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ABSTRACT:
Objectives: Final results of prospective randomized study to assess the influence of Tachosil® applied on mediastinum after systematic lymphadenectomy are presented.
Material and methods: In 28 patients from the Tachosil® group 1 to 3 large pieces of Tachosil® (mean 1.8, SD±0.7) were applied, while in 22 patient from the control group only coagulation was allowed. Clinical data were collected, and pleural concentrations of IL-6, IL-1ra and IL-8 on postoperative days 1, 2 and 3 were measured, with ELISA method.
Results: Both groups were well balanced according to sex, age, number of packyears of cigarettes, time from smoking cessation, pulmonary function test results, concomitant diseases, BMI index, number of N1 and N2 nodes resected, and perioperative serum concentrations of IL-6, IL-1ra and L-8. Postoperative complications occurred in 8/29 and 12/24 patients from each group (p=0.046), respectively, with no mortality. No differences in time of surgery, intraoperative blood loss, amount of drainage and drainage hemoglobin concentration on postoperative days 1, 2 and 3, time to drainage removal and number of blood units transfused between the groups were observed. The summarized postoperative pleural drainage was lower in the Tachosil® group (p=0.03). Concentration of IL-6 in pleural drainage was higher in the Tachosil® group on the first (p=0.01) and the second (p=0.03) postoperative days. Positive correlation between number of blood units transfused and pleural concentration of hemoglobin on subsequent postoperative days in the whole group but not in Tachosil® group was found.
Conclusions: This study showed a significant impact of application of Tachosil® on mediastinum after systematic lymphadenectomy on clinical outcome due to decreased amount of total postoperative drainage and decreased rate of complications. A higher concentration of IL-6 in pleural fluid on postoperative day 1 and 2 may reflect a minor local immune response to Tachosil®, without elevation of concentration of other cytokines and without negative impact on postoperative complications. Blood transfusions in patients from the Tachosil®

group were rather due to preoperative anemia and intraoperative blood loss, unlike in the control group where blood transfusions were rather due to postoperative blood loss with pleural drained fluid.

KEY WORDS: lung cancer, systematic lymphadenectomy, postoperative complications, IL-6, IL-1ra, IL-8, Tachosil

Introduction:

Mediastinal lymphadenectomy is considered a mandatory completion of pulmonary resection in lung cancer patients. According to recommendations [1], the most preferable technique of lymphadenectomy is systematic mediastinal lymphadenectomy which consists of removal of all fatty tissue from the paratracheal space (on the right side), aorto-pulmonary window and left inferior paratracheal spaces (station 4L – in case of left lung cancer), and from subcarinal and paraesophageal areas. On the left side, transection of superior pulmonary ligament is mandatory for removal of 4L lymph nodes. Alternatively, mediastinal lymph node sampling is also widely accepted, which consists of removal of 2-3 lymph nodes from every lymph node station. Other techniques of mediastinal lymphadenectomy are less popular, and by many considered either insufficiently radical or too extensive [2]. Systematic lymphadenectomy is believed to be more accurate than systematic sampling in staging and more radical in terms of removal of all metastatic sites. However, many controversies develop due to recommendations to widely perform this technique, because of an elevated risk of intraoperative injuries of recurrent laryngeal nerve, pulmonary artery and aortic arch. Systematic lymphadenectomy is also believed to extend the time of surgery [3] and time of postoperative drainage [4], both of them possibly resulting in an increased risk of postoperative complications. However, numerous studies comparing systematic lymphadenectomy with systematic sampling did not show the statistically significant difference in development of postoperative complications and postoperative immune response, only a positive correlation between the number of resected mediastinal lymph nodes and concentration of cytokines in pleural drained fluid was found [5]. To decrease the time and volume of postoperative pleural drainage after systematic lymphadenectomy many surgeons use hemostatic material on mediastinum also due to recommendations from earlier studies which were assessed the role of Tachocomb® after less extensive mediastinal lymphadenectomy [6]. However, the value of using hemostatic material on mediastinum after systematic mediastinal lymphadenectomy has never been assessed before. Before starting this study we observed that implementation of systematic lymphadenectomy instead of systematic sampling in our hospital resulted in an increased amount of total postoperative drainage and in prolonged postoperative drainage. The primary end-point of this study was to assess the volume and time of total postoperative drainage, and the secondary end-point was to assess the rate of complications in two study groups. In this prospective randomized study we applied on mediastinum Tachosil®, which has been proven in numerous studies to be safe and effective for decreasing air leak and bleeding [7, 8]. Tachosil® is a hemostatic tissue sealant consisting of human fibrinogen and human thrombin coated onto an equine collagen sponge which is used in surgery to stop leakage of fluids (blood, urine, cerebrospinal fluid, pancreatic fluid, etc.) or the air leak. The standard technique of administration of Tachosil® recommended by producer consists of placing the dry or premoistened product on the place of leakage and compressing it with moistened gauze [9].

