and phrenic nerves

We established the positional point of the vagus nerve and phrenic nerve on the clavicle as U; the point of the vagus nerve and phrenic nerve on the plane of the superior border of the heart as M; the point of the vagus nerve and phrenic nerve on the plane of the inferior border of portal pulmonary arteries as T; the middle point of T and the terminal point of the phrenic nerve and vagus nerve on the plane of diaphragmatic muscle as W (T and W were located on the branch of esophageal plexus) (Figure 1).

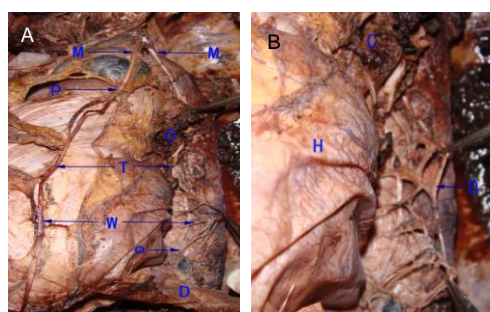


Figure 1 Anatomical features of the vagus nerve and phrenic nerve in the thoracic cavity (A), and the distribution of the esophageal plexus of the vagus nerve (B).

O: Branch of esophageal plexus; P: phrenic nerve; C: hilum of lung; H: pericardium; D: diaphragmatic muscle; M: M point; T: T point; W: W point.

The length of the phrenic nerve in the thoracic cavity on the left side was greater than that on the right side ($P < 0.01$; Table 1). The distance from the T point was about 7 cm to the diaphragm and was about 10 cm to clavicle level (Table 1). The width and thickness of the phrenic and vagus nerves were similar (Table 2), which would be suitable for end-to-end anastomosis. At U point, M point, T point, and W point, the distance between the phrenic nerve and vagus nerve ranged from 1.26 ± 0.43 cm to 5.54 ± 1.57 cm (Table 3), which was relatively fixed between subjects. The phrenic nerve was long enough to allow tensionless transfer to the branch of esophageal plexus.

Table 1 Length (cm) of the phrenic nerve and the distance from the T point to the diaphragm and to the clavicle level

Item	Left	Right
The length of phrenic nerve	18.79 ± 2.47	16.24 ± 1.97^a
The distance between T point and diaphragm	7.19 ± 2.11	6.82 ± 1.50
The distance between T point and clavicle level	10.91 ± 1.94	10.40 ± 2.23

Data are expressed as mean \pm SD from 12 cadavers. ^a $P < 0.01$, vs. the left side (*t*-test); T point: the point of the vagus nerve and phrenic nerve on the plane of the inferior border of portal pulmonary arteries.

Table 2 Width, thickness, and motor fiber and total nerve fiber counts at points (U, M, T, and W) in the phrenic nerve and vagus nerve

Site	Width (mm)	Thickness (mm)	Motor fiber counts (n)	Nerve fiber counts (n)
Vagus nerve				
U point	2.47 ± 0.56	0.60 ± 0.19	953 ± 336	$2\ 432 \pm 761$
M point	2.70 ± 0.56	0.60 ± 0.22	$1\ 094 \pm 187$	$2\ 799 \pm 272$
T point	2.63 ± 0.72	0.57 ± 0.19	$1\ 409 \pm 359$	$3\ 421 \pm 289$
W point	1.39 ± 0.14	0.23 ± 0.06	$1\ 716 \pm 362$	$4\ 473 \pm 653$
Phrenic nerve				
U point	1.81 ± 0.43	0.40 ± 0.13	$3\ 078 \pm 684$	$3\ 437 \pm 642$
M point	1.78 ± 0.44	0.41 ± 0.11	$3\ 633 \pm 668$	$3\ 938 \pm 630$
T point	1.78 ± 0.32	0.44 ± 0.14	$4\ 097 \pm 729$	$4\ 433 \pm 721$
W point	1.81 ± 0.32^a	0.44 ± 0.15^a	$4\ 794 \pm 638$	$5\ 071 \pm 723$

Data are expressed as mean \pm SD, there were 24 vagus and phrenic nerves (12 cadavers). ^a $P < 0.01$, vs. W point of vagus nerve (Kruskal-Wallis test).

