

# Comparative Quantitative Characteristic of Inflammatory Reaction After Pleurodesis Using Solutions of 4% Sodium Bicarbonate and 6% Hydrogen Peroxide in the Experiment

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*The purpose of this study is to perform a comparative analysis of the quantitative characteristics of the inflammatory response in the tissues of the lungs, pleura and adjacent subpleural areas of the chest wall to chemical pleurodesis using solutions of 4% sodium bicarbonate and 6% hydrogen peroxide in the experiment. An experiment was performed on 150 experimental animals (rats) in which spontaneous pneumothorax was modeled. Subsequently, after 1 hour, a solution of 4% sodium bicarbonate or 6% hydrogen peroxide was sprayed. The calculation of key statistical parameters of the presented data was carried out using descriptive statistic methods. The studied samples were evaluated using the Kruskal-Wallis test. Differences in the compared samples were considered significant at  $p \leq 0.05$ . In both comparison groups, the predominance of neutrophils in the first days of the experiment was revealed, which indicates an acute inflammatory reaction to the chemical agent introduction. In the following days of the experiment, a decrease in the number of neutrophilic leukocytes was observed, simultaneously with an increase in the number of lymphocytes, macrophages, histiocytes, which indicated the transition of acute to chronic inflammation. It was established that the pleurodesis method has a statistically significant effect on the number of free cellular elements involved in the inflammatory reaction, despite the stereotype of the very dynamics of free cellular elements in all comparison groups. During chemical pleurodesis using a 6% hydrogen peroxide solution, the signs of acute inflammatory reaction from the pleura and the adjacent chest wall areas in experimental animals are shorter in time and not so intense compared to the similar group where a solution of 4% sodium bicarbonate was used.*

**Keywords:** *non-specific spontaneous pneumothorax, chemical pleurodesis, sodium bicarbonate, hydrogen peroxide, pleura.*

Today non-specific spontaneous pneumothorax is detected among 6.2-7.1% of patients with non-specific lung diseases. There is a noticeable tendency for a steady increase in the incidence of this disease, which according to current data is about 15 patients per 100 thousand people per year [8; 4]. A large number of authors note in their works that the prevalence of this disease is: 7.4 - 18 cases per 100 thousand men and 1.2 - 6 cases per 100

thousand women per year. In 94.5% of cases spontaneous pneumothorax is a complication of bullous emphysema and COPD [2; 3; 10]. The choice of the method for preventing the relapse of spontaneous pneumothorax remains important so far [9].

Many authors find it necessary to perform chemical pleurodesis in the event of nonspecific spontaneous pneumothorax, especially if it is repeated. A number of authors recommend to use various chemical agents: talc, achromycin, 96% alcohol solution, 40% glucose solution, olive oil, hypertonic sodium chloride solution, and many other substances that have their own advantages and disadvantages [6; 5].

In modern thoracic surgery, the question of choosing the optimal chemical agent for pleurodesis does not have a clear answer [7; 1].

The purpose of this study is to perform a comparative analysis of the quantitative features of the inflammatory response in the tissues of the lungs, pleura and adjacent subpleural areas of the chest wall to chemical pleurodesis with solutions of 4% sodium bicarbonate and 6% hydrogen peroxide in the experiment.

## Materials and Methods

An experiment was performed on 150 conventional laboratory WISTAR rats weighing 160-180 grams, 10 animals in a subgroup depending on the time of the experiment, thus 50 animals in each study group, including the control group.

Spontaneous pneumothorax was simulated in laboratory rats by the introduction of air in a volume of 2 ml through a Veress needle under ether anesthesia.

1 hour later one of the injections in the volume of 1.0 ml was sprayed (with solutions of 4% sodium bicarbonate and 6% hydrogen peroxide) using a Veress needle also under ether anesthesia, and air was removed from the pleural cavity. Then the animals were observed and removed from the experiment in groups on the 3<sup>rd</sup>, 5<sup>th</sup>, 7<sup>th</sup>, 10<sup>th</sup>, 30<sup>th</sup> days. The experimental studies were performed in accordance with the "Rules for the Use of Experimental Animals in Researches" (Appendix to the Order of the USSR Ministry of Health dated 08.08.1977 No. 755) and the European Convention for the Protection of the Rights of Vertebrate Animals Used for Experimental or other Scientific Purposes (ETS No. 123 dated 18.03.1986 ) (10).

Samples from the experimental animals' chest organs and tissues were collected for histological examination. Pieces of lungs with adjacent sections of the chest wall were fixed in 10% neutral formalin, followed by the preparation of paraffin sections 6-7  $\mu\text{m}$  thick, which were stained with hematoxylin-eosin for observation purposes after removing paraffin.

During histological examination of the pleural membranes and adjacent sections of the lungs, a comparative analysis of the inflammatory reactions severity was performed depending on the medication used during pleurodesis.

