THE EUROPEAN JOURNAL
OF
lymphology
and related problems
VOLUME 22 • No. 62 • 2011
INDEXED IN EXCERPTA MEDICA

THE STUDY OF THE COMPOSITION OF THE LYMPH THROUGH ANALYSIS OF SECONDARY LYMPHOCELE

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ABSTRACT

The quantitative composition of the sap has been poorly studied because of difficulties related to methods of sampling biological fluids. The AA. performed a study of lymphatic components found out in collected lymph arising from surgical operations caused or not by cancer. In the same patients were determined the values of some biochemical and cellular components in serum and were monitored quantitative changes, as a result of physical and pharmacological treatments carried out in three weeks after the first haemo-lymphatic sampling. The study revealed a substantial coincidence of the qualitative components between blood and lymph (total protein, albumin, alpha and gamma globulin, Ca, Na, K, Fe, Cl, P, Leukocytes, Interleuchina 1, interleuchina 6, TNF alpha), but with some significant quantitative differences. Some important quantitative changes in lymphatic components have also been highlighted (especially in relation to conditions of inflammation) after specific treatments.

Key Words: Lymph composition. Lymphocele.

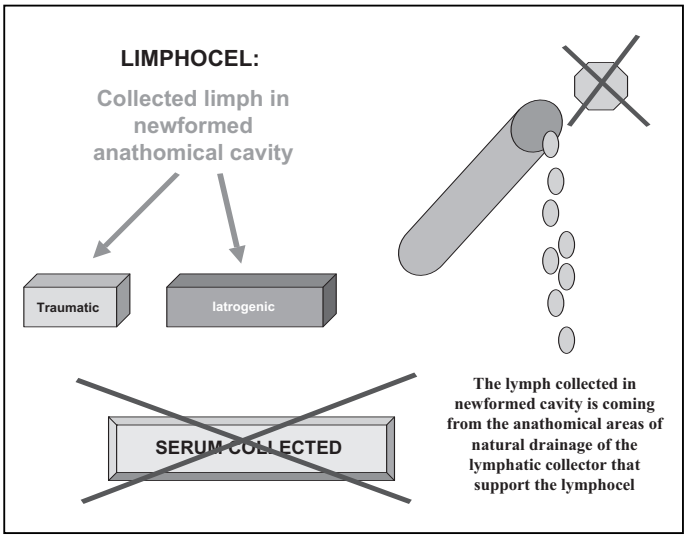


Figure 1: Diagram of lesion lymphocele.

INTRODUCTION

The qualitative and quantitative composition of the sap has been poorly studied as it appears from an examination of the literature [1,2,3,4]. It's easy to understand that this condition comes from the difficulties that arise in clinical practice, such as obtaining "in vivo" of lymphatic fluid. The study arose from the consideration that the sap is collected in a cavity formed as a result of neo-surgical procedure performed to complete the criteria of respect for oncologic radicality (lymphadenectomy) with persistence of one or more open afferent lymphatics collectors to the lymph nodes removed (as not related), or as a result of surgical procedure that causes unreasonable damage with secondary lymphatic routes with the same leakage of lymph, similarly to the "normal" lymph (Fig. 1).
It is in fact extracellular fluid that comes to the newly formed cavity itself, originated in the interstitial spaces of tissues and similarly to the lymph that normally flows through the lymphatic system coming from the healthy tissue intact. As known, the qualitative components, but also some quantitative biochemical and cellular components well known in the blood, are still poorly defined in lymph [5,6]. On such a basis it was suggested to study the quantitative and qualitative composition of these fluids, comparing with the study of blood sampling of the same components in in the same patients and in the same clinical stages of evolution.

MATERIAL AND METHODS

We studied 20 patients (males and females aged between 32 and 76 years) carrying lymphocele (Figs 2b and second), whose origin was not earlier than 15 days from acute state (Table 1).
The amounts of collected "evacuated" ranged from 25 to 785 cc.

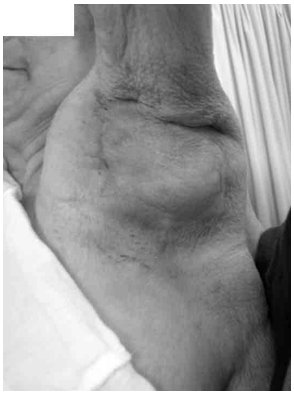


Figure 2a: lymphocele after axillary-femoral bypass.

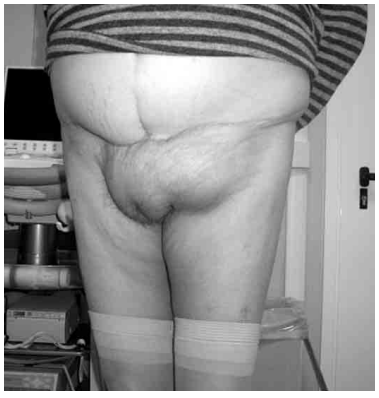


Figure 2b: inguinal lymphocele post vulvectomy for cancer.

Table 1. Scheme of lymphocele examined as a function of the underlying disease and some clinical aspects

Cause	P. without lymphedema	P. with lymphedema	P. with lymphedema and inflammation	Amount of fluid drained
VULVA CANCER		1	1	150 – 785 cc.
MELANOMA	1	2	2	30 – 120 cc.
BREAST CANCER	2	3	1	35 – 250 cc.
AXILLO-FEMORAL BYPASS			1	45 cc.
OVARIC UTERUS CANCER	1	2	1	40 – 320 cc.
MERKEL GLUTEUS			1	180 cc.
NOSE-PHARYNGEAL		1		20 cc.

Patients were performed ultrasound-guided sampling of the fluid collection in the newly formed (Fig. 3a and 3b) at time 0, time 1 (after seven days after first sampling), Time 2 (after 14 days from the first sampling) and time 3 (after 21 days from the first sampling). At the same time patients performed and were studied throught corresponding blood samples from a arm vein.

The plasma samples were performed at 8.00 am, in patients who were fasting and at rest for at least 30 minutes. The following biochemical and cellular components have been studied, both in blood and lymph samples in all subjects, from quantitative point of view, (Table 2).

