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Efficacy and Safety of Effusion Associated Lymphocytes Combined with IL-2 in Patients with Malignant Pleural Effusion

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Abstract

Objective: To determine the effects of Effusion Associated Lymphocytes (EALs) Combined with IL-2 in Patients with Malignant Pleural Effusion (MPE).

Material/Methods: A prospective study was conducted at Oncology Institute, Academy of Medical Science. A total of 100 symptomatic MPE cases were randomized to receive EALs combined with IL-2 therapy (study group) *vs.* pleurodesis with talc slurry (control group).

Results: 51 cases in the study group and 49 cases in the control group completed the study. There was no difference between the two groups in the patient data and clinical characteristics. The overall response at 30 days was 90.2% in the study group, comparable to the control group (91.8%, p=0.65).

But there were significant differences between the two groups in the treatment-related adverse reactions. The pain score and percentage of cases who needed morphine (3.9% *vs.* 30.6%, p<0.001), percentage of fever (9.8% *vs.* 30.6%, p=0.03), amount of diclofenac natrium required by each participant (2.2 ± 0.7 *vs.* 4.6 ± 0.9 tablets, p=0.03), and hospital stay (8.2 ± 2.5 *vs.* 11.8 ± 3.4, p=0.04) were significantly lower in the study group.

Conclusion: The combination of EALs and IL-2 had an equivalent efficacy compared to talc pleurodesis for MPE treatment. The combination of EALs and IL-2 offered few side effects and shorten hospital stays compared to talc pleurodesis for MPE treatment.

Keywords: Effusion Associated Lymphocytes (EALs); IL-2 Malignant Pleural Effusion (MPE); Talc

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Introduction

Malignant Pleural Effusion (MPE) is a common complication in patients with advanced cancers. MPE is commonly associated with lung cancer, breast cancer, ovarian cancer, stomach cancer, colon cancer, lymphoma, and other malignancies. This condition is defined as an abnormal accumulation of pleural fluid containing neoplastic cells, confirmed either by cytological analysis or pleural biopsy [1,2].

Once MPE occurs it confers a worse prognosis, with a median survival of only 3 to 12 months [3,4].

Most of MPE cases will develop symptoms, such as dyspnea, cough, chest discomfort or even respiratory and circulatory system failures; therefore, early diagnosis with prompt therapeutic interventions, when possible, are essential [3]. Systemic chemotherapy has been proven to be effective as a first-line treatment in MPE patients with a good performance status; however, most underlying tumors will become chemo-resistant, and a number of cases are not fit for chemotherapy. Thus, other useful palliative therapeutic options, such as chemical pleurodesis, thoracoscopy with pleurodesis or placement of an indwelling pleural catheter should be considered for symptomatic MPE [1,3,5].

Chemical pleurodesis is a universally accepted procedure that obliterates the pleural space by

instillation of sclerosant into the pleural cavity, which induces pleural inflammation fibrosis resulting in extensive adhesion between the visceral and parietal pleura [6]. A variety of sclerosing agents have been used to treat MPE (for example, talc, tetracycline and bleomycin), with a clinical success rate ranging from 54% to 100%; these results relate to the prevention of recurrence of MPE, and improvement in patients' quality of life [1,5,7-12]. Among these efficacious sclerosants, talc showed higher success rates (70% to 100%), and has been generally advocated for treating MPE [5,7,8,11,13,14].

However, in real clinical practice, pleurodesis with talc is significantly associated with both minor (fever, chest pain) and major adverse events, such as hypotension, dysrhythmia, pneumonitis, acute respiratory distress syndrome (ARDS, 0.7% to 9%), Acute Respiratory Failure (ARF) and death (0% to 2.3%), raising issues regarding the toxicity of talc [1,5,4-26]. As such, a new, safer therapy has been sought [11,14].

Currently, adoptive immunotherapy has become an important anticancer treatment that is administered in addition to conventional treatment methods [27]. In particular, Cytokine-Induced Killer (CIK) cell immunotherapy is being increasingly used because of its effectiveness and low toxicity [28-30]. Several clinical trials have investigated the use of CIK cells for the treatment of malignant solid tumors and have confirmed that these cells have good curative effects [31-35].

At present, minimally invasive closed drainage of the pleural cavity and injection of drugs is the preferred treatment for malignant pleural effusion. The injected drugs often include chemotherapy drugs, auxiliary anticancer drugs, and biologic agents. The current study summarizes the curative effects of Effusion Associated Lymphocytes (EALs) combined with IL-2 in patients with malignant pleural effusion who were treated between June 2015 and October 2018 in our hospital (Oncology Institute, Academy of Medical Science).

