Introduction
The clinical application of fetal membranes has been studied for many years. The human amnion is a fetal membrane composed of mesodermal tissue containing collagen fibers and fetal fibroblasts, thick basement membrane and single cuboidal epithelium. The amnion is thin and elastic. Amniotic epithelial cells produce and release growth factors such as epidermal growth factor (EGF), transformic growth factor alpha (TGF-alpha), prostaglandin E2 (PGE2), insulin-like growth factor (IGF), vasoactive endothelial growth factor (VEGF), hepatocyte growth factor (HGF), endothelin and tissue inhibitors of metalloproteinases 1 and 2 [1]. The human amnion is a readily available and inexpensive allograft with low antigenicity, high antimicrobial potential and the ability to stimulate epithelialization. It had been used as a biological dressing in treatments of burns as early as 1913 [2].

Preserved amnion grafts have been widely used in ophthalmology for the treatment of deep ulcerations of the cornea and sclera [3,4] and the treatment of conjunctive melanoma [5], in otolaryngology for replacing the nasal mucosa in Rendu-Osler-Weber disease, as tympanic membrane grafts, for treatment of severe epistaxis and for covering head and neck sites after flap necrosis [6,7]. It has also been used as a biological dressing in the treatment of burns [8-11], varicose ulcers, decubitus ulcers and open infected wounds [12]. Amnion allografts are also used in the moduation of peripheral nerve regeneration [13] and in reconstructive surgery to reconstruct the vagina following resection for diffuse carcinoma in situ (CIS) as well as for severe, erosive vulvovaginal burns [14].

Postoperative air leakage (due to both peripheral or alveolar air leak) is the most frequent complication and a major cause of morbidity post lung surgery. A prolonged air leakage is defined as an air leakage lasting 7 days or more [15,16]. This complication may result in additional costs due to the increased morbidity and prolonged hospital stay [17].

Pulmonary surgery often requires the separation of lung adhesions of the lung parenchyma during non-anatomic lung resections or the separation of the pulmonary fissure during lobectomy. The free edge of the separated lung tissue is a common site of air leakage. The presence of pleural adhesions increases the risk of air leakage [18]. Air leakage resulting from pulmonary staple lines can be caused by several factors: tissue fragility, excessive tissue tension and surgical technique. Lowering the risk of air leakage may be achieved by careful suturing, stapling, electrocautery and other methods. Various sealants have been used to reduce alveolar air leakage: fibrin glue [19,20] and fibrin sealant [21], synthetic sealant...
[22], bovine pericardium [23], expanded polytetrafluoroethylene [24], purified human collagen membrane-polyglycolic acid sheet [25] or the wall of the resected bulla [26].

To the best of our knowledge, there is no data on the application of human amnion grafts for alveolar air leakage prevention. The purpose of the present study was to develop a new method for treating lung and pleural injuries and to examine the efficacy, safety, and usefulness of amniotic membrane allografts as biological dressings in thoracic surgery.

Patients and methods
Human amnion grafts were used as a biological dressing to minimize or prevent air leakage post lung surgery. The amnion allografts were prepared at the Central Tissue Bank in Warsaw, according to the procedure described previously [1]. After separation of a chorion from the amniotic sac, the amnion membrane was placed on a sterile polyester net acting as a support and cut to suitable sizes. Allografts were preserved by deep-freezing (-72°C) and subsequently radiation-sterilized with a dose of 35 kGy using 10 MeV electron accelerator [1].

Human amnion grafts were used in the following clinical situations: (i) to treat visceral pleura and lung injury after pleurolysis (in particular, during rethoracotomies), (ii) to prevent air leakage from pulmonary staple lines after non-anatomic lung resection and anatomic separation of lung parenchyma and (iii) to prevent air leakage from the sites of metastases resection with NdYag laser.

Between September 2004 and March 2005, amnion allografts were applied to 20 patients (aged 24-73; mean 53.1; 12 female and 8 male). The age of the female patients varied between 45 and 69 years (mean: 57.9) and that of male patients, between 24 and 73 years (mean: 46). The surgeries were performed for primary lung cancer (2) or multiple metastases from other neoplasms: bone and soft tissue sarcomas (6), colorectal cancer (5), testicular cancer (2), salivary gland cancer (2), renal cancer (1), thymic carcinoma (1), and solitary fibrous tumor (1). Eleven thoracotomies and nine rethoracotomies were performed: 15 metastasectomies (from 1 to 12 metastases resected), 2 lobectomies and 2 residual tumor resections. The resection of metastases in 7 patients was performed with laser NdYag.