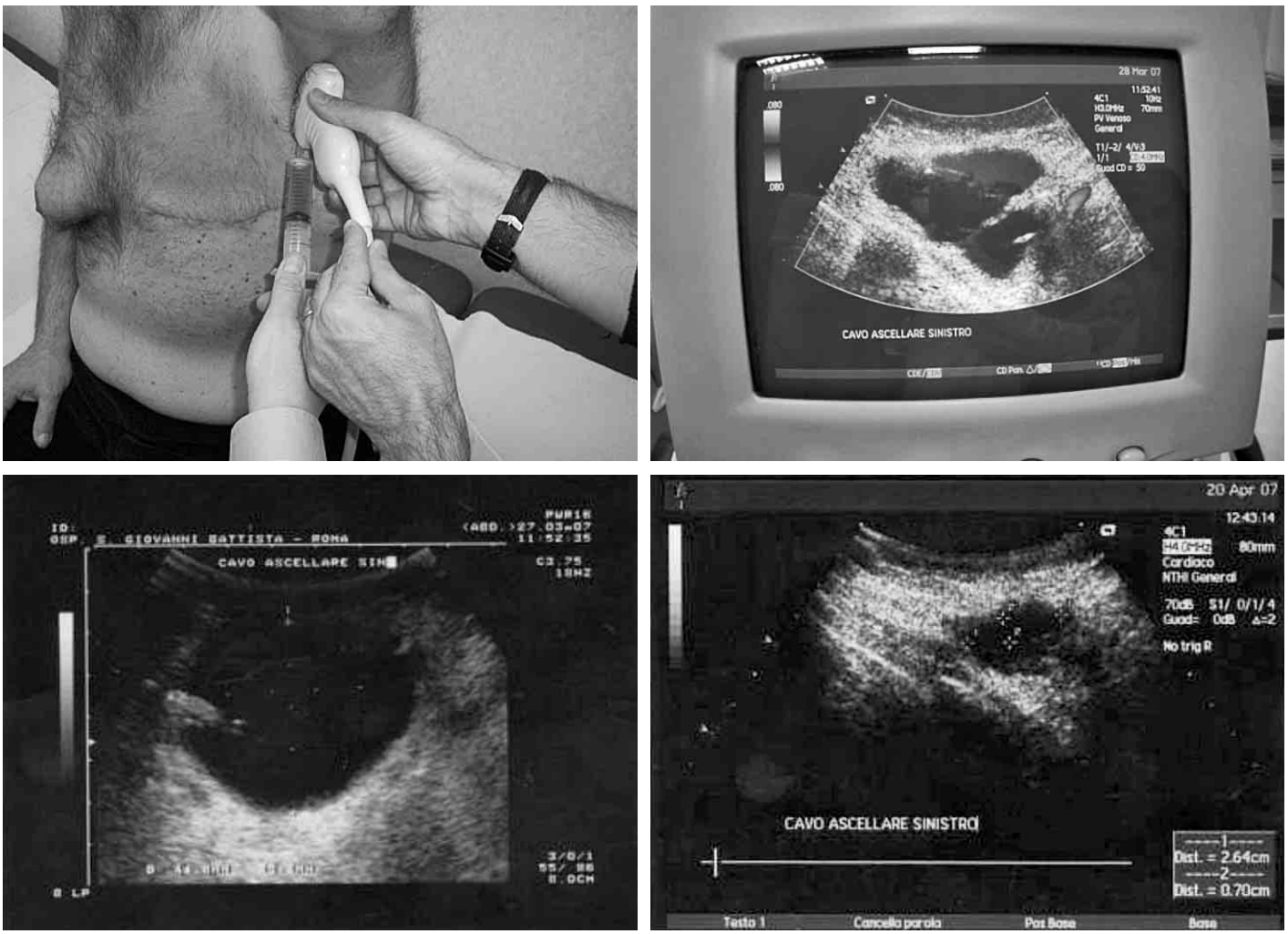


Figure 3a, 3b, 3c, 3d: Exhibit ultrasound before and after evacuation of axillary lymphocele

Table 2. Mean values of plasma concentrations (those lymph are not known).

Component	Lymphatic Component *Concentration known*	Average Blood Component *Concentration known*
Total proteins	?	7.0 gr./dl.
Albumin	?	4,2 gr./dl.
Alfa2 globulins	?	0,8 gr./dl.
Gamma Globulins	?	1,2 gr./dl.
Calcium	?	8,4-10,2 mg/100mL
Fosforum	?	2,3-4,1 mg/100 mL (adults) 4,5-5,5 mg/100 mL (children)
Clorum	?	98-106 mEq/L
Sodium	?	136-146 mEq/L
Potassium	?	3,5-4,5 mEq/L
Leucocytes	?	6.500 cc.
Interleukin1	?	6,1 pg/ml.
Interleukin6	?	5,2 pg/ml.
TNF-alfa	?	4,2 pg/ml.

Patients were divided into 3 groups: Group A (patients): absence of lymphedema and the absence of tissue inflammation in the tissues of origin of lymph collection, Group B (patients): the presence of recent onset of lymphedema with absence of tissue inflammation in the tissues of origin; Group C: absence or presence of recent-onset lymphedema with coexisting acute tissue inflammation. Patients in group A were not treated with any type of therapy. Patients in groups B and C were treated with physical decongestive treatment (manual lymphatic drainage, pressure sequential low pressure - 30-40 mmHg - ultrasounds, physiotherapy active and passive) for the entire period of clinical observation with multi-layer bandage inelastic at the end of each of the three weekly sessions (Fig. 4). All patients performed an homeopathic drug, **Lymdiaral®**: 4 ml. im./die + 20 gtt x 3/die for all the three weeks.

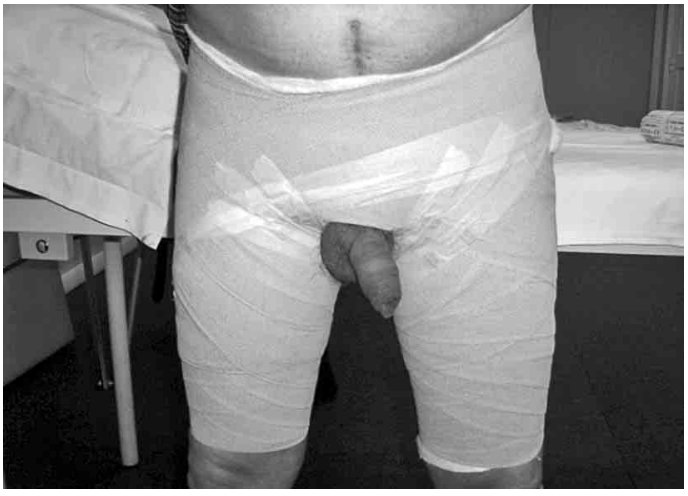


Figure 4: Wrap with secondary lymphedema and lymphocele in a subject with prostate K.

RESULTS

In the collected lymphatic fluids there was a qualitative composition similar to plasma. There were, however, substantial differences in some of the components examined, as shown in Table 3.

Table 3. Results of the quantitative values found in the lymphatic fluids at baseline (T0).