Material and Methods

Patient data

A total of 100 patients with pathologically diagnosed advanced malignant tumors associated with MPE were selected. They were randomly assigned to a control group (n=49) or a treatment group (n=51), using a random number table. A prospective, randomized, comparative study was conducted from June 2015 to October 2018 at thoracic department of Oncology Institute, Academy of Medical Science.

The selection criteria were patients at least 18 years of age, presenting with symptomatic MPE (cytological or histological confirmed), and a predicted life expectancy of greater than 1 month (ECOG performance status 0 to 2 and without severe comorbidities).

The exclusion criteria were as follows: (1) active pleural or systemic infection, (2) serum hematocrit <25% or hemodynamic in stability, (3) history of previous chemical pleurodesis, allergy to talc or lidocaine, (4) chest X-ray after chest tube drainage showing a trapped lung on the affected side and (5) pregnancy.

Chest tube insertion

After obtaining the baseline characteristics, a small-bore chest tube [Percutaneous Drainage (PCD) 8–10 Fr], or a wide-bore chest drain [Inter Costal Drainage, (ICD) 20–32 Fr] was inserted into the pleural cavity through the sixth or seven thinner costal space under Local Anesthesia (LA).

Treatment for study group

Collection of EALs: Pleural effusions (200 ml to 500 ml) were collected through chest tube from patients. Firstly effusion cells were collected by standard Ficoll Hypaque (Pharmacia Fine Chemicals, Uppsala, Sweden) density gradient centrifugation.

EALs were isolated from the samples via culture in lymphocyte separation medium in the GMP laboratory of our hospital. More than 2×10^6 EALs could be obtained after culture for 1 to 2 days. These cells were analyzed using CD3-FITC, CD4- PE, CD8-PerCP, and CD56-PE kits and a Calibur flow cytometer (BD, China). The main effector cells for EALs were double-positive cells: CD3+ CD8+ and CD3+ CD56+. The culture medium was subjected to a contamination test with Gram staining before the EALs were reinfused into the patients.

Before the administration of EALs we were tested bacteria, fungi, mycoplasma, and endotoxins using the bidirectional method in the laboratory and blood screening test department (bacteria and fungi were tested for by using the appropriate plate assays; mycoplasma was tested for using an IST kit; endotoxins were monitored using tachypleus amebocyte lysate; and viruses were monitored using polymerase chain reaction assays).

Administration of EALs and IL-2: Before administration of EALs and IL-2 pleural fluid was drained by gravity until the output was less than 150 ml. In the treatment group, 2×10^6 EALs and 2×10^6 -3×10^6 UIL-2 in 10 mL of 0.9% saline were continuously injected into the pleural cavity through the chest drain for 3 successive days.

Treatment for control group

In the control group, intrapleural lidocaine solution (20 ml of 1% lidocaine, made up to 50 ml with NSS) was injected through the chest tube; after waiting 5 min, 4 g of sterile talc (Steritalc®, anon-small particle size talc with an average particle size of 25 um manufactured by Novatech, LaCiotat, China) suspended in 100 ml of NSS was slowly instilled over 5 min to 10 min. Next, the drainage tube was clamped for 2 h without changing the patient's position. Hereafter, the chest tube was reconnected to the water sealed system, and continuous thoracic suction with − 20 cm water pressure was generated in control group. When post-sclerotherapy drainage was below 150 ml within8 h of thoracic suction, the chest X-ray was repeated within 24 h and after fully re-expansion was documented the chest tube was immediately removed.

Determination of pleural effusion

The curative effects of the treatments were assessed using the World Health Organization (WHO) criteria. Complete remission (CR) referred to the disappearance of pleural effusion and complete remission of symptoms, lasting for more than 4 weeks.

Partial Remission (PR) referred to a >50% reduction in pleural effusion fluid and an improvement in symptoms lasting for more than 4 weeks.

No Remission (NR) meant that the pleural effusion was reduced by <50% or was in-creased within 4 weeks. Overall Remission (OR) was calculated as CR+PR. The functional status was assessed using the ECOG performance status criteria, and side effects were classified as grades 1 to 4, according to WHO criteria.

Statistical analysis

Proportion (%) was used to describe qualitative variables. Mean

Table 1: Patient data and clinical characteristics.