The study was approved on March 25, 2009 by the Ethics Committee of Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University in Torun (KB 46/2009). The study was supported financially by Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University in Torun.

Material and methods:

Patients subjected to anatomical resection of preoperatively diagnosed or suspected non-small cell lung cancer (NSCLC) were asked to give an informed consent to participate in the study. Preoperative diagnosis and staging included bronchoscopy, abdominal ultrasound and positron emission tomography with computerized tomography (PET-CT) to exclude distant metastases and concomitant extrapulmonary cancer. Patients after neoadjuvant chemotherapy, patients preoperatively treated with systemic steroids or other immunosuppressive medicines as well as patients with concomitant diseases which could influence the postoperative immune response (e.g. insulin dependent diabetes) were excluded. All patients received 4 doses of 2 grams of cefamandole every 6 hours, as perioperative antibiotic prophylaxis, and daily

dalteparin 5000IU preoperatively and until discharge from the hospital as antithrombotic prophylaxis. If pleural adhesions involving more than 20 cm^2 of parietal pleura were found during the surgery, patients were excluded from the study. Randomization was performed intraoperatively, after completion of systematic lymphadenectomy. Fifty-three patients (16 women, 37 men, age 65.3±7.5 years) were accrued and intraoperatively found eligible for the study. In 29 patients (9 women, 20 men, age 65.9±7.3 years) from the Tachosil® group 1 to 3 large pieces of Tachosil® (mean 1.8±0.7) were applied on mediastinum. In 24 patients from the control group (7 women, 17 men, age 64.6±7.9 years) only coagulation was allowed. Forty-six lobectomies (including 6 VATS lobectomies and 1 sleeve lobectomy), 3 bilobectomies, 3 pneumonectomies (including 1 intrapericardial pneumonectomy) and 1 anatomical segmentectomy were performed. Final pathology revealed 27 squamous cell carcinomas, 13 adenocarcinomas, 4 large-cell carcinomas, 2 large cell neuroendocrine carcinomas, 5 other or mixed type pulmonary neoplasms, 1 metastatic breast cancer and 1 metastatic endometrial cancer. In all cases systematic mediastinal lymphadenectomy was performed. Pleural drainage was discontinued after it was less than 100mL per day. In some cases, mostly from the control group, the last drain was removed even when daily drainage was 100-200mL, but lasted for more than 6 days, to avoid pleural infection. Clinical data were collected and intraoperative serum concentrations (at the beginning and at the end of operation) as well as pleural concentration of Interleukin 6 (IL-6), Interleukin 1 receptor antagonist (IL-1ra) and Interleukin 8 (IL-8) on postoperative days 1, 2 and 3 were measured, with ELISA method. These three cytokines were selected because they have been proven to be the most effective in the measurement of postoperative immune response in previous studies [5, [10]]. After obtaining, venous blood and pleural fluid were cooled to 4ºC, centrifuged at a speed 2500/min for 10 minutes, and then preserved in temperature minus 72ºC until further investigations. Concentration of human IL-6, IL-8 and IL-1ra in serum and pleural fluid was determined by commercially available sandwich enzyme-linked immunosorbent assay (ELISA) sets: (OptEIA Set from BD Biosciences Pharmingen for IL-6, IL-8, and DuoSet from R&D Systems for IL-1ra/IL-1F3) according to the manufacturer's instructions. Cytokines' concentration was determined by measuring the optical density at 450 nm by microtiter plate reader (iEMS Reader MF, Labsystems). The lowest detection limits were: 4.6 pg/mL for IL-6; 3.1 pg/mL for IL-8 and 39.0 pg/mL for IL-1ra.