• Total proteins	3.1 g/dl	(2,2 – 3,9)
• Albumin	62,8%	(56,6 – 78,1)
• A2 globulins	6,0%	(3,4 – 12,2)
• Beta globulins	9,8%	(7.0 – 20.1)
• Gamma globulins	7,5%	(6,4 – 12,9)
• Na	141 mEq/l	(139 – 145)
• Ca	7,32 mEq/l	(6,65 – 7,54)
• P	3,92 mEq/l	(2,8 – 4,4)
• Cl	107 mEq/l	(96 – 111)
• Leucocytes	2.150 /microl	(828 – 15.300)

In particular, the average concentration of total proteins was lesser than 45% of the corresponding mean plasma concentration ($p < 0.001$). The percentage of individual globulins concentration was substantially comparable to serum values.
The substantial difference between the electrolyte concentration between lymphatic and blood sample collection was found for Calcium, whose average value was less than 79% of the corresponding plasma concentration ($p < 0.001$) (Graphics 6, 7, 8, 9,16, 17, 18, 19).
Leukocytes were present with an average concentration of about 1/3 compared to plasma.
In subsequent samplings, there were no changes in some of the components between lymph and blood (both biochemical and corpuscular), selected in the individual groups such as analytically shown in the 26 graphics below with the evolutionary merger of some of the lymph and plasma components from the times 0 and 3 times in individual groups studied.
In particular, it was observed for the following components:

– Total Protein: In patients of Group A has been a stagnation of the values found in both the blood sample collection in which lymphatic those of group B in a gradual and continuing reduction of the values in lymph compared with blood sampling, those in group C a slight increase in time constant of the values found in the lymph compared with blood (Graphics 1, 11, 21).

– Albumin: In patients of group A was found to steadily declining values in the lymph fluid; those in group B there was a mean increase of lymphatic albumin in the first ten days with subsequent return to baseline at time 3; while in patients of group C there was a slight increase lymphatic constant values from time 0 to time 3 (Graphics 2, 12, 22).

– Alpha2 globulins: The alpha2 globulins show a similar behavior in all three groups examined in advance of tests, in particular there is a continued gradual reduction of the values found in subsequent samples of all three groups (Graphics 3,13,23).

– Globulins: Even for gamma globulin there was an increase of lymphatic concentration in subsequent samplings in particular in patients of group B.

– Leukocytes: White blood cells show a substantial constancy of values in controls lymphatic success of the group, in groups B and especially C, there was a gradual increase in the lymphatic concentration in the controls until the next time 3 (Graphics 10, 20, 24).

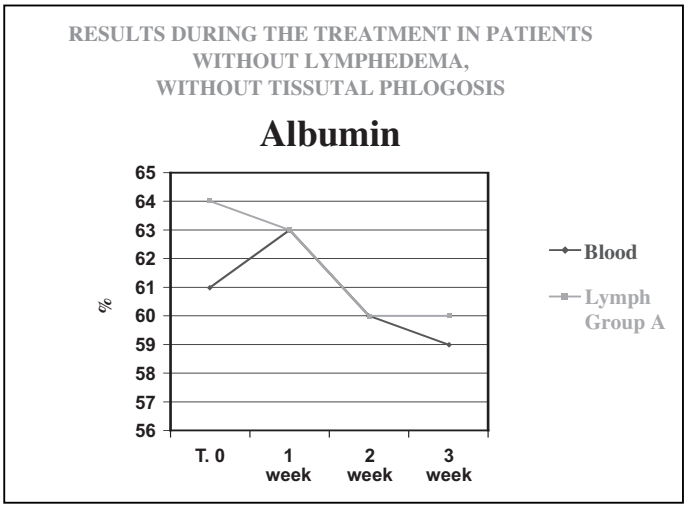
– Interleuchina1: The behavior of interleukin 1 shows higher basal values in group C than groups A and B, with a subsequent slow decline in values with the progress of treatment (Time 3).

– Leukocytes: White blood cells show a substantial constancy of values in controls lymphatic success of the group, in groups B and especially C, there was a gradual increase in the lymphatic concentration in the controls until the next time 3.

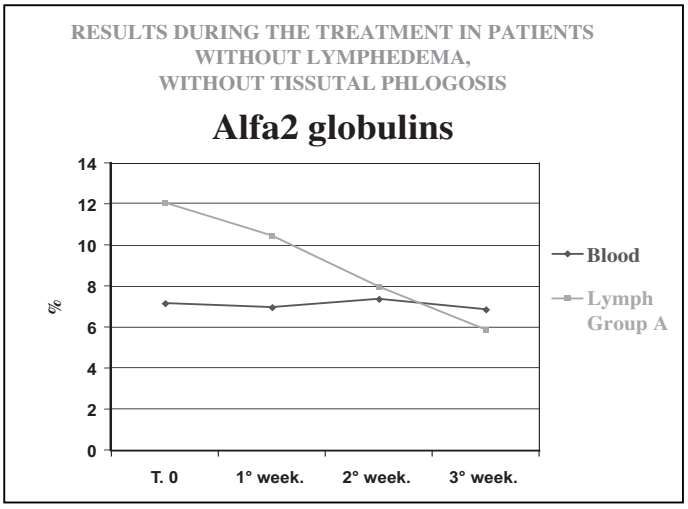
– Interleuchina1: The behavior of interleukin 1 shows high basal values in group C than groups A and B, with a subsequent slow decline in values with the progress of treatment (Time 3) (Graphic 25).

– Interleuchina6: The values obtained show high concentrations in the initial lymphatic groups B and C. Il6 tends to decline in absolute values prominently in the subsequent serial controls (Graphic 26).

– TNFalpha: The TNF alpha does not show substantial changes in the controls after serial value in the three studied groups (Graphic 25). [11]