The morphological pattern of inflammation was evaluated by counting free cell populations in the lung tissue (lymphocytes, macrophages, neutrophils, histiocytes) and was the main criterion for evaluating the comparative effectiveness of chemical agents (solutions of 4% sodium bicarbonate and 6% hydrogen peroxide) used for pleurodesis in rats.

The main statistical parameters of the studied data were evaluated by descriptive statistics methods. Comparison of the studied samples was carried out using non-parametric analysis of variance according to the Kruskal-Wallis method. Differences in the compared samples were considered significant at  $p \leq 0.05$

## Results and Discussion

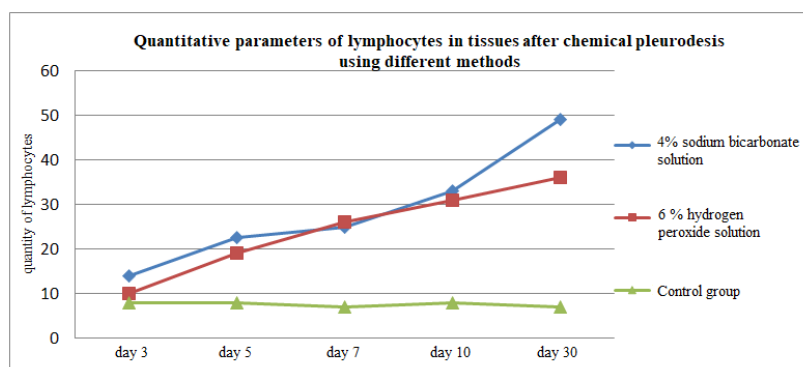
Data on the level of job satisfaction of The results of studying the number of free cellular elements in tissues after chemical pleurodesis with 4% sodium bicarbonate solution and 6% hydrogen peroxide solution compared with the control group are performed in Table 1 (data

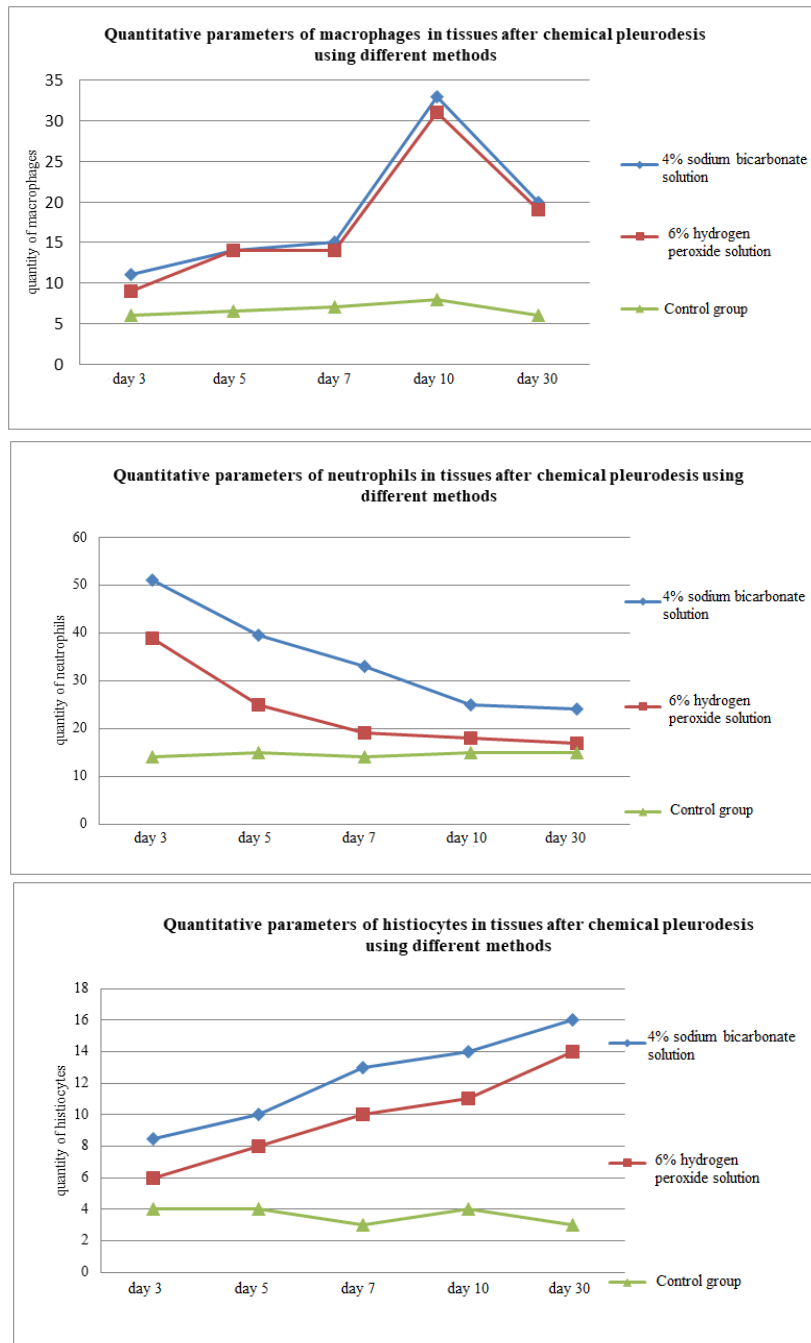
are presented with rounded numbers).

