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Multiparametric analysis of crystallogenic properties of blood serum of healthy people and patients with burn disease

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ABSTRACT

The aim of the study was to assess some of the biophysical properties of dehydrated biological fluids in patients with thermal trauma. Own and initiated by the basic substance (0.9% sodium chloride solution) crystallization of blood serum of 32 patients with burn disease (contact burn, the area of skin lesions - 15-45% of the body surface; acute toxemia phase; age 21-40 years), as well as 30 healthy people of comparable age were studied. It is shown that in thermal injury undergo significant changes all outcome measures of biocrystalloscopic and impedance parameters in relation to their own and initiated the crystallogenesis of the blood serum. These changes are manifested in both time and quality parameters. Thus, burn disease in the stage of acute toxemia is characterized by slowing and inhibition of dehydration structuring of samples, increased degree of destruction of the formed elements, relative narrowing of the boundary zone, etc., which is confirmed by the data of registration of acoustic impedance. Based on these results, it is assumed that the visualization and study of the impedance characteristics of the dynamics of drying of serum droplets can be a convenient tool for diagnosing the patient's condition in combustiology.

Keywords: *Blood serum, crystallization, acoustic mechanical impedance, thermal trauma*

1. Introduction

Burn disease is a complex and multifaceted set of pathological reactions, prevalent among which at the organizational level are metabolic changes [1]. This is due to the leading role in its development of burn endotoxemia, provided by the massive flow of organic toxic substances into the systemic bloodstream [1-3]. The spectrum of these toxins includes both intermediate (interpreted and detectable as "medium-mass molecules") and terminal (urea, creatinine) metabolites [2, 4]. Their presence can significantly transform not only the component composition of biological substrates, but also to ensure the transformation of their biophysical properties. The latter circumstance can be useful in terms of assessing the depth, severity and degree of reversibility of metabolic changes of the body in thermal injury.

On the other hand, the choice of conditions (or model effects) on the biological medium in which the study of their physical and chemical properties will be carried out is of fundamental importance. One of the most closely studied options is dehydration self-organization of biological substrates [5-8]. However, it is important to emphasize that currently the common approach to the interpretation of crystallogenic properties of a biological material is given by a qualitative description provided with photography of the bounds [8, 9], whereas quantitative methods study of biocrystalloscopy are underutilized [5-7]. Other chemical and physical methods in biocrystallomics are used only by a limited number of specialists [10, 11].

It is important to emphasize that the character of crystallogenic roperties of biosubstrates in patients with burn disease has not been studied [6, 12]. Therefore, the aim of this study was evaluation of some biophysical properties by dehydration (drying) of biological media in patients with thermal lesions.

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2. Material and Methods

We have investigated own and initiated crystallization of the blood serum of 32 patients with burn disease (the contact burn, the affected area of the skin - 15-45% of the body surface; acute phase toxemia; age 21-40 years) and 30 healthy individuals of comparable age. Drugs of biological media were prepared using our own methodology [5-7, 13]. Own crystallization of blood serum (crystalloscopic test) was tested by drying of biological fluid on horizontal plane (standard microscopic glass slide) without any physical and chemical stimulators. Initiated crystallization was studied in teziographic test by co-drying of biological fluid and basic substance (stimulator of crystallization) in proportion 1 : 1 on one slide. We used 0.9% sodium chloride solution as the basic substance for teziographic test.

During the whole time of drying of micro-preparations the dynamics of their biophysical parameters were recorded. This complex included the study of the acoustic-mechanical impedance of the drying droplet, as well as a minute-by-minute assessment of the main quantitative parameters of teziocrystalloscopy. Drying was performed in the laboratory conditions (temperature - 23-25°C, humidity - 45-60%) without thermal stimulation of crystal formation.

Measurements of acoustic mechanical impedance (AMI) were performed using a special hardware and software complex provided by Institute of Applied Physics of Russian Academy of Sciences [11, 13, 14]. The volume of samples subjected to AMI analysis was 5 μl. In order to minimize errors, the AMI curve was registered three times. The duration of the signal registration was fixed (30 minutes).