Results were expressed as median and first and third quartile or mean values±SD. To evaluate statistical significance of difference of cytokine concentration in serum at the beginning and at the end of surgery as well as in pleural fluid on subsequent postoperative days between two study groups Mann-Whitney U-test test was used. Student t-test for categorical parameters was used. Differences between groups without and with Tachosil® were analyzed with Mann-Whitney U-test. Impact of administration of Tachosil® and elevated concentrations of selected cytokines in pleural fluid on the risk of development of complications was analyzed using logistic regression method. Association between continuous variables was assessed using Spearman rank correlation coefficient. Frequency of complications between groups was tested with Chi^2 test. Impact of analyzed factors on probability of development of complications was assessed by the area under curve (ROC) inspection. Results are presented as odds ratio with 95% confidence intervals. Computations were performed with SPSS 12.0 statistical package.

Results:

Both groups were well balanced according to sex, age, number of packyears of cigarettes smoked, time from smoking cessation, pulmonary function test results, frequency of concomitant diseases, body-mass index (BMI), number of N1 and N2 lymphatic nodes resected, number of N1 and N2 positive lymphatic nodes and preoperative serum concentrations of IL-6, IL-1ra and IL-8. No differences in time of surgery, intraoperative blood loss, amount of drainage on three subsequent postoperative days and drainage hemoglobin concentration on three subsequent postoperative days, time to drainage removal (in days), number of blood units transfused and time of hospital stay and postoperative serum

concentrations of IL-6, IL-1ra and IL-8 between the groups were observed. The summarized postoperative pleural drainage was lower in the Tachosil® group (1289.1±641.4, vs. 1987±1462mL, p=0.03) (Figure 1). Postoperative complications occurred in 8/29 (27.6%) and 12/24 (50%) patients from Tachosil® and control group, respectively (p=0.046, Chi^2 test), with no mortality. Respiratory complications (predominantly respiratory insufficiency requiring bronchoaspiration) were observed in 6 patients from the Tachosil® group and in 7 patients from the control group. Infectious complications were observed only in 3 patients from the control group. Cardiac arrhythmia occurred in 1 patient from the Tachosil® group and in 4 patients from the control group. Postoperative bleeding requiring reoperation occurred only in 1 patient from the Tachosil® group. One patient from the Tachosil® group required reoperation (mentioned above) and 1 patient from the control group had postoperative cerebral ischemia. Two patients from the Tachosil® group had 2 complications and 1 patient from the control group had 3 complications.

Mean time of hospital stay was in the whole group of patients was 8.6±3.3 days, in the Tachosil® group 8.1±2.6 days and in the control group 9.4±4.1 days, p=0.3. No significant differences of IL-1ra and IL-8 in pleural fluid on postoperative days 1, 2 and 3 between the groups were observed. Concentration of IL-6 in pleural drainage was higher in the Tachosil® group on the first [188790 (119135-290380) vs 104053(80286-176356)pg/mL, p=0.01] and the second [117155 (75505-184670) vs 68205 (43268-113625)pg/mL, p=0.03] postoperative days (Figure 2, 3, 4).

