ORIGINAL PAPER

RJME
Romanian Journal of Morphology & Embryology
http://www.rjme.ro/

New ways of bronchial stump closure after lung resection: experimental study

V. BUD[1)], B. A. SUCIU[2)], V. BUTIURCA[3)], KLARA BRÎNZANIUC[2)], RUXANDRA COPOTOIU[4)], C. COPOTOIU[1)], ANCA SIN[5)]

[1)] *1st Surgical Clinic, University County Hospital, Targu Mures*
[2)] *Department of Anatomy and Embryology*
[3)] *Student*
University of Medicine and Pharmacy of Targu Mures
[4)] *ICU*
[5)] *Department of Pathologic Anatomy*
University County Hospital, Targu Mures

Abstract

Introduction: Lung surgery has long been associated with a high rate of mortality and morbidity. Bronchial stump fistula is one of the leading causes of high morbidity and high mortality rate. The objectives of our study are to asses the effect of new ways of closure the bronchial stump after lung resections. *Materials and Methods*: For our study, we used eighteen adult rabbits. We performed left trans-thoracic inferior lobectomy to each animal because left lung functional capacities are smaller than right lung functional capacities. The bronchial stump was closed in three different ways: simple running suture of 5.0 monofilament polypropylene (Sweet procedure), running suture of 5.0 monofilament polypropylene (horizontal "U" shape model) and Vycril patch, running suture of 5.0 monofilament polypropylene (horizontal "U" shape model) and bovine pericardium patch. All surviving rabbits were sacrificed in 30 day postoperatively. *Results*: In our study, we did not notice any deaths among our experimental animals and we did not observe any bronchial stump fistula. In the group of rabbits in which the bronchial stump was closed using a patch of heterologous bovine pericardium, the histological exams revealed a cvasinormal bronchial wall with a normal bronchial epithelium and basal membrane integrity and no inflammatory lympho-plasmocytary infiltrate cell, absence of macrophages at this level. *Conclusions*: Using the heterologous bovine pericardium patch to reinforce the bronchial stump could be beneficial because of the faster healing and scarring process. To our knowledge, no studies have previously evaluated this procedure of bronchial stump closure. Our findings could therefore, serve as an impetus for further research in this area.

Keywords: bronchial stump, fistula, bovine heterologous pericardium.

Introduction

Lung surgery has long been associated with a high rate of complications including mortality and long-term morbidity. Bronchial stump fistula is a life-threading complication after lung surgery, with a reported incidence of 0.8–15% [1–3] and with a reported mortality rate range between 25 to 75% [4, 5].

The management of bronchial stump fistula is difficult and depends on the choice of the approach. Treatment options include a variety of strategies and surgical procedures consist in two separate steps: re-closure of the stump and sterilization of the pleural cavity. More than 100 procedures have been published in the past for bronchial stump closure including standard ipsilateral re-thoracothomy, trans-sternal approach developed by Abruzzini P [6], trans-pericardial route through an anterior thoracothomy reported by Padhi RK and Lynn RB [7], and recently transcervical approach with the aid of video-assisted mediastinoscopy [8].

Bronchial stump closure with staples seems to be protective against bronchial stump fistulas when compared with suture closure [2].

The method of bronchial stump closure, primary *vs.* patch, has been a matter of considerable debate and recent articles indicate patch closure independently associated with a decreased incidence of recurrent bronchial fistula [5, 9].

While many patients, disease and technical risk factors for the development of bronchial fistulas have been identified, bronchial stump reinforcement has been shown to significantly reduce the incidence of this complication. Parietal pleura, intercostals muscle, pectoralis major muscle, azygos vein, Vycril and Dacron patch are the various patch materials commonly used [9–11].

On the other hand, processed bovine pericardium has been used as a patch material in intracardiac, neuro-surgery and vascular procedures for more than two decades with excellent results. It exhibits characteristics of an ideal patch material, including non-thrombogenicity, sufficient mechanical strength biocompatibility, off-the-shelf availability, and cost comparable to other synthetic materials. However, debate continues on which patch material best serves this purpose and currently the ideal way for bronchial stump closure is not yet discovered.

Herein we present the effect of a kind of new approach for bronchial stump closure.