The range of indicators of visualestimation of microspecimens of blood serum were included: for crystalloscopic test – index structure, crystallizability, facia destruction degree and clearity of marginal belt. For teziography we used main teziographic coefficient Q, belt coefficient P, facia destruction degree and clearity of marginal belt [5-7].

Statistical data processing and correlation analysis were performed using Microsoft Excel 2007 spreadsheets, as well as the program Primer of biostatistics 4.03.

3. Results and discussion

The study of the features of free and initiated crystal formation of biosubstrates in thermal trauma allowed us to establish that in the early period of burn disease, the crystallogenic properties and the initiatory potential of blood serum undergo significant transformations that, from our point of view, are destabilizing (Fig. 1 and 2). Thus, and in accordance with the data of figure 1, the General trend found with respect to the crystal formation of the considered biological environment of patients with thermal trauma is a significant slowdown in the rate of dehydration self-organization of the biomaterial. Thus, the stage of the visible beginning of structure formation in drying samples of healthy people begins with 7-8 minutes (without thermal stimulation), whereas in burn disease the signs of crystallization are found only on 17-19 minutes from the moment of applying the biofluid on the glass.

The qualitative and quantitative characteristics of the crystalloscopic sample also differ significantly. In particular, during the whole period of observation, significantly lower level of the index of structure, crystallizability and clarity of the marginal protein zone in comparison with the "pattern" revealed for the control group ($p<0.05$) was revealed in serum micro-preparations.

A slightly different dynamic was obtained in assessing the degree of destruction of facies: in patients with burn disease, despite the much later time of crystallization, the rate of growth of destructive changes is incomparably higher than the control figures, and from the 25th minute the level of this indicator exceeds the values established for healthy people.

The revealed changes were reflected in the analysis of the AMI curve of the control group and patients with thermal lesions (Fig. 2).

Taking into account the information shown in figure 2, we can confirm the thesis of slowing the rate of crystallization of biological fluid in burn disease, as well as the transformation of its dynamics.

The next stage of the study was the evaluation of the initiating properties of the biological substrate in respect of isotonic and pH-free medium of the crystal-0.9% sodium chloride solution.

Considering the nature of the initiatory process of blood serum inert crystal, it is possible, as well as for its crystallogenic properties, to note the significant transformation of the dynamics of dehydration of the biomaterial for all major performance indicators (Fig. 3) caused by the accumulation of toxic substances into the biological medium [2, 4]. The inhibitory effect of these substances led to decreasing of studied parameters of the facias of patients with burn disease is compared to levels of control subjects. The potential mechanism of this phenomenon, from our point of view, is due to the fact that in endotoxicosis of moderate severity the concentration of medium - and low-molecular organic compounds-metabolites stabilizes the biological medium in non-crystalline form (Sol, gel). This explains the low level of the main teziographic coefficient throughout the observation period. Existing patients reporting severe hypoproteinemia reduces the values of coefficient zones and width of the regional protein zone, and can also indirectly (because of dysproteinemia and relative hypoproteinemia) to reduce the triggering properties of the biosubstrate in thermal injury. All of the above explains the high rate and severity of destructive (destabilizing) processes in the drying drops of blood serum.

Study of the impedance metric of the biological environment confirmed the General trend in the depression of crystallogenic and initiating properties of biofluids in