In the whole group of patients a positive correlation between percent of body weight loss and the diameter of primary tumor (p=0.01), as well as negative correlation between body weight loss and pleural concentration of IL-1ra on postoperative day 2 and 3 and pleural concentration of IL-8 on day 2 was observed (p=0.01, p=0.001 and p=0.02, respectively). This correlation was also found in the Tachosil® group for IL-1ra on day 2 (p=0.01) and in the control group for IL-1ra on day 1 (p=0.03) as well as for IL-8 on day 2 (p=0.04).

In the whole group of patients a positive correlation between ECOG performance status and amount of postoperative drainage on postoperative day 3 (p=0.0001) as well as amount (p=0.0003) and time (p=0.0003) of total postoperative drainage was found. Positive correlation between total postoperative drainage and postoperative drainage on postoperative days 2 (p<0.000001) and 3 (p<0.000001) was found. Positive correlation between concentration of hemoglobin in blood before surgery and pleural concentration of hemoglobin on subsequent postoperative days was found (p=0.01, p=0.04 and p=0.02, respectively). Positive correlation between postoperative WBC in peripheral blood and amount of postoperative drainage on day 1 (p=0.03) as well as total postoperative drainage (p=0.02) was found. A negative correlation between pleural concentration of hemoglobin on day 3 and IL-1ra concentration on subsequent postoperative days was found (p=0.04, p=0.05 and p=0.01, respectively). The latter was also confirmed for day 2 and 3 (p=0.05, p=0.05, respectively) in the Tachosil® group, but not in the control group. No correlation between number of N1 and N2 positive lymph nodes and concentration of cytokines on subsequent postoperative days was found.

In the whole group, a positive correlation between IL-6 pleural concentration on day 3 and number of resected N2 nodes (p=0.03) was found, while in the Tachosil® group and in the control group the positive correlation between IL-6 pleural concentration on day 2 and number of resected N2 nodes was found (p=0.04 and p=0.04, respectively).

In the entire group, the number of blood units transfused positively correlated with pleural hemoglobin concentration on three subsequent postoperative days (p=0.05, p=0.01, p=0.01) and with total amount and duration of pleural drainage (p=0.01, p=0.002). In the Tachosil® group no such correlation was observed (p=0.23, p=0.57. p=0.35, p=0.35 and p=0.052, respectively). For the control group a positive correlation between number of blood units transfused and pleural hemoglobin concentration on day 2, total amount and duration of pleural drainage was found (p=0.02, p=0.01 and p=0.04, respectively). This suggests that blood transfusions in patients from the Tachosil® group were rather

due to preoperative anemia and intraoperative blood loss than due to postoperative blood loss with pleural fluid.

In the whole group, serum IL-6 concentration before surgery (IL-6S0) positively correlated with preoperative serum concentrations of IL-8 and IL-1ra (p=0.04 and p=0.02). Postoperative serum concentrations of IL-6 positively correlated only with postoperative IL-8 serum concentration (p=0.001). Pre- and postoperative serum concentration of cytokines did not correlate with pleural concentration of all three cytokines on subsequent postoperative days. Pleural IL-6 concentration on day 1 (IL-6P1) correlated positively with IL-6 concentration on day 2 (IL-6P2) and 3 (IL-6P3) (p=0.000001, p=0.01, respectively), with IL-1ra concentration on day 1 (IL-1raP1) and 2 (IL-1raP2) (p=0.02, p=0.03, respectively), as well as with IL-8 pleural concentration on day 2 (IL-8P2) (p=0.03). In the Tachosil® group IL-6P1 correlated positively only with IL-6P2 and IL-8P2 (p=0.02, p=0.01, respectively), while in the control group it correlated positively with IL-6P2, IL-6P3 and IL-1raP2 (p=00000.1, p=0.001 and p=0.04, respectively). This suggests, that elevation of pleural concentration of IL-6 may be partly due to immunological reaction to Tachosil®, while pleural concentration of IL-8 does not depend upon local immunological reaction to Tachosil®.