Materials and Methods

The experimental protocol was approved by the Ethics Committee of the University of Medicine and Pharmacy of Târgu Mureş, Romania, and all rabbits were cared for in compliance with the European Guide for the Care and Use of Laboratory Animals.

Eighteen adult male white rabbits weighing 2.8 to 4 kg (weighed at the Animal Care Unit, University of Medicine and Pharmacy of Târgu Mureş) were used in this study. The animals were housed in individual cages at room temperature and were fed solid chow and water *ad libitum*.

The animals were anesthetized by intramuscular injection of Xylazine (5 mg/kg body weight) and Ketamine (35 mg/body weight).

We performed left trans-thoracic inferior lobectomy to each animal and the technique was similar in all cases. We choose to perform left trans-thoracic lobectomy because left lung functional capacities are smaller than right lung functional capacities. Tracheal intubation was performed using a 4 mm (internal diameter) endotraheal tube with no cuff. Intra-operative hydratation was maintained with Ringer solution (10 mL/kg body weight) to the auricular vein and ventilation was maintained using pressure cycle ventilator.

For the bronchial stump, closure rabbits were divided into three groups according to closure stump technique:

- Group A: six rabbits; bronchial stump was closed using a running suture of 5.0 monofilament polypropylene (Sweet procedure);
- Group B: six rabbits; bronchial stump was closed using a running suture of 5.0 monofilament polypropylene (horizontal "U" shape model) and Vycril patch;
- Group C: six rabbits; bronchial stump was closed using a running suture of 5.0 monofilament polypropylene (horizontal "U" shape model) and bovine pericardium patch.

No drainage tube was detained in the left thorax, because we did not have the possibility to keep the rabbits into fixed posture. All surviving rabbits were sacrificed in 30 day postoperatively.

The healing process was evaluated by histological examination at autopsy. Histopathological analysis was performed using Hematoxylin–Eosin and van Gieson staining.

Reid index was used to evaluate the ratio of the thickness of the mucous gland layer to the thickness of the wall between the epithelium and cartilage.

Results

The intraoperative aspect of the bronchial stump is shown in Figure 1.

All rabbits maintained good blood flow and spontaneous ventilation and all survived until the 30th postoperative day and no fistula was observed. Microscopically findings were consistent with the macroscopically histological findings and did not reveal any occurrence of bronchial stump fistula. Autopsy revealed that the left lung incompletely inflated for all rabbits caused by the absence of left thorax drainage. Pleural histological findings: inflammatory changes were more evident for animals included in Groups A and B, compared to Group C.

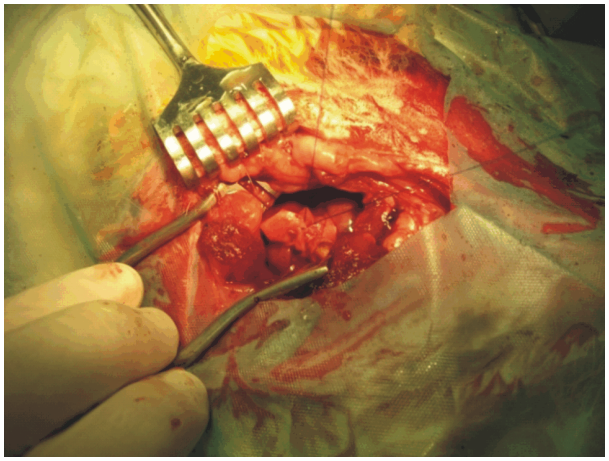

Figure 1 – ***Intraoperative aspect of the bronchial stump (closed using heterologous bovine pericardium).***

Animals of Group A

For animals included in Group A, the 30-day post-operative histopathological exam revealed the thickness of the epithelium and under-epithelial lymphoid infiltrate and unification at the chorion with an under-mucosal layer. Another constant finding was basal membrane damage, lymphocytes passing the bronchial internal lumen and bronchial epithelial desquamation. The lympho-plasmocytary inflammatory infiltrate was observed at the bronchial wall level and often was associated with epithelial cells inclusion into the lympho-plasmocytary cell mass. We noted that the lung parenchyma was surrounded by areas of athelectasis, along with alveolar macrophages. Areas of obliterans fibrosis of blood vessels were observed, also we observed a process of neo-formation of perivascular lymph nodes that is more evident due to the healing process. The hyperplasia of lymphoid nodules represents a marker of the inflammatory reaction (Figures 2 and 3).