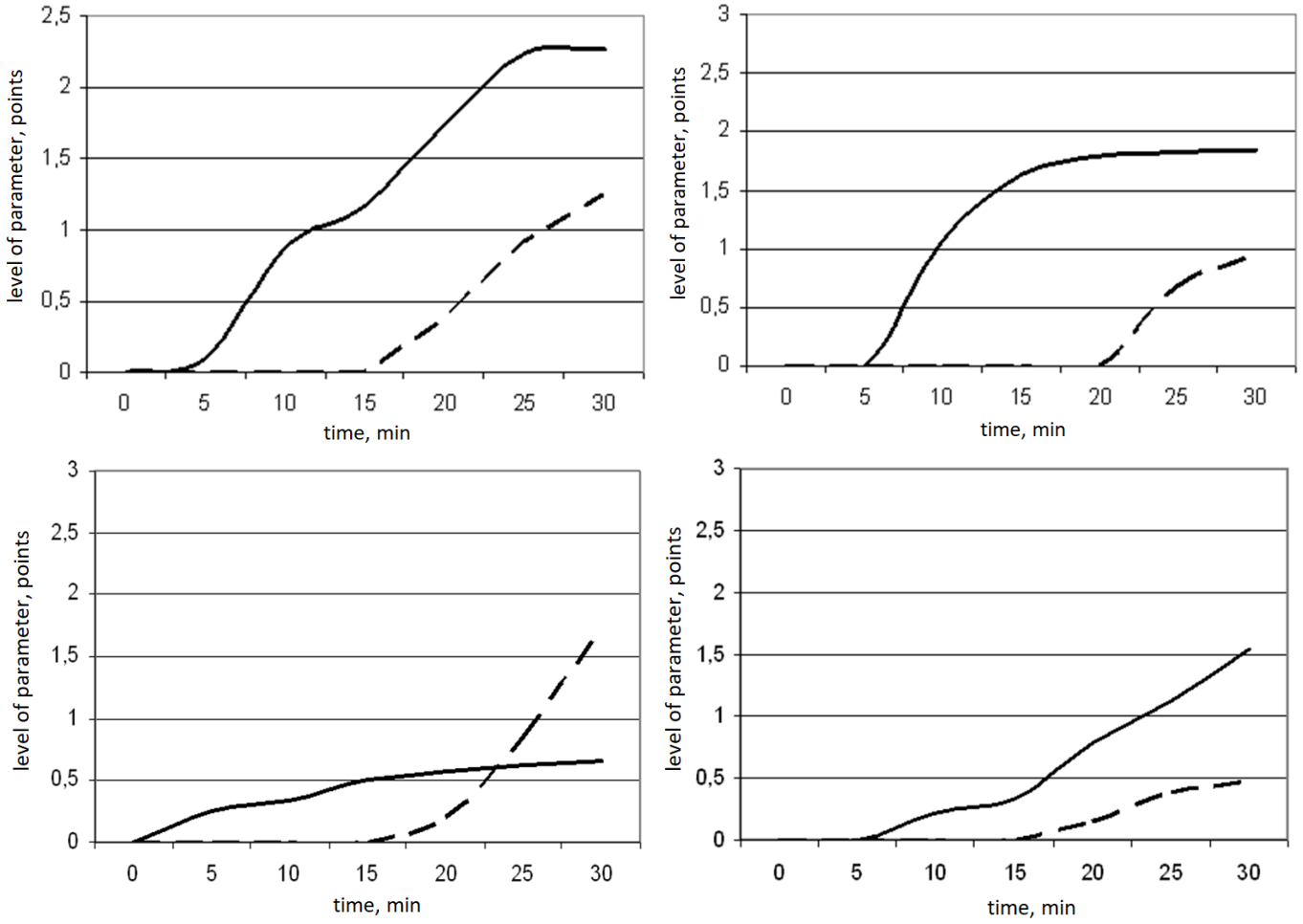

Figure 1 | Dynamics of main parameters of crystalloscopic facias of healthy people (n=30) and burned (n=32) persons (solid line indicates healthy people; punture line indicate patients with burn disease)