U: The point of vagus nerve and phrenic nerve on the plane of the clavicle;

M: the point of the vagus nerve and phrenic nerve on the plane of the superior border of the heart;

T: the point of the vagus nerve and phrenic nerve on the plane of the inferior border of portal pulmonary arteries;

W: the middle point of T and the terminal point of the phrenic nerve and vagus nerve on the plane of diaphragmatic muscle.

Table 3 Distance (cm) between the phrenic nerve and vagus nerve at points (U, M, T, and W) in the thoracic cavity

Site	Left	Right
U point	1.26±0.43	1.75±0.37 ^a
M point	2.71±1.67	3.22±0.47
T point	4.92±1.49	4.42±0.79
W point	5.54±1.57	4.34±0.72

Data are expressed as mean ± SD from 12 cadavers.

^a*P* < 0.01, vs. the left side (*t*-test);

U: The point of the vagus nerve and phrenic nerve on the plane of the clavicle;

M: the point of the vagus nerve and phrenic nerve on the plane of the superior border of the heart;

T: the point of the vagus nerve and phrenic nerve on the plane of the inferior border of portal pulmonary arteries;

W: the middle point of T and the terminal point of the phrenic nerve and vagus nerve on the plane of diaphragmatic muscle.

Numbers of motor nerve fibers and total nerve fibers in the phrenic nerve and vagus nerve

Measurements taken from points U, M, T, and W showed that the total number of nerve fibers in the vagus nerve ranged from 2 432 ± 761 to 4 473 ± 653 and the number of motor fibers ranged from 953 ± 336 to 1 716 ± 362. The total number of nerve fibers in the phrenic nerve ranged from 3 437 ± 642 to 5 071 ± 723, and the number of motor fibers ranged from 3 078 ± 684 to 4 794 ± 638 (Table 2, Figure 2). There was a gradually increasing trend in motor fiber number from the U point to the W point in the vagus nerve.

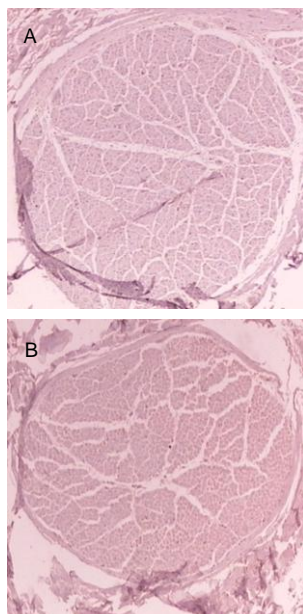


Figure 2 Motor nerve fibres of the vagus nerve (A) and phrenic nerve (B) at the W point (stained using the streptavidin-peroxidase method, × 40).

The phrenic nerve exhibited a similar trend, but with larger changes in the amount of motor fibers at each point. The quantity of nerve fibers in the two nerves was similar. One axon in the proximal donor nerve regenerated three or four collaterals and grew into the receptor nerve^[22].

DISCUSSION

The vagus nerve is the longest and most widely spread brain nerve. It contains four types of fibers: general visceral motor fibers (parasympathetic fibers), special visceral motor fibers, general visceral sensory fibers, and general somatic sensory fibers. It forms the cardiac plexus, the pulmonary plexus, and the esophageal plexus in the thoracic cavity. In our study, the branches of esophageal plexus show a parallel or reticulate form. Our results show that the width, thickness, and total number of nerve fibers is similar between the branch of esophageal plexus and phrenic nerve. Therefore, it would be possible to select one branch from esophageal plexus to neurotize the phrenic nerve and restore the function of the diaphragmatic muscle, whilst minimally affecting the vagus nerve.