Because their immunological effects, the presence of the lymphocytes represents a non-specific stimulating factor of the glycoproteins and collagen synthesis. Also, we observed a small edema and loose extracellular matrix with rare inflammatory cells (Figure 4).

Reid index value was higher than 0.4 for subjects included in this group.

Animals of Group B

For the animals included in Group B, the 30-day postoperative histopathological exam revealed lympho-plasmocytary inflammatory infiltrate, hyperplasia of bronchial epithelium and also erosion and necrosis. Often, the inflammatory infiltrate caused bronchial muscle wall rupture; the presence of granulation tissue in bronchial stump at the 30th postoperative day proved

that the healing process was not completed. For rabbits included in Group B, a constant finding was the appearance of an intense hyper-mucigenetic reaction (Figures 5 and 6).

Reid index value was higher than 0.4 for subjects included in this group and bronchial gland hyperplasia was also present.

Figure 6 show gland hyperplasia demonstrated by the existence of a hyper-mucigenic intense reaction for subjects included in Group B. Sometimes, the granulation tissue invades the bronchi lumen, the lymphocytes and the plasma cells proliferates in the damaged bronchial wall. Also, we observed a pseudo-adenomatous phenomenon when the glandular ducts rich the bronchial lumen, passing through the bronchial epithelium. Another phenomenon that is present in the repairing process is the hyperplasia of the muscular fibers.

Animals of Group C

For the animals included in Group C, histological exams revealed a near to intact bronchial wall with an intact bronchial epithelium and basal membrane integrity and no inflammatory lympho-plasmocytary infiltrate cell, absence of macrophages at this level. The Reid index was less than 0.4 and the glands were normal among animals included in Group C. Common findings among animals included in Group C were a new chondrogenesis and a new osteogenesis process, which represent a restructuring pulmonary process plus pulmonary fibrosis (Figure 7).

New chondrogenesis and new osteogenesis process and Reid index less than 0.4 was found only for subjects included in Group C but not for those included in Groups A and B.

We noticed that in the restructuring pulmonary process are involved both mesenchymal elements and epithelial elements. The suturing zone represents for a while a zone of excitation for the surrounding pulmonary parenchyma, due to the products, which are released from the granulation tissue. The absence of macrophages on the preparation studied represents a sign of the completion of the restructuring process.

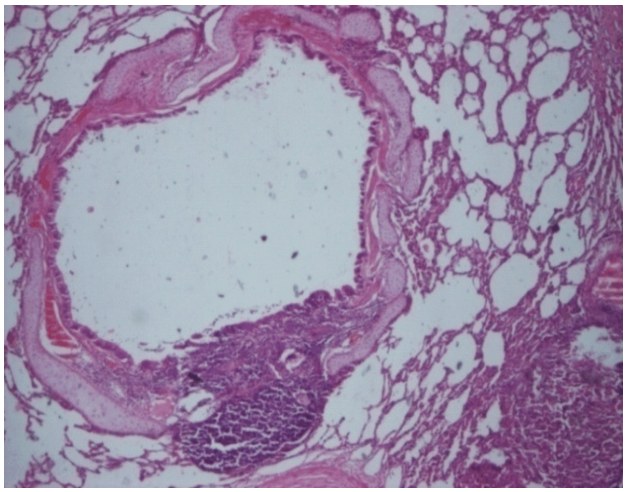

Figure 2 – ***Bronchial stump closed using Sweet procedure: perivascular lymph nodes, lymphocytes in the lumen of the bronchi, bronchial epithelial damage (HE stain, ob. 4×).***