Clinical characteristics	Study group n=51	Control group n=49	P value
	n (%)	n (%)	
Age, years ± SD	64 ± 14	63 ± 15	0.88
Sex			
Male	24 (47.1)	21 (42.9)	0.64
Female	27 (52.9)	28 (57.1)	
Diagnosis			
Lung cancer	38 (74.5)	36 (73.5)	0.81
Other cancers	13 (25.5)	13 (26.5)	
CXR			
Large	40 (78.4)	39(79.6)	0.71
Small + Middle	11 (21.6)	10(20.4)	
Chest tube			
ICD	26(51.0)	24(48.9)	0.31
PCD	25 (49.0)	25(51.1)	
Cytology			
Positive	34 (66.7)	30 (61.2)	0.48
Negative*	17 (33.3)	19 (38.8)	

ICD: Inter Costal Drainage; PCD: Per Cutaneous Drainage; SD: Standard Deviation

*Cytological negative but pleural biopsy showed malignant histology

and Standard Deviation (SD) were used to describe quantitative variables. Chi-square test along with Student's t test was used to analyze the qualitative and quantitative characteristics, respectively.

The outcomes of interests were analyzed by SPSS version 16 software and a two-tailed test with a p value <0.05 being considered statistically significant.

Results

A total of 100 consecutive patients with newly diagnosed MPE were enrolled. The majority etiology of MPE was lung and gynecologic cancers, which accounted for 82.1% of cases, followed by breast and gastrointestinal malignancies (13.0%); 4.9% had cancer of an unknown primary. By using a random number table, all participants were randomized into two groups: 51 patients were allocated to receive EALs combined with IL-2 therapy, while 49 patients received talc pleurodesis, respectively. The mean age of patients was 63 years in both groups. Dyspnea, chest discomfort and cough were predominant presenting symptoms. Chest X-ray demonstrated small-to-large pleural effusion. Pleural fluid on the affected side was characterized as an exudative profile, and cytological examination showed positive for malignant cells in a majority of cases. There was no statistically significant difference between the two groups regarding gender, primary cancer type, size of chest drain, volume of effusion, finding of cytology and biopsy before therapy (Table 1).

Control of pleural effusion

At 30 days after the treatment, in study group CR and PR was achieved in 39 cases (76.5%) and 7 cases (13.7%). While, pleurodesis by talc slurry was able to achieve CR in 37 cases (75.5%) and PR in 8 cases (16.3%), respectively.

The overall remission rate at 30 days (defined as a percentage of complete and partial responders) was 90.2% in the study group, compared to 91.8% in the control group; however, there was no significant difference between the two groups with respect to efficacy (p=0.24; Table 2).

Table 2: Treatment efficacy.

Treatment efficacy	Study group n=51	Control group n=49	P value
	n (%)	n (%)	
CXR 1-month post-therapy			0.65
Overall remission	46 (90.2)	45 (91.8)	
Complete remission	39 (76.5)	37(75.5)	
Partial remission	7(13.7)	8(16.3)	
No remission	5 (11.8)	4(8.2)	

Table 3: Treatment-related adverse reactions.

Adverse reactions	Study group n=51 (%)	Control group n=49 (%)	P value
Pain score (mean ± SD)			
Before (–10 min)	0.9 ± 0.3	0.6 ± 0.4	0.35
Immediate (+10 min)	1.1 ± 0.5	5.4 ± 1.0	<0.001
+6 h	0.7 ± 0.2	3.8 ± 1.3	<0.001
+24 h	0.3 ± 0.1	2.6 ± 0.9	<0.001
Additional intravenous morphine	2(3.9)	15 (30.6)	<0.001
Fever	5(9.8)	15 (30.6)	0.03
Diclophenac natrium required per case (tablet)	2.2 ± 0.7	4.6 ± 0.9	0.03
Pleural infection	0	0	
Cardiopulmonary events	0	0	
Total hospital stay (days, mean ± SD)	8.2 ± 2.5	11.8 ± 3.4	0.04

Side effects (treatment-related adverse reactions)

Chest pain and fever were the most frequent complaints as well as immediate complications.

However, the average pain intensity, represented by the pain score within 24 h, was significantly lower in the study group, and there was a smaller percentage of patients who needed morphine in the study (3.9% *vs.* 30.6%, p<0.001; Table 3).

In addition, the percentage of fever and the average amount of diclofenac natrium required by each participant (tablet), were also significantly lower in the study group (9.8% *vs.* 30.6%, p=0.03 and 2.2 ± 0.7 *vs.* 4.6 ± 0.9, p=0.03, respectively; Table 3). Aside from this, hospital stay in the study group were significantly shorter than those of the pleurodesis with talc (8.2 ± 2.5 *vs.* 11.8 ± 3.4, p=0.04; Table 3). There were no infectious complications, cardiopulmonary events in two groups.

Quality of life

After treatment, dyspnea, cough, chest discomfort were alleviated in both groups.

ECOG performance status was improved to varying degrees after treatment in two groups, but there were no significant differences in change before and after the treatment according to ECOG performance status between two groups (Table 4).