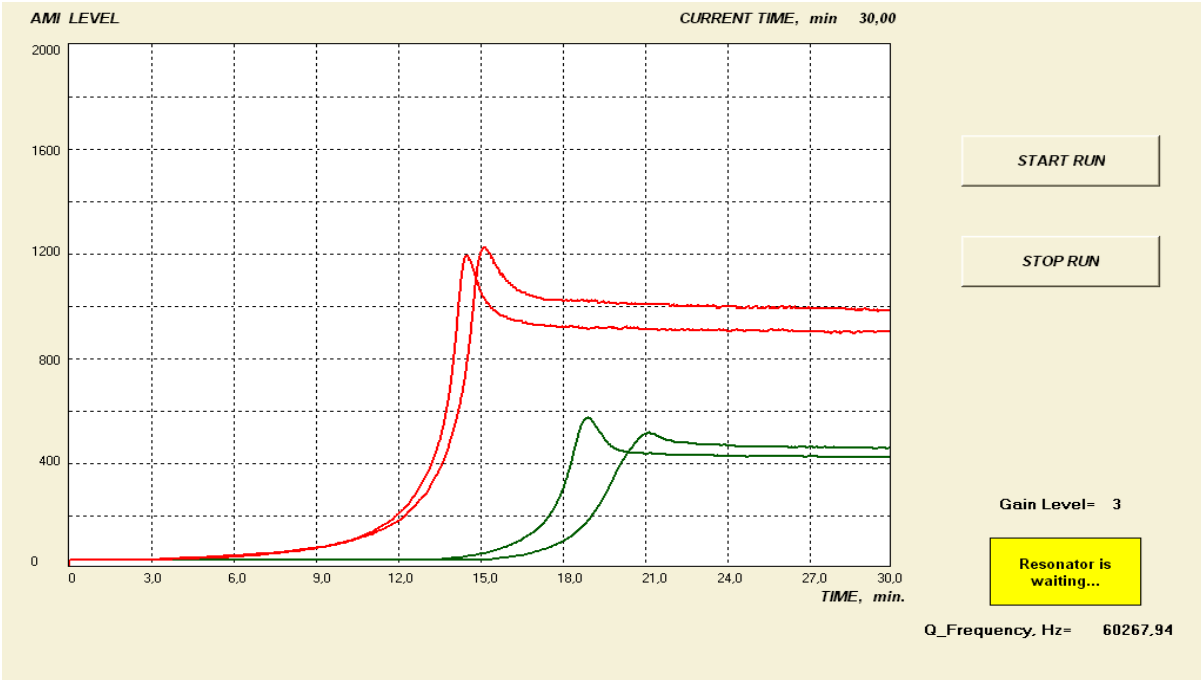

Figure 2 | Example of curves of acoustic and mechanical impedance of drying drops of blood serum from healthy people (1) and burned persons (2)