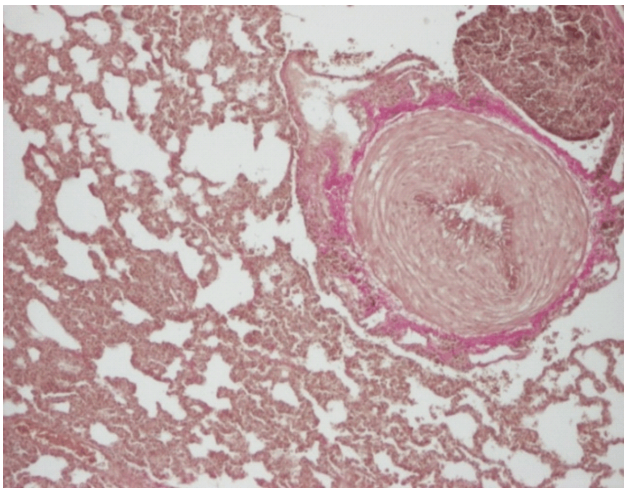

Figure 3 – ***Bronchial stump closed using Sweet procedure: perivascular fibrotic changes, perivascular lymph node, areas of athelectasis (van Gieson stain, ob. 4×).***

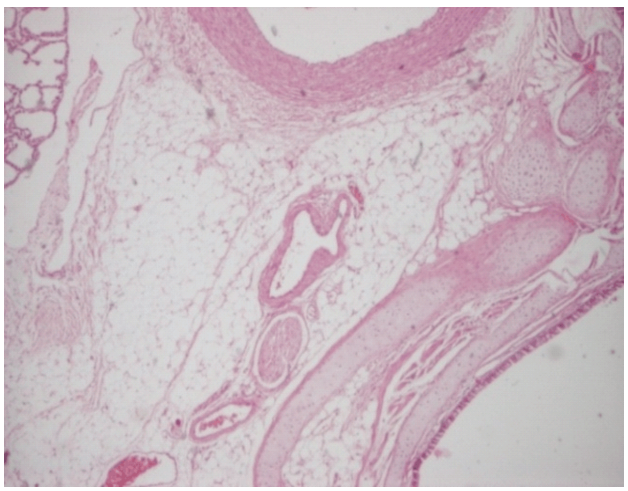

Figure 4 – ***Bronchial stump closed using Sweet procedure: edema, rare inflammatory cells, loose extra-cellular matrix (HE stain, ob. 4×).***

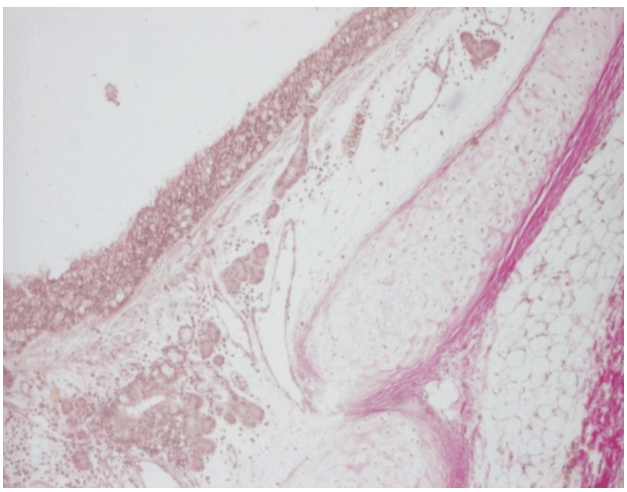

Figure 5 – ***Bronchial stump closed using Vycril patch: hyperplasia of the bronchial epithelium, hyperplasia of the bronchial glands in the submucosa (van Gieson stain, ob. 4×).***

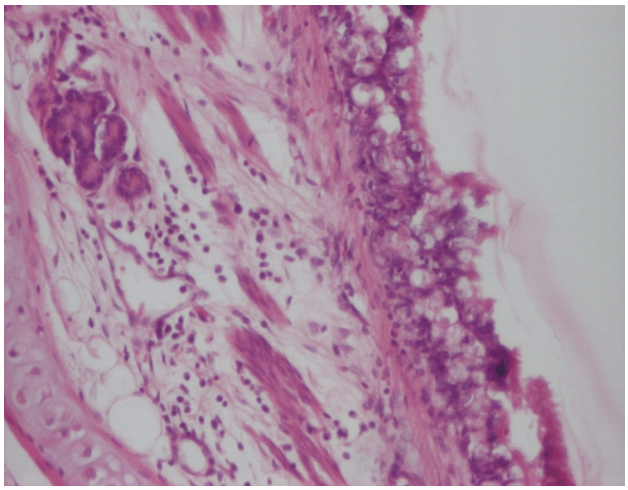

Figure 6 – ***Bronchial stump closed using Vycril patch: hypertrophy of the bronchial epithelium, mucigenic reaction, hyperplasia of the muscle fibers, hyperplasia of the bronchial glands, in the left part of the figure we can observe small mucous acins and the hyperplasia of the bronchial glands from the damaged bronchial wall (HE stain, ob. 10×).***