Results from fiber counts showed that motor fiber content exhibited an increase from points U to W in the vagus nerve, but increased less than in the phrenic nerve. Jiang *et al*^[21] found that the ratio of regenerative myelinated axon number to proximal donor axon number was approximately 3.3 as an estimated maximum value, in an immediate repair model after peripheral nerve injury. This means that one axon in the proximal donor nerve can regenerate three or four collaterals and grow into the receptor nerve when the space in the receptor nerve is large enough. Thus, there is little impact on the vagus nerve as it grows into the phrenic nerve dominating the diaphragm.

The reported speed of axonal regeneration is about 1–2 mm/day^[22-23] generally. Research on phrenic nerve transfer to brachial plexus root injuries showed that it took about one year, on average, to restore the power of the biceps muscle to Grade 3 (M3) in patients who received phrenic nerve transfer to the musculocutaneous nerve^[24]. Xu *et al*^[25] pointed out that the vascularizing procedure has little clinical value in full-length phrenic nerve transfers in patients with brachial plexus injury. It can also provide sufficient nutrition for the target muscle to recover function. Our results showed that the distance between the diaphragm and the transfer point of the phrenic nerve and esophageal plexus was approximately 7.19 ± 2.11 cm in the left side and 6.82 ± 1.5 cm in the right side. Compared with procedures performed in the neck, there is potential for a reduction in distance of approximately 10.91 ± 1.94 cm in the left side and 10.4 ± 2.23 cm in the right side. Specifically, the distance of axonal regeneration could be greatly shortened and the re-innervated span of the target organ could be reduced. This is because the closer the target organ and the

anastomosis are, the more easily the function of the target organ recovers^[20]. Thus, our method has the potential to significantly recover the function of diaphragmatic muscle.

Zheng *et al*^[26] studied the reconstruction of the bladder reflex arc after SCI. The authors performed anastomosis between the proximal L5 ventral root (somatic motor) and the distal S2 ventral root (visceral parasympathetic), and confirmed that somatic motor nerves can grow into the visceral parasympathetic nerves, as shown by electrophysiological and horseradish peroxidase labeling experiments. Leïter *et al*^[27] verified that the electrical activity of the phrenic and vagus nerves are positively related during the inspiratory phase. Respiratory activity coincided with electrical activity.

To minimize the effect on patients, we propose to complete nerve transfer using video-assisted thoracoscopy. In the thorax cavity, the phrenic nerve can be easily located and separated by video-assisted thoracoscopy^[25]. However, the esophageal plexus is behind the heart and esophagus, which can be hard to locate. Our results here have shown that the beginning of esophageal plexus is relatively fixed. The distance between the nerves was 4.92 ± 1.49 cm on the left side and 4.42 ± 0.79 cm on the right side at the T point, and 5.54 ± 1.57 cm on the left side and 4.34 ± 0.72 cm on the right side at the W point. Upon location of the phrenic nerve, it should be possible to locate the esophageal plexus at the T point and W point. The operation could potentially be performed at the W point or below.

A good restoration strategy could enormously improve the quality of life in patients with upper cervical SCI. This study has confirmed the feasibility of neural regeneration of the phrenic nerve and vagus nerve anatomically. The procedure has the potential advantages of being able to reduce the span of regeneration and limit trauma for the patient. Following surgery, the donor nerve could provide spontaneous breathing to the patient, who could then live independently without need for ventilation. Further studies are needed to determine whether the nerve transfer procedure is able to benefit patients.

MATERIALS AND METHODS

Design

A neuroanatomical study.

Time and setting

The experiment was conducted at the Department of Neuroanatomy, Qiqihaer Medical University, China in September 2010.

Materials

A total of 12 formalin-fixed cadavers (nine males, three females) were supplied by the Department of Anatomy, Qiqihaer Medical University.

Methods

Measurements

A total of 12 formalin-fixed cadavers were dissected in this study. The vagus and phrenic nerves were dissected

carefully and measured by vernier caliper (accuracy 0.02 mm; Guilin Guanglu Measuring Instrument Co., Ltd., Guilin, China). (1) The width and thickness of the point of the vagus and phrenic nerves were measured using a vernier caliper. (2) The distances between the clavicle level and the endpoint of bilateral phrenic nerve were measured. The distances from the T point to the clavicle level of the phrenic nerve and the terminal point of the phrenic nerve were measured in the same way. (3) The distance between the phrenic nerve and vagus nerve at the U, M, T, W points were measured.