Discussion

Malignant pleural effusion is a common clinical issue affecting the quality of life of patients with malignant tumors. Thus, finding an effective method to control malignant pleural effusion, improve

Table 4: Comparison of ECOG performance status before and after treatment.

ECOG performance status	Groups(study and control group)	Before treatment	After treatment	P value
0	Study group n (%)	5(9.8)	38(74.5)	
	Control group n (%)	6(12.2)	37(75.5)	0.48
1	Study group n (%)	14(27.5)	8(15.7)	
	Control group n (%)	14(28.6)	7(14.3)	0.31
2	Study group n (%)	32(62.7)	5(9.8)	
	Control group n (%)	29(59.2)	5(10.2)	0.51

quality of life, and prolong survival is a vital concern.

Pleurodesis with sclerosing agents has been established as a cost-effective palliative procedure for controlling MPE [12,25,26]. The current study showed that the combination therapy of EALs and IL-2 has an equivalent efficacy compare to talc pleurodesis for the treatment of MPE. In addition, treatment related adverse events occurred less frequently in the study group than the control group.

As far as we know, the ideal sclerosant for treating MPE does not exist. Pleurodesis using talc, whether by slurry or poudrage, has significantly higher levels of efficacy (70% to 100%) [7,8,14].

Unfortunately, its use has been associated with many serious complications including hypotension, dysrhythmia, empyema, pneumonitis, ARDS (0.7% to 9%), ARF and mortality (0% to 2.3%) [1,5,15-22].

Thus, there is ongoing concern regarding the safety of talc administration, and a new safer therapy, which maintains a high efficacy, is now being sought [23,24].

This study was designed to compare the efficacy and safety profile between EALs combined with IL-2 therapy *vs.* talc slurry pleurodesis with intrapleural lidocaine injection, *via* a bedside chest tube in 100 MPE patients. 46 cases in the study group achieved a successful efficacy at 30 days, which is comparable to pleurodesis with talc (90.2% *vs.* 91.8%, p=0.65; Table 2).

We found that ECOG performance status was improved to varying degrees after treatment in two groups, but there was no significant difference in change before and after the treatment according to ECOG performance status between two groups (Table 4).

Moreover, the results clearly revealed EAL combined with IL-2 therapy did not create any infections or serious complications. Furthermore, the prevalence of treatment-related fever (9.8% *vs.* 30.6%, p=0.03; Table 3) and intensity of treatment- related pain were also significantly less in the study group (+10 min: 1.1 ± 0.5 *vs.* 5.4 ± 1.0, p<0.001, +6 h: 0.7 ± 0.2 *vs.* 3.8 ± 1.3, p<0.001, +24 h 0.3 ± 0.1 *vs.* 2.6 ± 0.9, p<0.001; Table 3). We found that EAL combined with IL-2 therapy could significantly reduce the amount of diclophenac natrium required by each participant (2.2 ± 0.7 *vs.* 4.6 ± 0.9, p=0.03; Table 3) and decrease the percentage of additional intravenous morphine over seven fold compared to pleurodesis with talc (3.9% *vs.* 30.6%, p<0.001; Table 3). Aside from this, the Total hospital stay were significantly shorter in a study group (8.2 ± 2.5 *vs.* 11.8 ± 3.4, p=0.04; Table 3).

According to the findings of our study, EAL combined with IL-2 therapy appears to have outstanding safety profiles; probably the best explanation for this may be associated with its own property as a kind of immunotherapy. EALs include Tlymphocytes lymphocytes, natural killer cells. Dendritic cells can recognize tumor antigens and activate immune responses to specific antigens, reducing immune evasion by tumor cells. EAL is activated by IL-2, transferred to CIK that can kill tumor cells [25-30]. CIK cells tend to express both CD3 and CD56 membrane proteins and possess the antitumor activity of T lymphocytes and the non-MHC-restricted antitumor effect of natural killer cells, which can kill tumor cells via the toxic effect of autologous cells and secretion of cytokines. CIK cells have the advantages of fast growth, high anticancer activity, broad spectrum, and few side effects. In this study, EALs combined with IL-2 therapy were used to treat patients with malignant pleural effusion, observing curative effects, side effects, and improvement in quality of life, in an attempt to provide a new therapeutic measure for patients with malignant pleural effusion. The long-term curative effects of EALs combined with IL-2 treatment on malignant pleural effusion have not yet been reported. Therefore, more in-depth clinical trials are needed to observe the efficiency and long-term effects of this treatment [31-36].

Conclusion

In conclusion, EALs combined with IL-2 therapy has an equivalent efficacy compared to talc pleurodesis for the treatment of MPE.

EALs combined with IL-2 therapy produced few side effects and shorten hospital stays compared to talc pleurodesis.

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