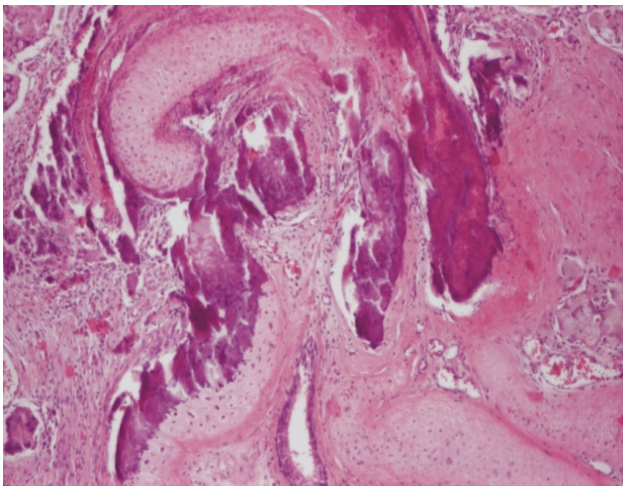

Figure 7 – ***Bronchial stump closed using bovine pericardium: restructuring pulmonary process, pulmonary fibrosis, neo-chondrogenesis, neo-osteogenesis (HE stain, ob. 4×).***

Discussion

Currently, medical literature identifies two main trends for suturing bronchial stump: manual *vs.* mechanical [12, 13]; however, debate continues on which patch material best serves this purpose. Proponents of autologous patch claim a lower incidence of postoperative thrombosis, immediate sealing, and no additional costs for synthetic material. Synthetic materials, in contrast, do not requires a separate harvest procedure, thereby eliminating longer operative time and associated postoperative morbidity and do not rupture but are associated with higher incidence of infection, poor vessels growth and bronchial stump fistula associated with mechanical factors incur the additional costs of the synthetic material.

Brewer LA 3rd *et al.* (1953) were the first ones who reported on their experimental and clinical work on the use of pedicled pericardial graft for reinforcement of bronchial closure in patients with pulmonary resection [14]; Rienhoff WF *et al.* published his results after he used parietal pleura for covering bronchial stump [10], Topolnitskiy EB *et al.* postpone the principles of outside contention of the bronchial stump covered with titanium alloy [15].

We choose to perform bronchial stump closure using Vycril patch because of the tissues good tolerability, immediate sealing and low costs. We performed bronchial stump closure using processed bovine pericardium based on our expertise in vascular reconstruction during heart surgery and also based on other author's results about using this material for the reconstruction of the trachea [16]; other authors show that the bovine pericardium in the human body undergoes a calcification process [17–21]. The calcification process at bronchial stump level can be favorable because it increases the resistance and prevents long-term bronchial fistula occurrence. Other authors, based on heart surgery expertise claim that bovine pericardium undergoes an epithelization process rather than calcification and this can be beneficial in the bronchial stump healing process [21]. Using a bovine pericardium patch has the advantage because the structure is not destroyed and provides resistance for the tissue that is implanted [22].

It is well known from literature that first stage of tissue healing is the inflammation process. The presence of the inflammatory process at the 30th postoperative day for rabbits included in Groups A and B revealed the fact that the healing process is incomplete compared to rabbits included in Group C. This observations show that for animals included in Group C the healing process is faster than for the other two groups studied. Another interesting finding in our study was the new osteo- and chondrogenesis process observed at the 30th postoperative day among animals included in Group C. We concluded that the bovine pericardium patch may increase stump resistance and could be beneficial in the prevention of bronchial stump fistula occurrence.

Different mechanisms involved in tissue repair, trigger chemical signs that cause the migration of the inflammatory cells, proliferation, differentiation, synthesis and degradation of the matrix proteins that interfere with the cellular response to the growth factor.