Immunohistochemistry and number of motor fibers

Specimens of the points (U, M, T and W) of the vagus nerve and phrenic nerve from 12 cadavers were selected. The chosen segments were stained using the streptavidin-peroxidase method^[28]. Motor fibers were stained using rabbit anti-human choline acetyltransferase monoclonal antibody (1:200; Dako, Carpinteria, CA, USA), and nerve fibers were stained using rabbit anti-human neurofibrin monoclonal antibody (1:200; Dako). Motor and nerve fibers were visualized using diaminobenzidine. Fibers were observed under the microscope. Motor and nerve fibers were quantified using the Motic Med CMIAS Pathological Image Analysis System (Beihang Motic Inc., Beijing, China).

Statistical analysis

Anatomical data and numbers of fibers are expressed as mean \pm SD. Differences in the anatomical data and the number of motor fibers were analyzed by one-way analysis of variance (ANOVA). The Student-Newman-Keuls test was used for comparison between groups. Additionally, the Wilcoxon rank-sum test was employed to compare values if the values did not satisfy the conditions of one-way ANOVA, and the Kruskal-Wallis test was used for the comparison between groups. Statistical significance was accepted at $P < 0.05$. Statistical analysis was conducted using SPSS 13.0 statistical software package (SPSS, Chicago, IL, USA).

Funding: This work was supported by the National Natural Science Foundation of China, No. 30571886.

Author contributions: All authors participated in the study design, performance, data analysis, and result assessment.

Conflicts of interest: None declared.

Ethical approval: The study was approved by Shanghai Changhai Hospital Ethics Committee, Second Military Medical University, China.

REFERENCES

- [1] Brown R, DiMarco AF, Hoit JD, et al. Respiratory dysfunction and management in spinal cord injury. *Respir Care*. 2006;51(8): 853-870.
- [2] DiMarco AF. Restoration of respiratory muscle function following spinal cord injury. Review of electrical and magnetic stimulation techniques. *Respir Physiol Neurobiol*. 2005;147(2-3):273-287.
- [3] DiMarco AF. Phrenic nerve stimulation in patients with spinal cord injury. *Respir Physiol Neurobiol*. 2009;169(2):200-209.