After marginal, anatomical or laser resection the sites of air leakage were localized intraoperatively. They were identified by air bubbling through the injured visceral pleura, the site of the metastasectomy or along the staple line. The resection sites were tested for air leakage under water (Fig. 1).

The sites of intensive air leakage were reduced by careful suturing with twisted or spiral sutures. The lung was then re-expanded and the amnion flap lying on the polyester net was placed on the sites of air leakage during lung ventilation with the pressure of 15-20 cm of H₂O (Fig. 2). The amnion flap was attached to the lung tissue by moderate compression and stabilized by a few knot sutures (Fig. 3).

During the postoperative period, the air leakage and the duration of chest tube air drainage were evaluated.

Results
Twenty patients were enrolled in the study. Massive pleural adhesions were observed in 10 patients (50%),
minor adhesions in 3 patients (15%) and no adhesions in 7 patients (35%). In all patients air leakage was identified intraoperatively before amnion graft application. The sites of air leakage were: injured visceral pleura – 13 (43.3%), the line of mechanical sutures – 11 (36.7%), site of metastasesectomy following NdYag resection – 4 (13.3%), bulla emphysematosa – 1 (3.3%), and divided pulmonary fissure – 1 (3.3%). After amnion graft application, no air leakage was noted in 6 cases (30%), traces in 11 cases (55%), and minor air leakage in 3 cases (15%). Chest X-rays performed immediately after surgery revealed a fully expanded lung in 18 cases (90%) and a non-expanded cavity in 2 cases (10%).

The duration of the postoperative air leakage varied from nil (4 cases – 20%) to 20 days (1 case – 5%). In 80% of patients the duration of the air leakage was less than 3 days. In 4 cases (20%) – no air leakage was observed, in 6 cases (30%) 24-hour, in 4 cases (20%) 2-day and in 2 cases (10%) 3-day air leakage was noted. Prolonged post surgical air leakage was caused by incomplete lung re-expansion after massive lung parenchyma resection.

Discussion

The separation of lung tissue (anatomical and non-anatomical resection) and the liberation of pleural adhesions (especially during rethoracotomy) may be the cause of lung and visceral pleura injury during thoracic surgery. These are probable reasons for the air leakage. Prolonged peripheral or alveolar air leakage is frequent and remains a significant complication that prolongs the duration of pleural drainage and the length of hospitalization. It also increases hospital costs [17-19]. Air leakage may also result in significant morbidity.

Careful application of staples and sutures diminishes, but does not eliminate air leakage. Various strategies have been employed to eliminate and/or diminish pulmonary air leakage. Most of them were developed to reduce the risk of air leakage and to shorten hospital stay after lung volume reduction surgery. Fibrin glue, fibrin or albumin sealant as well as other materials, such as bovine pericardium or emphysematous bulla, were used to buttress the staple line on thin and friable lung parenchyma. There is no evidence that the mentioned above sealants reinforce the staples or the manual sutures after multiple resections of metastases and the sites of metastasesectomy resection with NdYag laser. Another problem is the prevention of air leakage after liberation of multiple massive adhesion during rethoracotomies. The presence of pleural adhesions is one of the predictors of prolonged air leakage [18]. The liberation of massive adhesions during rethoracotomy causes multifocal pleural and lung injuries and the air leakage.

The ideal reinforcing material should possess several properties, such as be biocompatible, flexible, strong, airtight and cost-effective [23]. The material used in our study, deep-frozen and radiation-sterilized human amnion has all of these properties. Moreover, it resembles the visceral pleura and may be easily attached to the lung parenchyma. It allows coverage of the area of the lung parenchyma deprived of the visceral pleura after the adhesion liberation and lung decortication. The amnion membrane is elastic and may be placed over an expanded and ventilated area of the lung.

The use of human amnion allografts is simple and safe. There are reports suggesting that the amniotic membrane prevents adhesion formation [27,28].

Our preliminary results suggest that the use of amniotic membrane as a biological dressing applied for the prevention of air leakage in lung surgery is a safe, simple and effective method.

Address reprint requests to: Mariusz Żmięwski MD, Lung and Thoracic Tumor Department, The Maria Sklodowska-Curie Memorial Cancer Center & Institute of Oncology, 5 W.K. Roentgen Street, 02-781 Warsaw, Poland Phone/Fax: (+48 22) 643 93 85 E-mail: marioz@acn.waw.pl

References