The Reid index was used to evaluate the ratio of the thickness of the mucous gland layer to the thickness of the wall between the epithelium and the cartilage. The Reid index is increased in the presence of chronic bronchitis but not in the presence of the inflammatory process [23]. Chronic bronchitis is associated with hyperactivity of the bronchial glands and the increased Reid index [24]. The Reid index value was higher for animals included in Groups A and B compared to Group C. Increased Reid index value for subjects included in Groups A and B but not for those included in Group C allowed us to conclude that processed bovine pericardium increase stump resistance through an earlier inflammatory and sealing process.

The formation of an inadequate bronchial scar, an excessive secretion of some growth factors, an excessive

retraction of the repairing tissue can affect the healing of the bronchial stump. At a microscopic level, the repairing process and the integration of the heterologous pericardium depend on the cell–cell interaction, the cell–matrix interaction, the cell proliferation and the deposits of the extracellular matrix.

Conclusions

Using processed bovine pericardium patch to reinforce the bronchial stump could be beneficial because of the faster healing and scarring process. To our knowledge, no studies have previously evaluated this procedure of bronchial stump closure. Our findings could therefore, serve as an impetus for further research in this area.

Acknowledgments

This work was partially supported by the Sectoral Operational Programme Human Resources Development, financed from the European Social Fund and by the Romanian Government under the contract number POSDRU/89/1.5/S/64153.

References

[1] Klepetko W, Taghavi S, Pereszlenyi A, Bîrsan T, Groetzner J, Kupilik N, Artemiou O, Wolner E, *Impact of different coverage techniques on incidence of postpneumonectomy stump fistula*, Eur J Cardiothorac Surg, 1999, 15(6):758–763.

[2] Deschamps C, Bernard A, Nichols FC 3rd, Allen MS, Miller DL, Trastek VF, Jenkins GD, Pairolero PC, *Empyema and bronchopleural fistula after pneumonectomy: factors affecting incidence*, Ann Thorac Surg, 2001, 72(1):243–247; discussion 248.

[3] Algar FJ, Alvarez A, Aranda JL, Salvatierra A, Baamonde C, López-Pujol FJ, *Prediction of early bronchopleural fistula after pneumonectomy: a multivariate analysis*, Ann Thorac Surg, 2001, 72(5):1662–1667.

[4] Asamura H, Naruke T, Tsuchiya R, Goya T, Kondo H, Suemasu K, *Bronchopleural fistulas associated with lung cancer operations. Univariate and multivariate analysis of risk factors, management, and outcome*, J Thorac Cardiovasc Surg, 1992, 104(5):1456–1464.

[5] Taghavi S, Marta GM, Lang G, Seebacher G, Winkler G, Schmid K, Klepetko W, *Bronchial stump coverage with a pedicled pericardial flap: an effective method for prevention of postpneumonectomy bronchopleural fistula*, Ann Thorac Surg, 2005, 79(1):284–288.

[6] Abruzzini P, *Trattamento chirurgico delle fistole del bronco principale consecutive a pneumonectomia per tubercolosi*, Chir Torac, 1961, 14(3):165–171.

[7] Padhi RK, Lynn RB, *The management of bronchopleural fistulas*, J Thorac Cardiovasc Surg, 1960, 39:385–393.

[8] Azorin JF, Francisci MP, Tremblay B, Larmignat P, Carvaillo D, *Closure of a postpneumonectomy main bronchus fistula using video-assisted mediastinal surgery*, Chest, 1996, 109(4):1097–1098.

[9] Sfyridis PG, Kapetanakis EI, Baltayiannis NE, Bolanos NV, Anagnostopoulos DS, Markogiannakis A, Chatzimichalis A, *Bronchial stump buttressing with an intercostal muscle flap in diabetic patients*, Ann Thorac Surg, 2007, 84(3):967–971.

[10] Rienhoff WF, Gannon J, Sherman I, *Closure of the bronchus following total pneumectomy: experimental and clinical observations*, Ann Surg, 1942, 116(4):481–531.