Graphic 2



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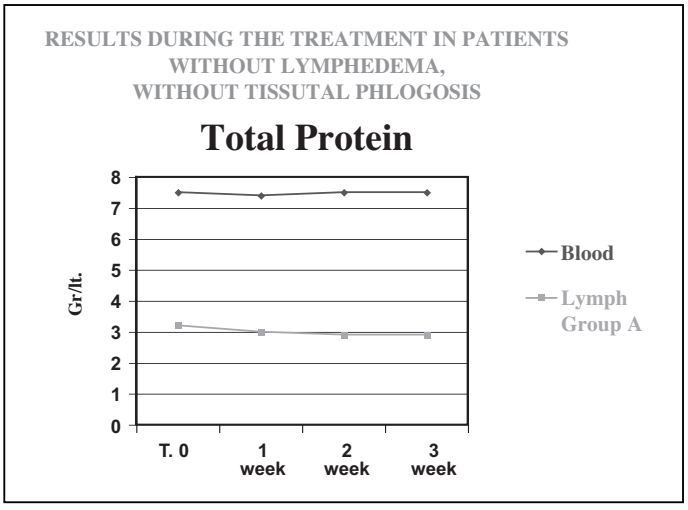
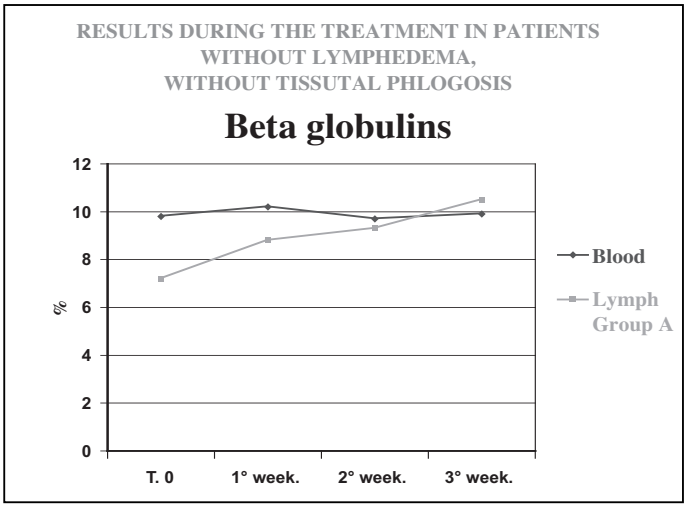


Graphic 1



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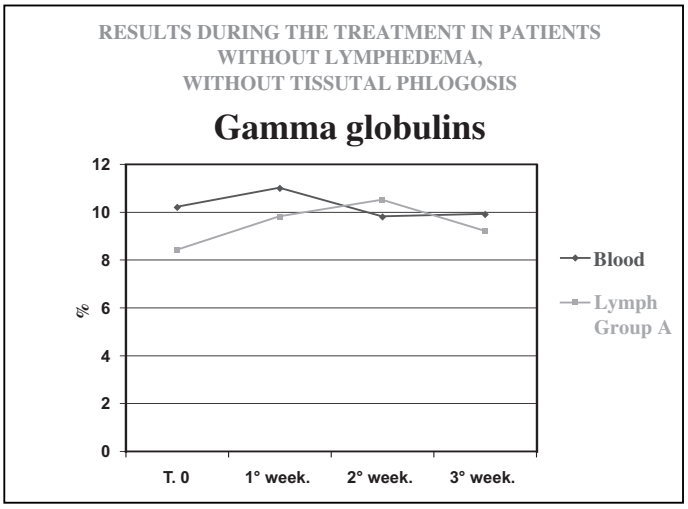

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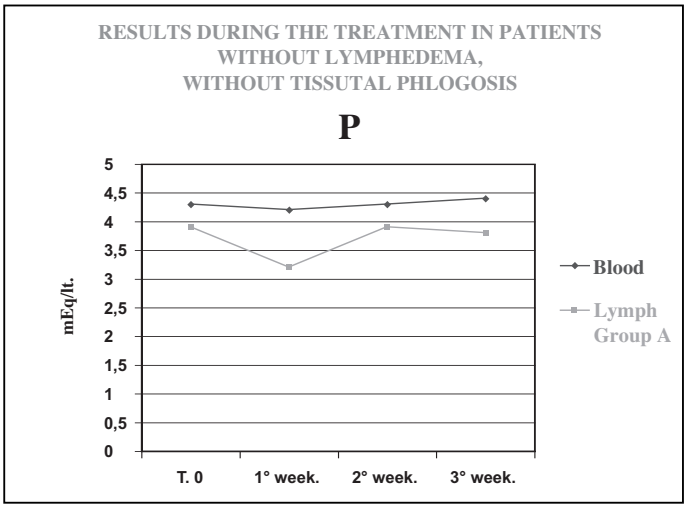

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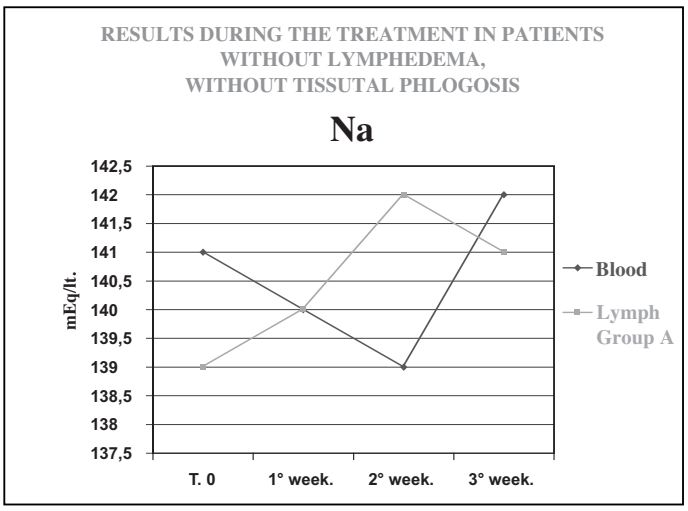

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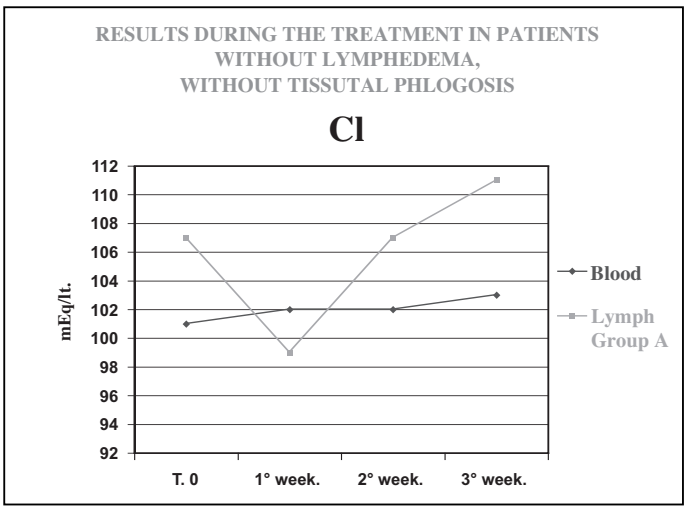