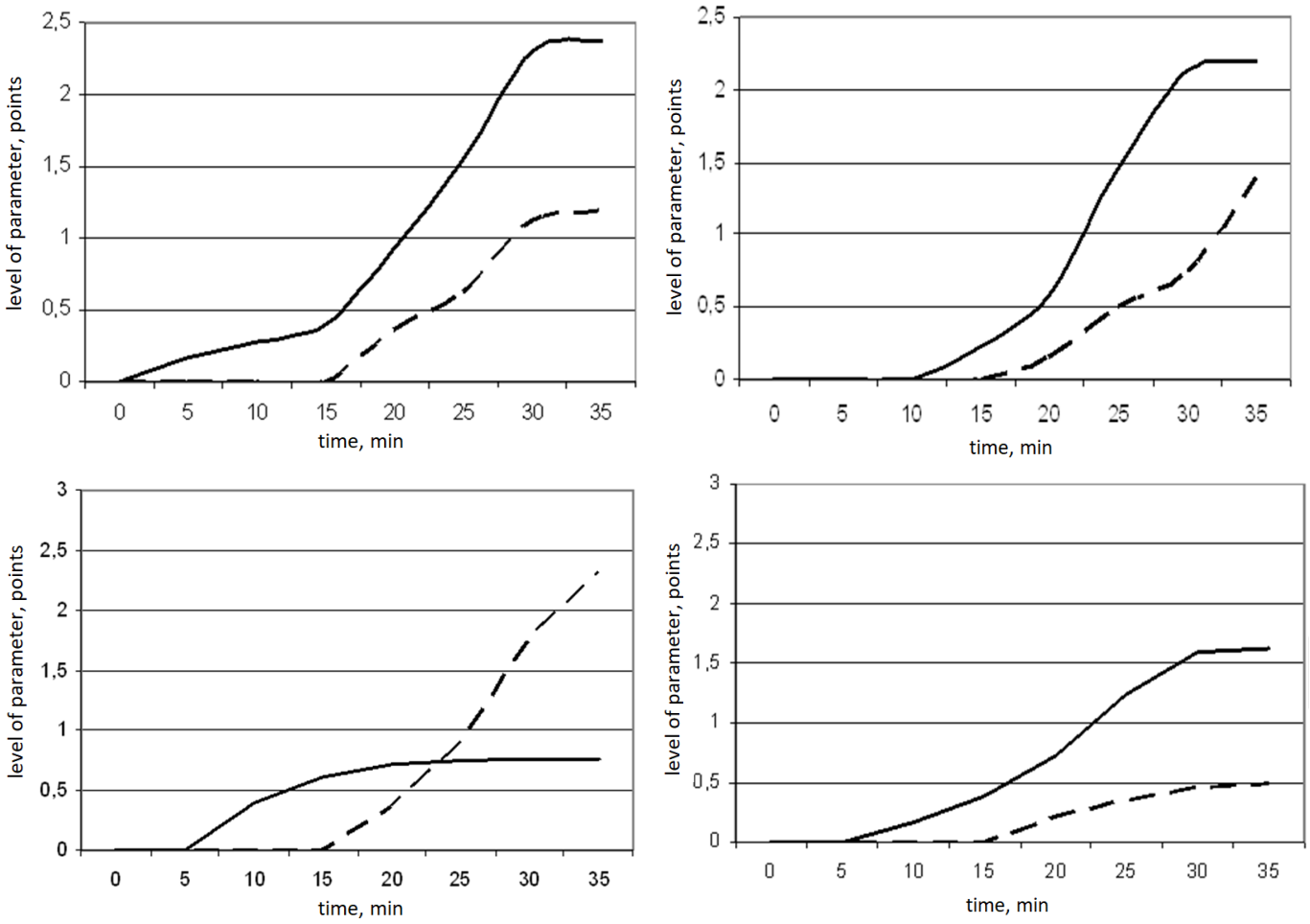

Figure 3 | Dynamics of main parameters of crystalloscopic facias of healthy people (n=30) and burned (n=32) persons (solid line indicates healthy people; punture line indicate patients with burn disease)