[11] Al-Mufarrej F, Margolis M, Strother E, Tempesta B, Gharagozloo F, *Bronchial stump reinforcement with an azygous vein flap*, J Cardiothorac Surg, 2009, 4:22.

[12] Dili GSPZA *et al.*, *Comparison of curative effects in different methods on handling bronchus stump during pneumonectomy*, Shaanxi Medical Journal, 2008-06, http://en.cnki.com.cn/Article_en/CJFDTOTAL-SXYZ200806023.htm.

[13] Panagopoulos ND, Apostolakis E, Koletsis E, Prokakis C, Hountis P, Sakellaropoulos G, Bellenis I, Dougenis D, *Low incidence of bronchopleural fistula after pneumonectomy for lung cancer*, Interact Cardiovasc Thorac Surg, 2009, 9(4):571–575.

[14] Brewer LA 3rd, King EL, Lilly LJ, Bai AF, *Bronchial closure in pulmonary resection: a clinical and experimental study using a pedicled pericardial graft reinforcement*, J Thorac Surg, 1953, 26(5):507–532.

[15] Topolnitskiy EB, Dambaev GTs, Fomina TI, *Morphological assessment of different ways of bronchial stump closure after pneumonectomy*, Bull Exp Biol Med, 2009, 148(2):257–261.

[16] Carter JJ, Evans D, Shah P, Ura M, *Iatrogenic tracheal rupture: bovine pericardial patch repair without flap reinforcement*, Interact Cardiovasc Thorac Surg, 2012, 14(4):502–503.

[17] Schoen FJ, Levy RJ, *Calcification of tissue heart valve substitutes: progress toward understanding and prevention*, Ann Thorac Surg, 2005, 79(3):1072–1080.

[18] Huang-Lee LL, Cheung DT, Nimni ME, *Biochemical changes and cytotoxicity associated with the degradation of polymeric glutaraldehyde derived crosslinks*, J Biomed Mater Res, 1990, 24(9):1185–1201.

[19] Konakci KZ, Bohle B, Blumer R, Hoetzenecker W, Roth G, Moser B, Boltz-Nitulescu G, Gorlitzer M, Klepetko W, Wolner E, Ankersmit HJ, *Alpha-Gal on bioprostheses: xenograft immune response in cardiac surgery*, Eur J Clin Invest, 2005, 35(1):17–23.

[20] Manji RA, Zhu LF, Nijjar NK, Rayner DC, Korbutt GS, Churchill TA, Rajotte RV, Koshal A, Ross DB, *Glutaraldehyde-fixed bioprosthetic heart valve conduits calcify and fail from xenograft rejection*, Circulation, 2006, 114(4):318–327.

[21] Guldner NW, Jasmund I, Zimmermann H, Heinlein M, Girndt B, Meier V, Flüss F, Rohde D, Gebert A, Sievers HH, *Detoxification and endothelialization of glutaraldehyde-fixed bovine pericardium with titanium coating: a new technology for cardiovascular tissue engineering*, Circulation, 2009, 119(12):1653–1660.

[22] Vaughn CC, Vaughn PL, Vaughn CC 3rd, Sawyer P, Manning M, Anderson D, Roseman L, Herbst TJ, *Tissue response to biomaterials used for staple-line reinforcement in lung resection: a comparison between expanded polytetrafluoroethylene and bovine pericardium*, Eur J Cardiothorac Surg, 1998, 13(3):259–265.

[23] Field WE, Davey EN, Reid L, Roe FJ, *Bronchial mucus gland hypertrophy: its relation to symptoms and environment*, Br J Dis Chest, 1966, 60(2):66–80.

[24] Mullen JB, Wright JL, Wiggs BR, Pare PD, Hogg JC, *Reassessment of inflammation of airways in chronic bronchitis*, Br Med J (Clin Res Ed), 1985, 292(6504):1235–1239.

Corresponding author

Bogdan Andrei Suciu, MD, PhD, Department of Anatomy and Embryology, University of Medicine and Pharmacy of Târgu Mureş, 38 Gheorghe Marinescu Street, 540139 Târgu Mureş, Romania; Phone +40365–407 474, +40744–796 343, e-mail: suciubogdanandrei@yahoo.com

Received: October 4th, 2012 *Accepted: January 20th, 2013*