 doi:10.20944/preprints201907.0155.v1

Article

Dielectric properties of serum in children with suspected immunodeficiency and suffering from recurrent respiratory infections

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Abstract: Despite considerable progress in the diagnosis of various diseases, an ideal, simple tool for diagnosing patients with respiratory tract infections has not yet been invented. Many simple diagnostic tests are widely available to most doctors, provided they are aware of the prevalence of primary immunodeficiency. Other, more accurate studies are available only to immunologists. The aim of the study was to investigate the occurrence of dependence between selected physical parameters of serum such as: electrical conductivity, electrical permeability, dielectric loss factor, and selected parameters of the immune system. In addition, we have also included the ionogram (Na, K, Cl, Ca, Mg) and glucose concentration. As a result of research, the statistically significant, but very weak correlations between impedance magnitude |Z| and platelet counts (PLT), mean platelet volume (MPV) and chloride ions (Cl-) were found. The statistically significant differences according |Z| between children with and without deficiency in parameters of the immune system were noticed. Values of |Z| are higher in the case of children without deficiency in parameters of the immune system.The method of impedance measurements presented in our work is significantly easier then biosensors presented by other scientists. Taking into account our results, it can be stated that this method is promising for fast and easy detection of immunological disorders.

Keywords: immune system; immunedeficiency; respiratory tract infections; children; impedance; serum; laboratory diagnostics

1. Introduction

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Despite considerable progress in the diagnosis of various diseases, an ideal, simple tool for diagnosing patients with recurrent respiratory tract infections has not yet been invented.

Recurrent respiratory infections in children constitute an important clinical problem in the practice of the primary care doctor as well as the specialist. While their recurrent nature is a common diagnostic problem. In children up to 5 years of age, the number of respiratory infections may reach 6-8 per year. Usually, as the child develops, the relapse rate drops, however, in some children, these infections are more frequent and / or have a more severe and prolonged course. This may result, among others, from geoclimatic conditions, lifestyle, and the maturation of the immune system (especially in humoral immunity) in developmental age.
One of the reasons for these ailments is a variety of abnormalities in the immune system, which may be of primary (innate) or secondary nature.

In addition, the incidence rate is not the only criterion indicating the need to qualify the child for a wider diagnosis to assess the causes of recurrence of infection. The occurrence of risk factors, clinical course of infections, possible complications, coexistence of upper and lower respiratory tract infections are also assessed. Important risk factors for recurrent infections, such as the presence of the child in day care places (nursery and kindergarten), exposure to tobacco smoke, and exposure to domestic and industrial sources of air pollution should also be taken into account. The possible co-occurrence of allergies, which affects the body's immune balance, is also important. The age of the child and the stages of development of the respiratory system should also be taken into account. The younger the child, the more functional and anatomical features of the respiratory tract, the greater the possibility of developing bronchial obstruction caused by a decrease in their patency, retention of secretions, impaired clearing of the airways and an increased risk of bacterial infections [1,2, 3].
Immunodeficiency is a common cause of recurrent respiratory infections, especially recurrent pneumonitis [4,5]. The immune deficiencies encountered in these cases include: dysgammaglobulinemia, hypogammagobulinemia, decreased immunity in the course of cancer and its treatment, deficiencies in complement components, as well as impaired cellular immunity.
In the case of such children, special emphasis should be placed on diagnostics. As a cause of complaints one should take into account the mentioned immunodeficiency, allergic diseases as well as defects of the respiratory and circulatory system, cystic fibrosis, primary ciliary dyskinesis and the presence of a foreign body in the respiratory tract.
Many simple diagnostic tests are widely available to most doctors, provided they are aware of the prevalence of primary immunodeficiency. Other, more accurate studies are available only to immunologists. Standard immunological diagnosis in children suffering from recurrent respiratory infections should include full blood counts, main immunoglobulin classes (IgG, IgA, IgM and IgE), complement hemolytic activity, granulocyte function assessment (neutrophils phagocytosis efficiency relative to Stapylococcus aureus 209P and the percentage of phagocytic neutrophils) and lymphocyte subpopulation.

All the time we are looking for new conditions that may underlie recurrent infections. Various screening assays are also being tested to quickly and easily demonstrate abnormalities in selected parameters of the immune system, so that detailed, cost-consuming and time-consuming immunoassays only be performed in justified cases.

The methods based on measurement of electrical parameters of blood or blood derivatives such as serum seems to be promising methods for the determination of immunoglobulin levels. These methods, which are low cost, easy in application, and noninvasive, has been successfully used previously to study the different parameters of blood. Complex bioimpedance measurements simultaneously with absorption spectroscopy were used for non-invasive measurement of blood glucose [6]. The biosensor for direct detection of cholesterol in human serum was presented by Aghaei and coauthors [7]. Also biosensors for immunoglobulin levels were reported in literature [8,9].

In biophysical literature, the three distinct dielectric dispersion regions are identified for blood. The α dispersion (frequency < 1 kHz) is associated with the diffusion process of ions. The β dispersion (frequency from 1 kHz to 10 MHz) is of Maxwell-Wagner type [10]. The γ dispersion (frequency > 1 GHz) is due to the reorientation of water molecules [11]. It should be emphasized that according some scientific reports, an α relaxation is absent in whole blood [12].

2. Experimental Section

Materials and Methods

This study was approved by the bioethical committee of Wroclaw Medical University approval number 331/2016. All procedures performed in studies involving human participants were 'in accordance' with the ethical standards in compliance with the Helsinki Declaration. Written informed consent was obtained from all patients participating in the study.