In the ROC (Receiver Operating Characteristic) curve analysis the probability of development of postoperative complications was higher with increased serum concentration of IL-6 at the end of surgery and increased amount of total postoperative drainage.

Discussion:

Systematic mediastinal lymphadenectomy is unquestionably one of the most accurate method of intraoperative mediastinal staging in lung cancer patients [1] and is believed to improve survival in stage II and III lung cancer, especially in patients with cancer of the right lung [11], although survival benefit in patients with left lung cancer was also observed [12]. However, its influence on 5-year survival in early stage cancer is equivocal [13]. Opponents of implementation of systematic lymphadenectomy for every case of lung cancer stress out that it increases the time of surgery [14], amount and time of total postoperative drainage [4] and is likely to cause postoperative cough [15]. Increased postoperative drainage, observed in many institutions, resulted in administration of different hemostatic materials on the mediastinum denuded of mediastinal pleura outside clinical trials. Administration of Tachosil® was proved to beneficial after groin [16], pelvic [17, 18] and axillary [19] lymphadenectomy. Whether administration of Tachosil® on mediastinum after systematic mediastinal lymphadenectomy helps to decrease the amount of postoperative drainage and whether it does not increase the postoperative immune response has never been assessed before. The study by Czerny et al. was performed with the use of Tachocomb® and before a widespread introduction of systematic lymphadenectomy and in their study lymphadenectomy was probably less extended than in ours which corresponds with a short time of postoperative drainage and low total postoperative drainage.

Many surgeons apply hemostatic products on mediastinum after mediastinal lymphadenectomy. These materials are expensive and are likely to increase local immune response. Tachosil®, one of the most effective hemostatic materials and as such often considered the reference product, is also one of the most expensive. The usefulness of other materials applied on mediastinum after lymphadenectomy as well as cost effectiveness of their application should also be assessed.

In our study patients from the Tachosil® group had non-significantly lower results of preoperative pulmonary function test and non-significantly longer time of surgery which could not be explained by mere time of application of Tachosil® on mediastinum which did not exceed 5 minutes. Despite these facts, a positive impact of application of Tachosil® on mediastinum was noted.

One patient from the Tachosil® group needed reoperation due to postoperative bleeding, which increased the pleural hemoglobin concentration in the Tachosil® group. It was the main reason why in the final results of this study we did not observe decreased pleural hemoglobin concentration in the Tachosil® group.

In the Tachosi® group, the time of pleural drainage was not significantly shorter than in the control group, despite total postoperative drainage was significantly lower in the Tachosil® group. This can be explained by removal of drainage even when it still exceeded 100mL per day, in case of prolonged pleural drainage, to avoid infection which was more common in the non-Tachosil® group.

Elevated concentration of IL-6 in pleural fluid in the Tachosil® group was not accompanied by an increased pleural concentration of other two cytokines. This suggests that Tachosil® does not cause a meaningful local immune response.

Conclusions:

The total volume and time of postoperative drainage in this study was several times higher than reported in the literature. We believe that this is the result of implementation of systematic lymphadenectomy. This study showed significant impact of application of Tachosil® on mediastinum after systematic lymphadenectomy on clinical outcome, due to due to postoperative blood loss with pleural fluid.