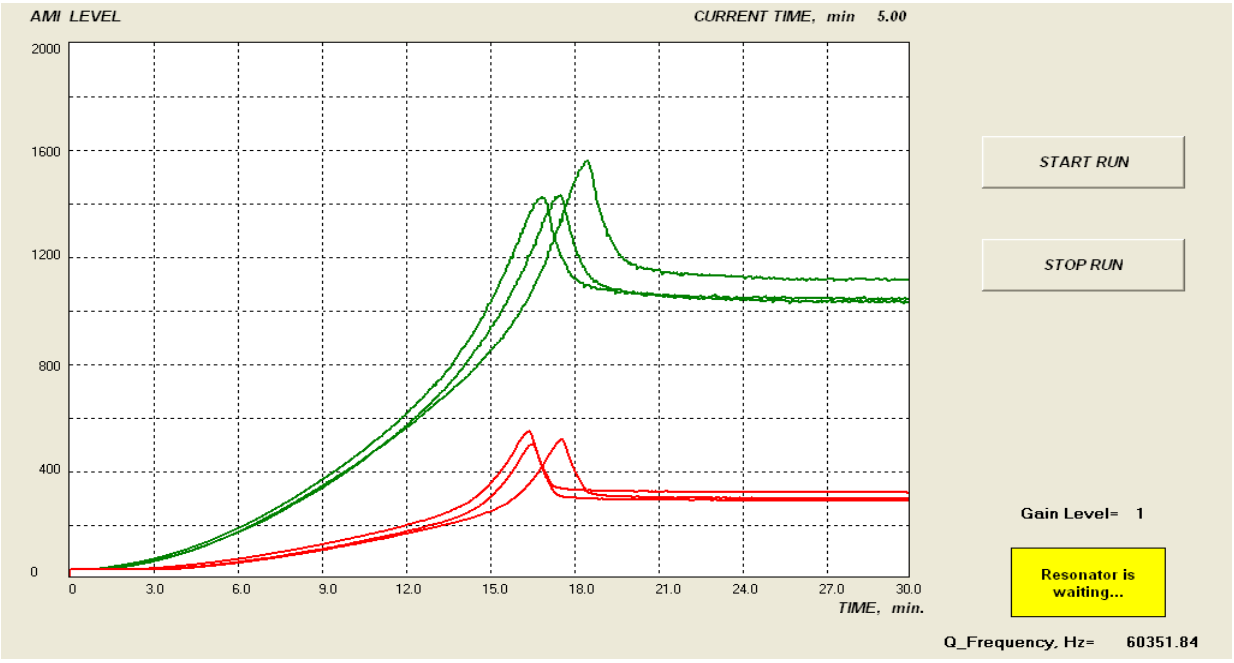

Figure 4 | Example of curves of acoustic and mechanical impedance of drying drops of blood serum from healthy people (1) and burned persons (2)

thermal injury. With respect to this method, this is reflected in the time and amplitude parameters (Fig. 4). It can be assumed that the appearance in the systemic circulation of substrates of burn intoxication (intermediate and terminal metabolites) in conjunction with changes in other components of the biofluid change its biophysical parameters, which can be reflected in the form of crystallodiagnostics of this pathological condition in the dynamics of management of patients of the profile under consideration.

The conducted correlation analysis within and between groups of indicators (visualization and acoustic-mechanical impedance) allowed to establish the presence of multiple correlations of average [0.3≤r≤0.7)] and high (r≥0.7) force (Fig. 5 and 6), which further confirms the reliability of the selected trends and consistency of changes in the biophysical properties of blood serum in thermal injury. These co-directional shifts give reason to conclude that in burn disease there are pronounced endotoxicosis-dependent rearrangements of the composition and properties of biofluids [1, 2].

Separately, it is necessary to emphasize the possibility of further transformation of physical and chemical characteristics of the studied biomaterial in the later postthermal period, as well as the importance of clarifying the contribution of the severity of metabolic disorders in the change of free and initiated crystal formation of body fluids in thermal lesions. All of the above is the subject of a new biomedical synthetic science – metabonomics, the essence of which is to study the possibility of monitoring the functional and metabolic status of a person on the basis of an integral or partial assessment of the characteristics of the biological medium. Thus, the biophysical metabonomics of burn disease through the methods of biocrystallomics can provide a large amount of information about the current state of the patient and its dynamics, which is valuable in terms of primary diagnosis of the severity of metabolic disorders and further metabolic control.

4. Conclusion

On the basis of the conducted studies it was found that the dynamics of dehydration self-organization of blood serum of healthy people and patients with thermal trauma varies significantly. In this case, we have shown the presence of relationships between the parameters of crystalloscopic test and registration of the acoustic impedance of drying droplets of this biofluid in burn disease. These facts allow to conclude that crystalloscopy of dried samples of biological fluids and study of its acoustic mechanical impedance can serve as informative tools biophysical metabonomic of thermal injury.

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References

[1] A. Vigani, C. A. Culler, Vet Clin North Am Small Anim Pract. 47 (2017) 1149-1163.
[2] O. M. Kovalenko, Klin Khir. 5 (2015) 77-80.
[3] P. I. Ramzy, J. P. Barret, D. N. Herndon, Crit.Care Clinics, 15

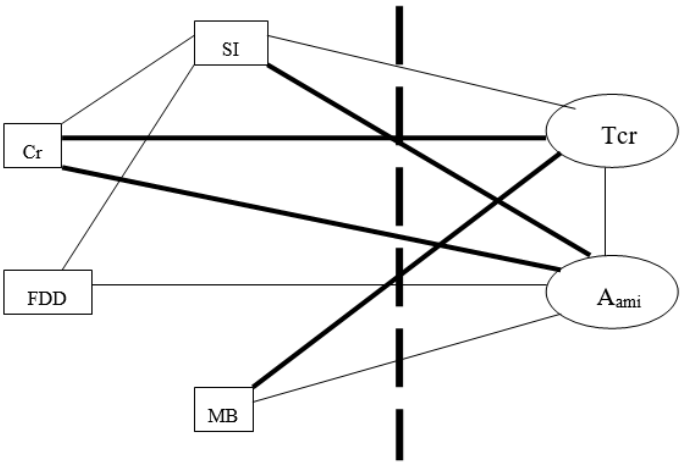

Figure 5 | Correlations between crystalloscopic (indicate as rectangles) and AMI-parameters (indicate as ovals) in facias of blood serum from healthy (n=30) people and burned (n=32) persons (SI – structure index, Cr - crystallizability, FDD – facia destructiob degree, MB – clearity of marginal belt, Tcr – time of crystallization beginning, Aami – maximal amplitude of the level of acoustic mechanical impedance; thin lines indicate moderate correlations, fat lines are strong correlations).