**Table 1.** Quantitative Indicators of Free Cellular Elements in Tissues after Chemical Pleurodesis using Various Methods (units in the field of vision; Me (Q<sub>25</sub>; Q<sub>75</sub>))

Study Period	Parameter	Study Groups		
		4 % sodium bicarbonate solution (n=50), Me (Q <sub>25</sub> ; Q <sub>75</sub> )	6% hydrogen peroxide solution (n=50), Me (Q <sub>25</sub> ; Q <sub>75</sub> )	Control group (n=50), Me (Q <sub>25</sub> ; Q <sub>75</sub> )
3 <sup>rd</sup> day	lymphocytes	14 (14;14)*	10 (9;10)*, #	8 (7;8)
	macrophages	11 (11;12)*	9 (9;10)*, #	6 (6;7)
	neutrophils	51 (51;52)*	39 (39;40)*, #	14 (14;14)
	histiocytes	8.5 (8;9)*	6 (7;8)*, #	4 (3;4)
5 <sup>th</sup> day	lymphocytes	22.5 (22;23)*	19(18;19)*, #	8 (8;9)
	macrophages	<b>14 (14;14)*</b>	<b>14 (13;14)*</b>	6.5(6;7)
	neutrophils	39.5 (39;40)*	25 (25;26)*, #	15(15;15)
	histiocytes	10 (10;11)*	8(7;8)*, #	4 (4;4)
7 <sup>th</sup> day	lymphocytes	25 (25;25)*	26 (26;27)*, #	7 (7;7)
	macrophages	15 (15;16)*	14 (14;14)*, #	7(7;7)
	neutrophils	33 (32;33)*	19 (18;19)*, #	14 (13;14)
	histiocytes	13 (13;13)*	10 (10;10)*, #	3 (3;3)
10 <sup>th</sup> day	lymphocytes	33 (32;34)*	31 (31;31)*, #	8 (7;8)
	macrophages	19 (18;19)*	16 (16;16)*, #	6 (6;6)
	neutrophils	25 (24;25)*	18 (18;18)*, #	15 (15;15)
	histiocytes	14 (14;14)*	11 (11;12)*, #	4 (4;5)
30 <sup>th</sup> day	lymphocytes	49 (48;49)*	36 (36;37)*, #	7(7;8)
	macrophages	20 (20;20)*	19 (18;19)*, #	6 (6;7)
	neutrophils	24 (24;24)*	17 (16;17)*, #	15(15;15)
	histiocytes	16 (16;17)*	14 (13;14)*, #	3(2;3)

Note: \*p<0.01 compared to the control group; \*\* p<0.01 between groups





**Figure 1.** Quantitative parameters of free cellular elements in tissues after chemical pleurodesis using different methods (units in the field of vision)

The performed statistical analysis of the data indicates that the factor – pleurodesis method, affects the response - the number of free cellular elements. Significant differences in all pairs were evaluated as substantial ( $p < 0.01$ ) using the Kruskal-Wallis test, except for the one where the number of macrophages on day 5 was compared between the studied medications ( $p = 0.6147$ ).

All comparison groups are characterized by a gradual increase in the number of lymphocytes, macrophages and histiocytes, starting from the minimum to maximum values, simultaneously with a gradual decrease in the number of neutrophils, starting from the maximum and ending with the minimum values. At the same time, the number of macrophages gradually decreases from day 10 to day 30, not reaching the level of day 7 in

the research. The predominance of neutrophilic leukocytes over other cell populations indicates acute inflammatory reaction to the administration of the medication, a further increase in the level of lymphocytes, macrophages, histiocytes with a simultaneous decrease in the number of neutrophils indicates the transition of acute to chronic inflammation.

As a result of comparing the number of analyzed free cellular elements in dynamics, it was found that when using a solution of 6% hydrogen peroxide as a chemical agent, the increase in the number of lymphocytes, macrophages and histiocytes was faster, but the total number was lower than in the compared groups after pleurodesis with a 4% solution sodium bicarbonate. During pleurodesis with a solution of 6% hydrogen peroxide, the minimum number of neutrophils and the fastest reduction in their number in the same time period were recorded. Slight fluctuations in the number of free cellular elements' initial level were detected in the control group.

### Conclusions

1. The stereotypic dynamics in the number of free cellular elements in all comparison groups was established, associated with the course of the inflammatory process and the transition of acute to chronic inflammation.

2. The performed Kruskal-Wallis analysis suggests that the pleurodesis method has a statistically significant effect on the number of free cellular elements involved in the inflammatory reaction.

3. During chemical pleurodesis using a 6% hydrogen peroxide solution, the acute inflammatory reaction from the pleura and adjacent chest wall sections in experimental animals was not only less in duration, but also lower in intensity, compared with a solution of 4% sodium bicarbonate.

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