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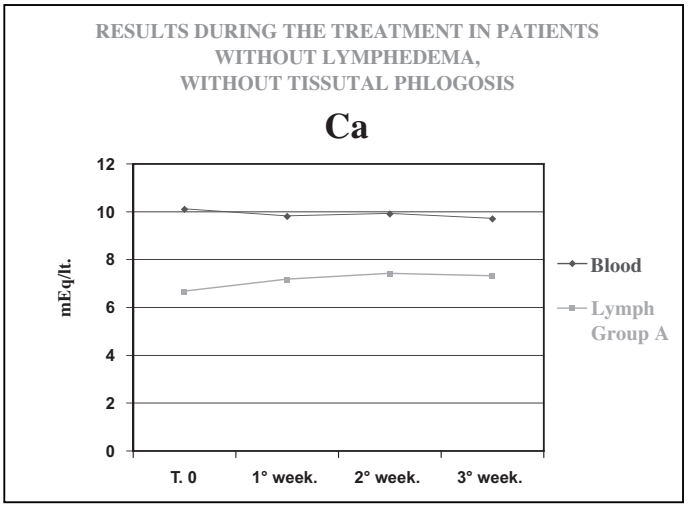

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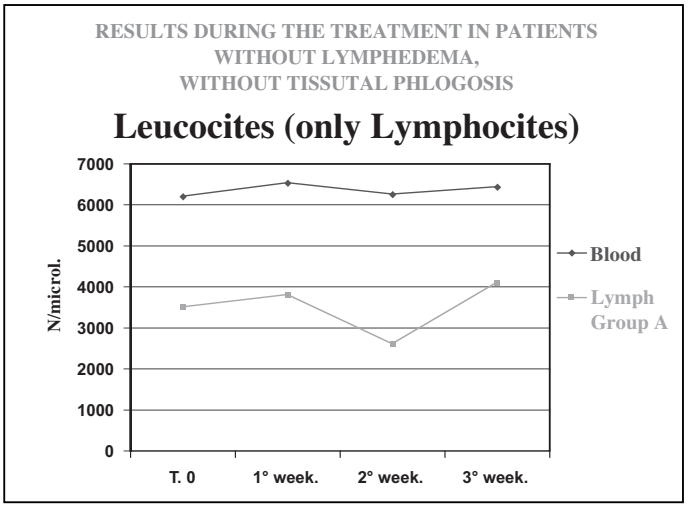

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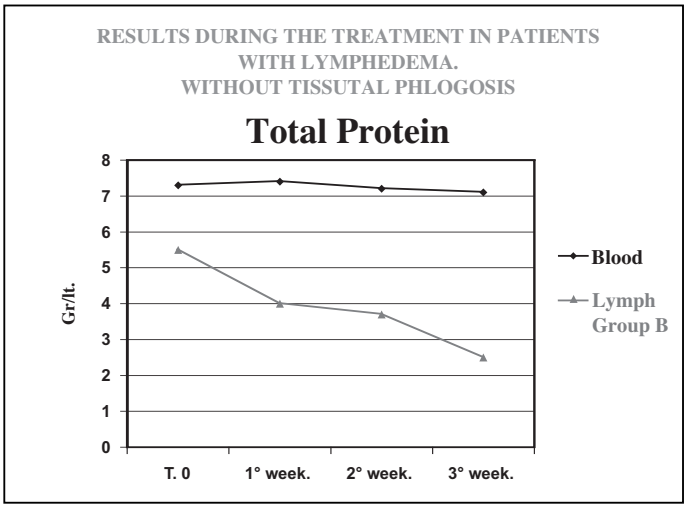

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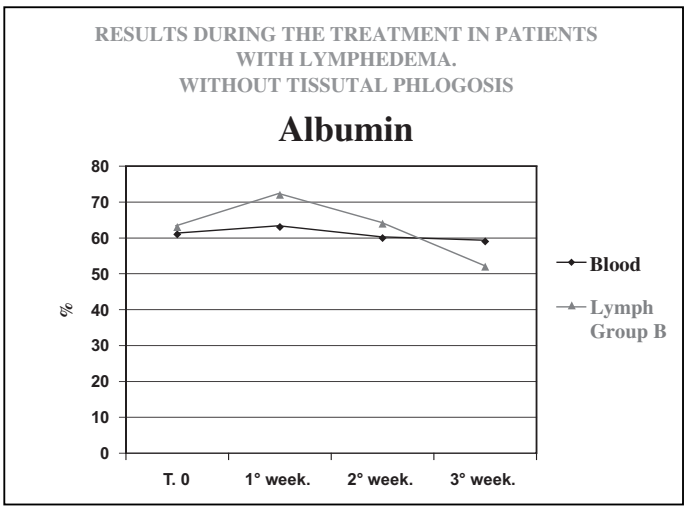

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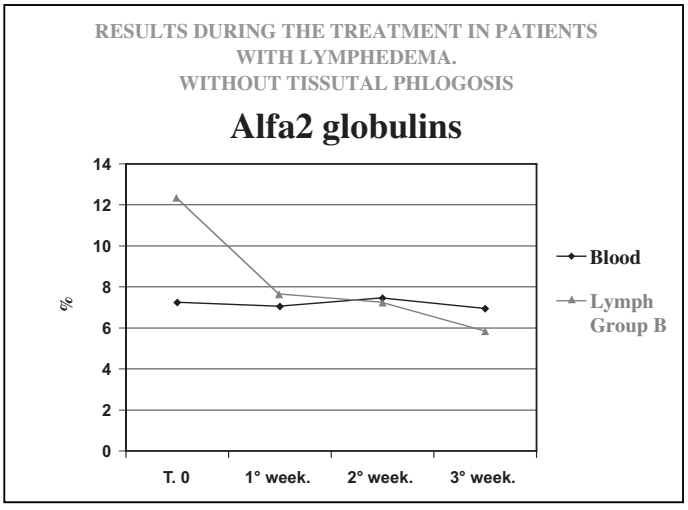

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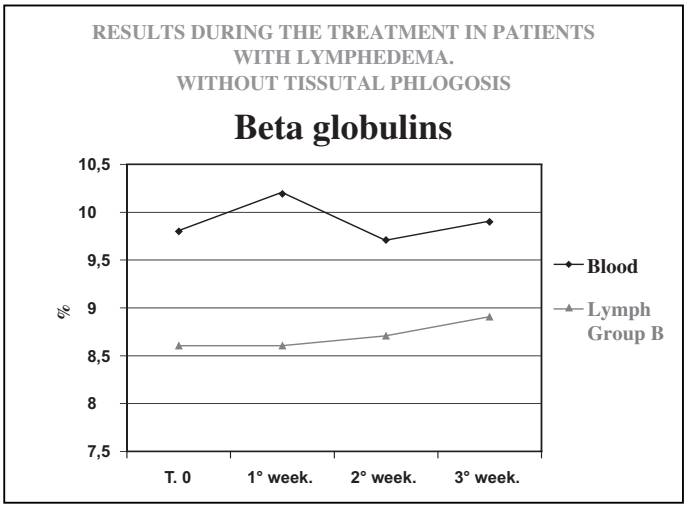