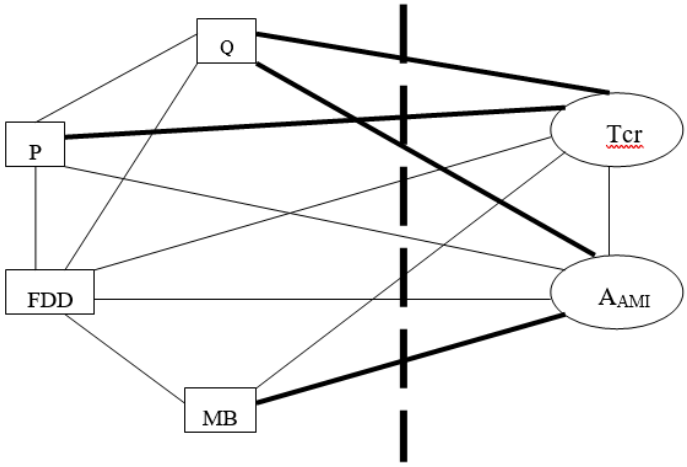

Figure 6 | Correlations between teziographic (indicate as rectangles) and AMI-parameters (indicate as ovals) in facias of blood serum from healthy (n=30) people and burned (n=32) persons (basic substance – 0.9% NaCl solution; Q – main teziographic coefficient, P – belt coefficient, FDD – facia destructiob degree, MB – clearity of marginal belt, Tcr – time of crystallization beginning, Aami – maximal amplitude of the level of acoustic mechanical impedance; thin lines indicate moderate correlations, fat lines are strong correlations)

(1999) 333-352.
[4] N. J. Prindeze, B. M. Amundsen, A. R. Pavlovich, D. W. Paul, B. C. Carney, L. T. Moffatt, J. W. Shupp, Diagn Microbiol Infect Dis. 79 (2014) 303-307.
[5] A. K. Martusevich, N. F. Kamakin, Bull Exp Biol Med. 143 (2007) 385-388.
[6] A. K. Martusevich, S. P. Peretiagin, I. E. Pogodin, Patol Fiziol Eksp Ter. 1 (2009) 30-32.
[7] A. K. Martusevich, N. F. Kamakin, Klin Lab Diagn. 6 (2007) 21-24.
[8] S. N. Shatokhina, V. N. Shabalin, M. E. Buzoverya, V. T. Punin, Scientific World Journal. 4 (2004) 657-661.
[9] V. N. Shabalin, S. N. Shatokhina, Klin Lab Diagn. 3 (2002) 25 -32.
[10] S. N. Shatokhina, V. V. Aleksandrin, I. S. Shatokhina, A. A. Kubatiev, V. N. Shabalin, Bull Exp Biol Med. 164 (2018) 366-370.
[11] T. A. Yakhno, J Colloid Interface Sci. 318 (2008) 225-230.
[12] A. K. Martusevich, S. P. Peretyagin, M. V. Ruchin, A. A. Struchkov, J. Biomedical Science and Engineering 11 (2018) 27-35.
[13] T. Yakhno, A. Sanin, A. Pelyushenko, V. Kazakov, O. Shaposhnikova, A. Chernov, V. Yakhno, C. Vacca, F. Falcione, B. Johnson, Biosens Bioelectron. 22 (2007) 2127-2131.
[14] T. A. Yakhno, V. G. Yakhno, A. G. Sanin, O. A. Sanina, A. S. Pelyushenko, N. A. Egorova, I. G. Terentiev, S. V. Smetanina, O. V. Korochkina, E. V. Yashukova, IEEE Engineering in Medicine and Biology Magazine 24 (2004) 96–104.