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Table 1. Comparison of study groups – student t-test

	whole group		Tachosil group (a)		control group (b)		P value a vs b
	mean	SD	Mean	SD	mean	SD	
Age (years)	65.4	7.5	65.9	7.3	64.6	7.9	0.5
weight (kg)	74.9	16.2	72.3	15.4	78.6	16.9	0.2
height (cm)	170.0	7.7	169.0	8.02	171.4	7.2	0.3
FVC % normal	84.1	21.6	81.1	24.2	89.2	15.5	0.3
FEV1 % normal	74.9	21.1	73.8	22.5	76.4	19.2	0.7
packyears smoked	39.1	19.7	38.9	22.5	39.4	16.4	0.9
Time of surgery (min)	171.6	50.1	181.0	58.1	159.8	35.4	0.1
intraoperative blood loss (mL)	396.9	239.4	365.5	232.6	442.5	248.0	0.3
postoperative drainage day 1 (mL)	535.0	348.1	524.3	358.1	547.9	343.0	0.8
postoperative drainage day 2 (mL)	399.7	320.3	363.1	169.2	444.0	440.0	0.4
postoperative drainage day 3 (mL)	222.4	151.7	196.6	100.8	258.1	200.0	0.2
Total drainage (mL)	1597.8	1127	1289.1	641.4	1987.0	1462.0	**0.03**
Total drainage (days)	5.0	2.6	4.7	2.1	5.4	3.2	0.3
Ht before surgery (%)	40.3	3.6	40.2	3.7	40.4	3.7	0.9
Ht at the end of surgery (%)	34.8	4.6	34.8	5.0	34.8	4.2	1
WBC before surgery	7.4	2.0	7.3	1.9	7.5	2.2	0.9
WBC at the end of surgery	12.3	4.0	12.7	4.0	11.9	4.1	0.5
tumor diameter (mm)	38.0	20.3	40.1	23.7	35.7	15.9	0.5
Time of hospital stay (days)	8.6	3.3	8.1	2.6	9.0	4.1	0.3

WBC = white blood count; Ht = hematocrite
FVC = forced expiratory capacity; FEV1 = forced expiratory volume at 1 second

Table 2. Comparison of study groups – Wilcoxon test.

	Median with Tachosil® (a)	Median without Tachosil® (b)	P value a vs b
weight loss (%)	0	0	0.58
ECOG PS	0	0	0.32
Time from smoking cessation	0.6	1	0.80
Pleural hemoglobin day 1 (g%)	2.0	0.9	0.18
Pleural hemoglobin day 2 (g%)	0.5	0.7	0.82
Pleural hemoglobin day 3 (g%)	0.5	0.3	**0.05**
N1 positive nodes	0	0	0.16
N1 resected nodes	6	5	0.54
N2 positive nodes	0	0	0.09
N2 resected nodes	18	14	0.26
Blood units transfused	0	0	0.48
IL6 pleural fluid day 1 (pg/mL)	188790.0	104052.5	**0.01**
IL6 pleural fluid day 2 (pg/mL)	117155.0	68205.0	**0.03**
IL6 pleural fluid day 3 (pg/mL)	41776.5	37345.0	0.42
IL1ra pleural fluid day 1 (pg/mL)	20274	15045.5	0.10
IL1ra pleural fluid day 2 (pg/mL)	12574.5	11306.5	0.59
IL1ra pleural fluid day 3 (pg/mL)	10013.65	12569	0.16
IL8 pleural fluid day 1 (pg/mL)	3968.8	4552.4	0.99
IL8 pleural fluid day 2 (pg/mL)	3829	3828.9	0.67
IL8 pleural fluid day 3 (pg/mL)	4070.1	7654.2	0.76

ECOG PS = Performance Status according to Eastern Cooperative Oncology Group
N1= intrapulmonary and hilar N2=ipsilateral mediastinal

LEGENDS TO FIGURES:

Figure 1. Intraoperative blood loss and total postoperative drainage in both study groups. P<0.05 for total postoperative drainage between study groups.

Figure 2. Concentration of IL-6 in pleural fluid on three subsequent postoperative days. P=0.01 on day 1 and P= 0.03 on day 2 between study groups.

Figure 3. Concentration of IL-8 in pleural fluid on three subsequent postoperative days. P>0.05 on three subsequent days between study groups.

Figure 4. Concentration of IL-1ra in pleural fluid on three subsequent postoperative days. P>0.05 on three subsequent days between study groups.

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