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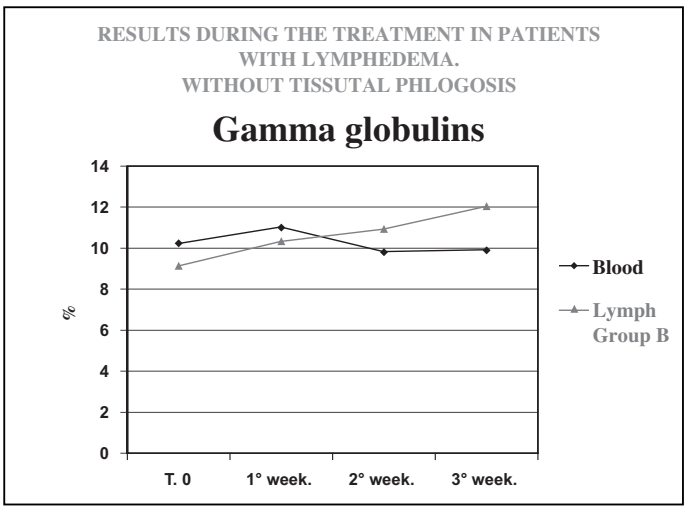

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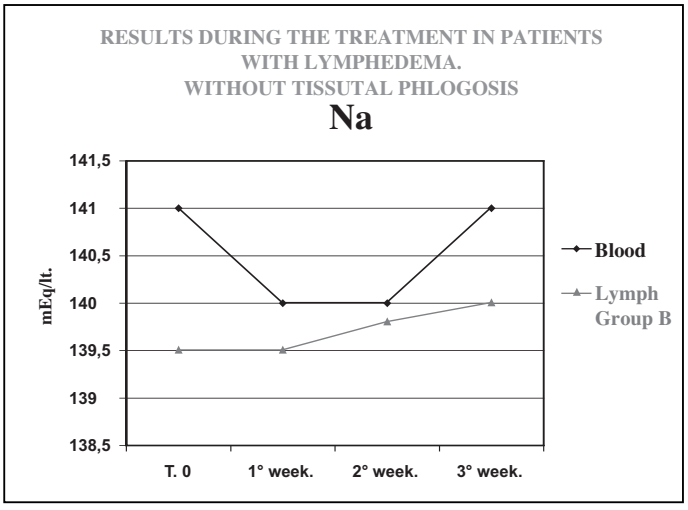

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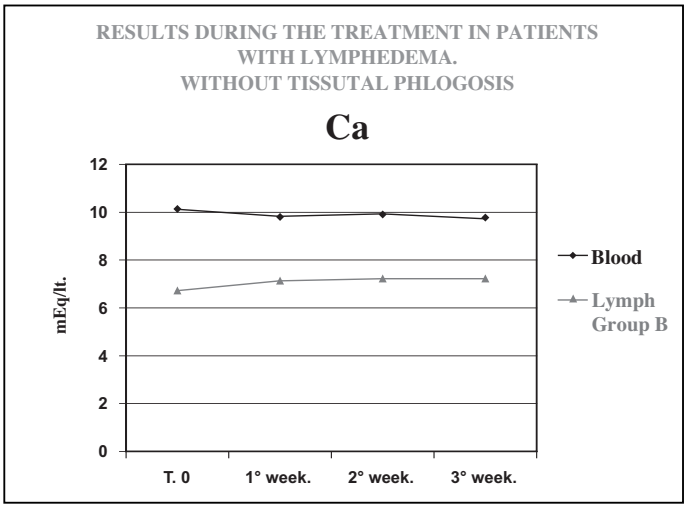

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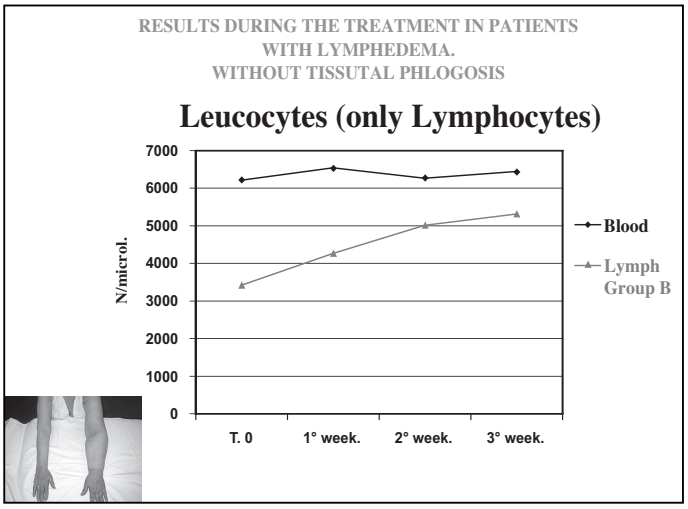

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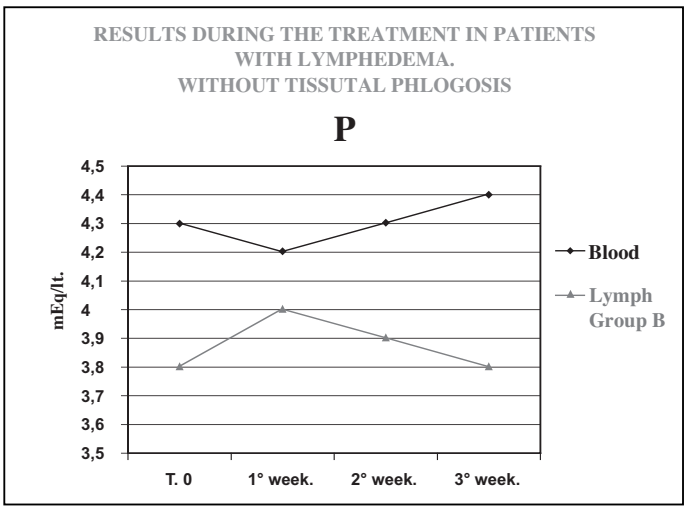

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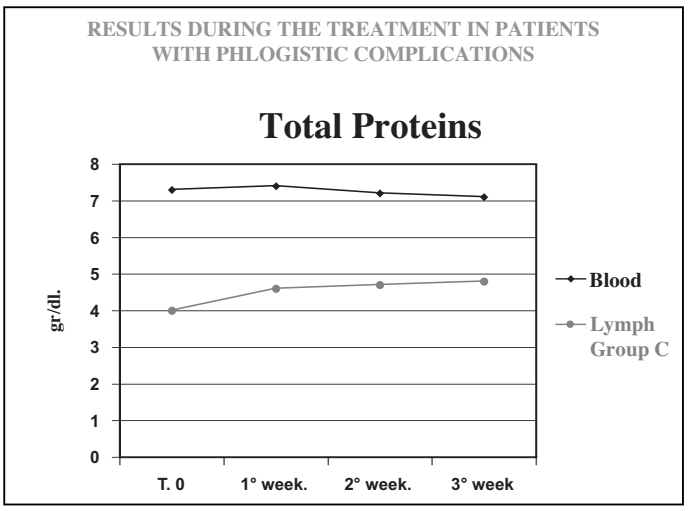