- [4] DiMarco AF, Onders RP, Ignagni A, et al. Inspiratory muscle pacing in spinal cord injury: case report and clinical commentary. *J Spinal Cord Med*. 2006;29(2):95-108.
- [5] Levine S, Nguyen T, Taylor N, et al. Rapid disuse atrophy of diaphragm fibers in mechanically ventilated humans. *N Engl J Med*. 2008;358(13):1327-1335.
- [6] National Spinal Cord Injury Statistical C. Spinal cord injury. Facts and figures at a glance. *J Spinal Cord Med*. 2005;28(4):379-380.
- [7] Watson PJ, Hixon TJ. Effects of abdominal trussing on breathing and speech in men with cervical spinal cord injury. *J Speech Lang Hear Res*. 2001;44(4):751-762.
- [8] Baer GA, Talonen PP, Hakkinen V, et al. Phrenic nerve stimulation in tetraplegia. A new regimen to condition the diaphragm for full-time respiration. *Scand J Rehabil Med*. 1990;22(2):107-111.
- [9] Series F, Verin E, Similowski T. Impediment in upper airway stabilizing forces assessed by phrenic nerve stimulation in sleep apnea patients. *Respir Res*. 2005;6:99.
- [10] Zimmer MB, Nantwi K, Goshgarian HG. Effect of spinal cord injury on the respiratory system: basic research and current clinical treatment options. *J Spinal Cord Med*. 2007;30(4):319-330.
- [11] Terzis JK, Kostopoulos VK. The surgical treatment of brachial plexus injuries in adults. *Plast Reconstr Surg*. 2007;119(4):73e-92e.
- [12] El-Gammal TA, El-Sayed A, Kotb MM. Surgical treatment of brachial plexus traction injuries in children, excluding obstetric palsy. *Microsurgery*. 2003;23(1):14-17.
- [13] Polentes J, Stamegna JC, Nieto-Sampedro M, et al. Phrenic rehabilitation and diaphragm recovery after cervical injury and transplantation of olfactory ensheathing cells. *Neurobiol Dis*. 2004;16(3):638-653.
- [14] Gauthier P, Baussart B, Stamegna JC, et al. Diaphragm recovery by laryngeal innervation after bilateral phrenicotomy or complete C2 spinal section in rats. *Neurobiol Dis*. 2006;24(1):53-66.
- [15] Gauthier P, Rega P, Lammari-Barreault N, et al. Functional reconnections established by central respiratory neurons regenerating axons into a nerve graft bridging the respiratory centers to the cervical spinal cord. *J Neurosci Res*. 2002;70(1):65-81.
- [16] Vinit S, Boulenguez P, Efthimiadi L, et al. Axotomized bulbospinal neurons express c-Jun after cervical spinal cord injury. *Neuroreport*. 2005;16(14):1535-1539.
- [17] Zhou XH, Jia LS, Yuan W, et al. Motor evoked potential and pathology research about this diaphragm after transposition of accessory nerve and phrenic nerve. *Zhongguo Jiaoxing Waike Zazhi*. 2007;15(14):1091-1093.
- [18] Zhou XH, Jia LS, Yuan W, et al. Histological study on reconstruction of respiratory function by transposition of accessory nerve to phrenic nerve following upper cervical cord injury. *Zhonghua Chuangshang Zazhi*. 2007;23(10):761-763.
- [19] Zhou XH, Ye XJ, Yuan W, et al. The histochemical study on transpositional nerve for respiratory function rehabilitation in rats model with upper cervical spinal cord injuries. *Jizhu Waike Zazhi*. 2007;5(2):106-108.
- [20] Pallini R, Fernandez E, Lauretti L, et al. Experimental repair of the oculomotor nerve: the anatomical paradigms of functional regeneration. *J Neurosurg*. 1992;77(5):768-777.
- [21] Jiang BG, Yin XF, Zhang DY, et al. Maximum number of collaterals developed by one axon during peripheral nerve regeneration and the influence of that number on reinnervation effects. *Eur Neurol*. 2007;58(1):12-20.
- [22] Maki H, Watanabe M, Tokita Y, et al. Axons of alpha ganglion cells regenerate faster than other types into a peripheral nerve graft in adult cats. *J Neurosci Res*. 2003;72(2):218-226.
- [23] Stoll G, Muller HW. Nerve injury, axonal degeneration and neural regeneration: basic insights. *Brain Pathol*. 1999;9(2):313-325.
- [24] Gu YD, Wu MM, Zhen YL, et al. Phrenic nerve transfer for brachial plexus motor neurotization. *Microsurgery*. 1989;10(4):287-289.
- [25] Xu WD, Xu JG, Gu YD. Comparative clinic study on vascularized and nonvascularized full-length phrenic nerve transfer. *Microsurgery*. 2005;25(1):16-20.
- [26] Zheng XY, Hou CL, Zhong HB, et al. Reconstructed bladder innervation below the level of spinal cord injury: the knee-tendon to bladder artificial reflex arc. *J Spinal Cord Med*. 2009;32(1):79-85.
- [27] Leiter JC, St-John WM. Phrenic, vagal and hypoglossal activities in rat: pre-inspiratory, inspiratory, expiratory components. *Respir Physiol Neurobiol*. 2004;142(2-3):115-126.
- [28] Marx SC, Kumar P, Dhalapathy S, et al. Distribution of sympathetic fiber areas of radial nerve in the forearm: an immunohistochemical study in cadavers. *Surg Radiol Anat*. 2010;32(9):865-871.

(Edited by He XJ, Chen TY/Qiu Y/Wang L)