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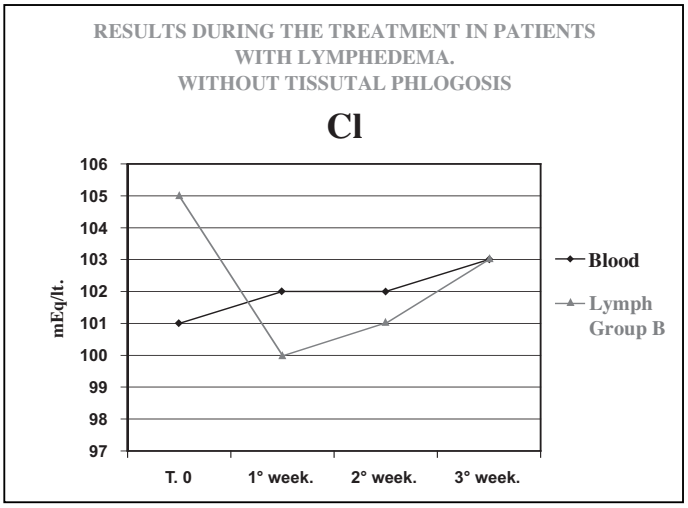

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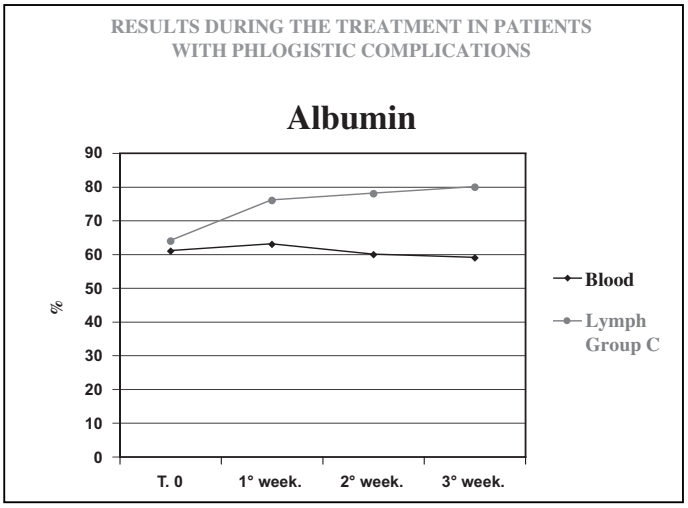

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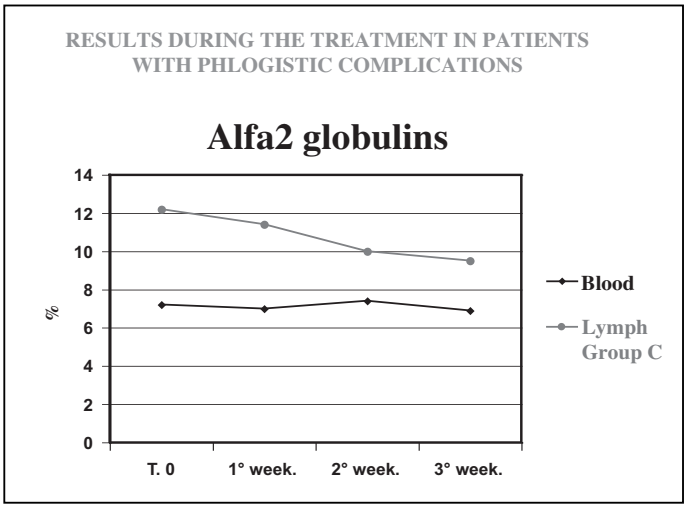

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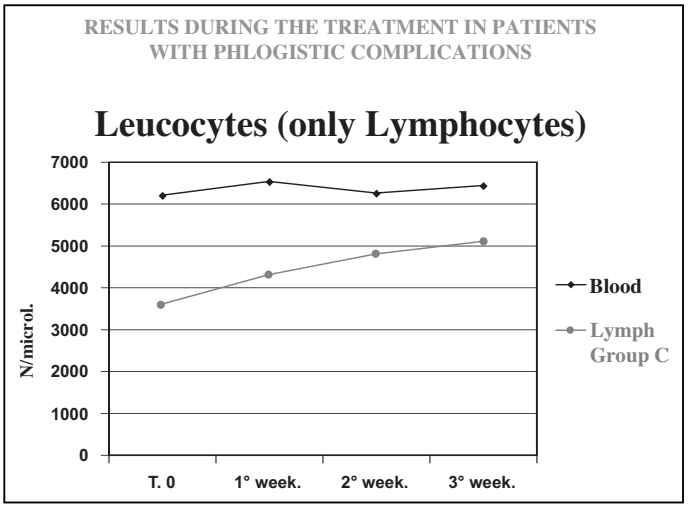

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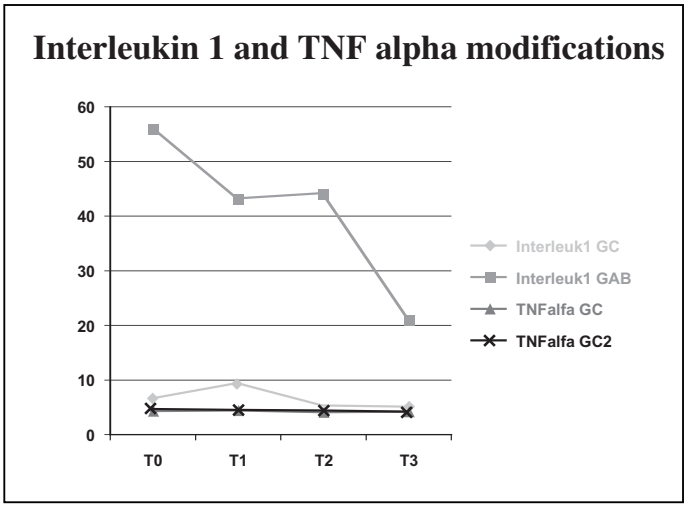

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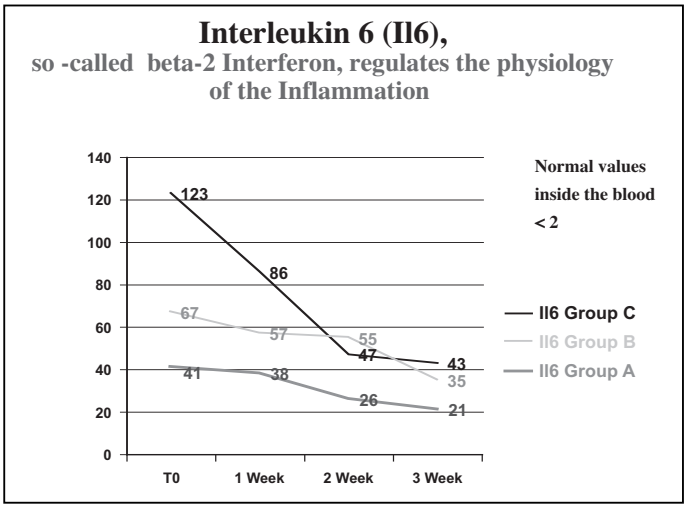

Graphic 26

DISCUSSION AND CONCLUSIONS.

The study shows, in general, the biochemical and cellular components that are found in collected lymph. Those were examined from the point of view of quality, comparable to those blood. Some of these components are essentially the same in terms of quantity, in both body fluids examined in the same subjects. However, there are some substantial differences between the two fluids for certain components (total protein, calcium and white blood cells) [7]. Another interesting observation is that the concentrations of certain biochemical and cellular components linked essentially to inflammation (total protein, alpha2 globulin, leukocytes) are reduced to their absolute values in both body fluids from the same individuals, following the clinical resolution of inflammation, but more marked in the lymph fluid.

Albumin, in particular, is increasing in the early stages of the processing performed in groups B and C, indicating that physical and / or pharmacological treatment are able to remove proteins in tissues from the interstitial spaces and conveyed through the lymphatic collectors to the natural "terminus". Among the group of interleukins studied (the average plasma concentration is unknown, but is completely unknown in the lymphatic fluid) [8,9,10] it was found a little change in the values of TNF-alpha (not statistically detectable) in all studied cases. Conversely, interleukin-1 values were significantly altered with the change of the clinical status in both liquids, but mainly in the lymphatic fluid. Interleukin-6, the more "labile" and the most sensitive, between interleukins, during inflammatory state, showed major changes in both body fluids, with the grater result in lymphocele generated from lymph coming from tissues and lymphedema in inflammatory state.

A final point that must be highlighted is the possibility that the physical treatment and / or medications may alter plasma concentrations of lymph and some of their components, especially those mainly related to inflammation, increased at 0. This study highlights the utility of the definition of quantitative components of the lymphatic and how some of these could be influenced by the treatments, even in the absence of acute inflammation in place. By

analyzing the concentration of leukocytes and, in particular interleukin-1 and interleukin-6, altered even in the absence of acute inflammation in place, it can be shown that these components are not altered in normal tissues (lymph from tissues and not lymphedematous inflammation), while are increased in lymphedematous inflammation (not sap from fabric lymphedematous and inflammation), greatly increased in tissue lymphedematous inflammation (lymphedematous lymph from tissues and inflammation), so the claim by reason of Ethel Földi that says "presence in lymphedema of inflammation are not in the presence of at least one chronic sterile inflammation".

REFERENCES.

1) Wu A.L., Clark S.B. and Holt P.R.: *Composition of lymph chylomicrons from proximal or distal rat small intestine*. American Journal of Clinical Nutrition, Vol. 33, 582-589, 1980. Copyright © 1980 by The American Society for Clinical Nutrition, Inc

2) Allan E., Dumont M.D. and John H. Mulholland, M.D.: *Flow Rate and Composition of thoracic-Duct Lymph in Patients with Cirrhosis*. N. Engl. J. Med., 1960; 263: 471-474 September 8, 1960.

3) Waldemar L., Olszewski. *Lymphatic Research and Biology*. June 2003, 1(3): 235-243

4) Hein W.R., McClure S.J., Miyasaka M.: *Cellular Composition of Peripheral Lymph and Skin of Sheep Defined by Monoclonal Antibodies*. Int. Arch. Allergy Immunol., 1987; 84: 241-246.

5) Hein W.R., Shelton J.N., Simpson-Morgan M.W., Seamark R.F. and Morris B.: *Flow and composition of lymph from the ovary and uterus of cows during pregnancy*. Journal of Reproduction and fertility (1988) 83: 309-323.

6) Robert K. Ockner, Faith B. Hughes and Kurt J. Isselbacher: *Very low density lipoproteins in intestinal lymph: origin, composition, and role in lipid transport in the fasting state*. J. Clin. Invest., 1969; 48(11): 2079-2088.

7) Földi M., Földi E.: *Földi's textbook of Lymphology*. Elsevier, May 2004.

8) Koçer, Abdulkadir MD, MSc; Koçer, Emel MD; Memişoğullari, Ramazan MD; Domaç, Füsun M. MD; Yüksel, Hatice MD: *Interleukin-6 Levels in Tension Headache Patients*. Clinical Journal of Pain: October 2010 - Volume 26 - Issue 8 - pp 690-693.

9) Gillian W. Cockerill, PhD; Tanya Y. Huehns, MD; Arjuna Weerasinghe, MD; Claire Stocker, PhD; Peter G. Lerch, MD; Norman E. Miller, MD, PhD; Dorian O. Haskard, MD, PhD: *Elevation of Plasma High-Density Lipoprotein Concentration Reduces Interleukin-1 – Induced Expression of E-Selectin in an In Vivo Model of Acute Inflammation*. Circulation. 2001; 103: 108.

10) Wilfried Dinh, Reiner Füth, Werner Nickl, Thomas Krahn, Peter Ellinghaus, Thomas Scheffold, Lars Bansemir, Alexander Bufe, Michael Coll Barroso and Mark Lankisch: *Elevated plasma levels of TNF-alpha and Interleukin-6 in patients with diastolic dysfunction and glucose metabolism disorders*. Cardiovascular Diabetology, 2009, 8: 58.

11) Michelini S., Cardone M., Failla A., Moneta G., Todisco R., Todini M., Albergati F.M.: *Study of lymph composition in post-surgical lymphoceleand its modifications by the treatment*. European Journal of Lymphology, 2010; Vol. 22, n° 60, pag 11.

ERRATA CORRIGE

In the last issue (Volume 21, No. 61, 2010) the authors of the article *"The role of diamagnetic pump (CTU mega 18) in the physical treatment of limbs lymphoedema. A clinical study"* were Marcello Izzo, Luigi Napolitano, Vincenzo Coscia, Antonio La Gatta, Fabrizio Mariani, Vincenzo Gasbarro