The research project included a group of 150 children (aged 1 to 18 years) referred to the Department of Clinical Immunology and Pediatrics with the suspicion of primary immunodeficiency. All of these children had in the interview recurrent infections of the upper and lower respiratory tract. A group of 20 children was selected from this group that were no deficient in any of the immunological parameters.

In this work, we did not focus on the qualitative analysis of data from medical history (including the type of infection, their frequency or severity of the course). The aim of the study was to investigate the occurrence of dependence between selected physical parameters of serum such as: electrical conductivity, electrical permeability, dielectric loss factor, and selected parameters of the immune system: whole blood count, concentrations of the main classes of immunoglobulins (IgG, IgA, IgM and IgE), complement hemolytic activity, phagocytic properties of neutrophils, and lymphocyte subpopulations (CD3 +, CD4 +, CD8 +, CD19 +, CD56 +). In addition, we have also included the ionogram (Na, K, Cl, Ca, Mg) and glucose concentration. The studies were carried out on the occasion of routine laboratory tests used for standard assessment of the immune system.

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Whole blood count were tested on the Sysmex XN2000 analyzer, immunoglobulins and biochemical parameters as well as ions on the Abbott-Architect analyzer, while the lymphocyte subpopulations on the Beckman Coulter analyzer.

Approximately 2.5 ml of whole blood samples were collected into tubes without the addition of anticoagulant, then, after the clot was formed, they were centrifuged at 1000 x g for 10 minutes using a laboratory centrifuge with cooling. The serum obtained in this process was used to measure its electrical properties.

The serum complex impedance measurements were carried out at a room temperature, with a parallel plate electrode system, at frequency in a range 10 Hz – 1 MHz and by using ATLAS 0441 HIA apparatus. Electrode system was cylindrical in shape and had volume of 2 ml.

Impedance (*Z*) is generally defined as the total opposition a material offers to the flow of an alternating current (AC) at a given frequency, and is represented as a complex quantity:

$$Z = ReZ + j \cdot ImZ \qquad (1)$$

where ReZ is a real part (resistance), *ImZ* is an imaginary part (reactance), and *j* is the imaginary unit ($j = \sqrt{-1}$).

The magnitude of the impedance is defined as follows:

$$|Z| = \sqrt{(ReZ)^2 + (ImZ)^2} \qquad (2)$$

Statistical analyses were carried out with Statistica v.10 environment (StatSoft, Poland). A p-value <0.05 defined statistically significant differences. The distribution of data was evaluated by the Kolmogorov-Smirnov and Shapiro tests and when the data didn't follow a normal distribution, the Kruskal-Wallis and Mann–Whitney U nonparametric tests were used for determination of significant differences between serum samples according to their impedance. Correlations were established using Pearson's correlation coefficients.

3. Results

To better describe the immunologically patient population, the number of patients with deficiencies in specific immune system parameters was determined. In table 1, the percentage of patients with deficiency in each parameter is presented.

Table 1. The percentage of patients with deficiency in certain parameters of the immune system.

Parameter	IgG	IgA	IgM	CD3+	CD4+	CD8+	CD19+	CD56+	phagocytosis efficiency	percent phagocytosis
Percentage of patients	12.3	7.1	14.1	4.1	29.2	56.6	2.6	8.8	28.3	75.2

neutrophils phagocytosis efficiency relative to Stapylococcus aureus 209P and the percentage of phagocytic neutrophils

As an effect of impedance spectroscopy, for each patient, the complex impedance of serum was measured in the frequency range from 10 Hz to 1 MHz. Figures 1 and 2 show the frequency dependence of the impedance magnitude |Z| for five patients. For better readability, these dependencies are presented in two frequency ranges. From 10 Hz to 1 kHz for α dispersion region (see fig. 1) and from 1 kHz to 1 MHz for β dispersion region (see fig. 2).

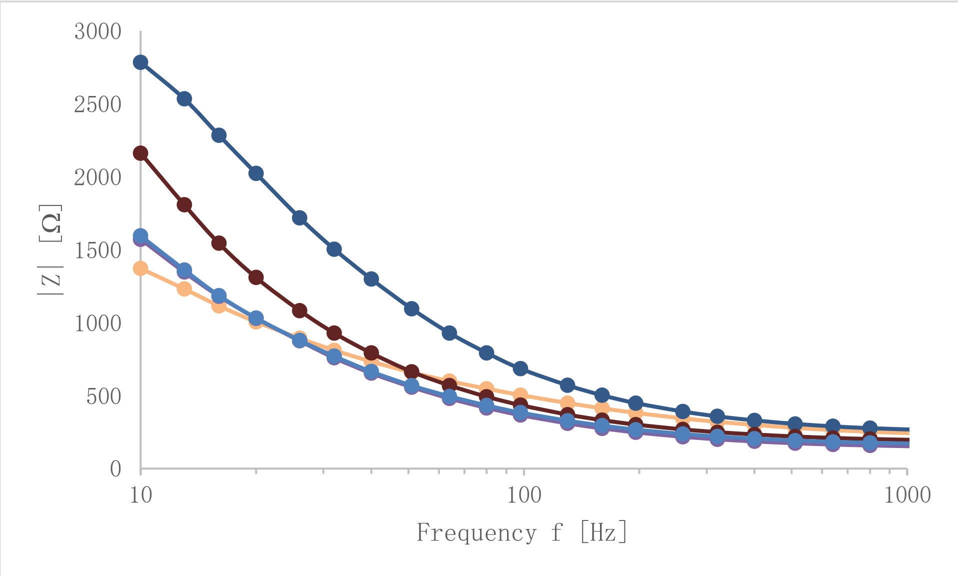

Fig. 1. Bode Plot of serum (α dispersion region)

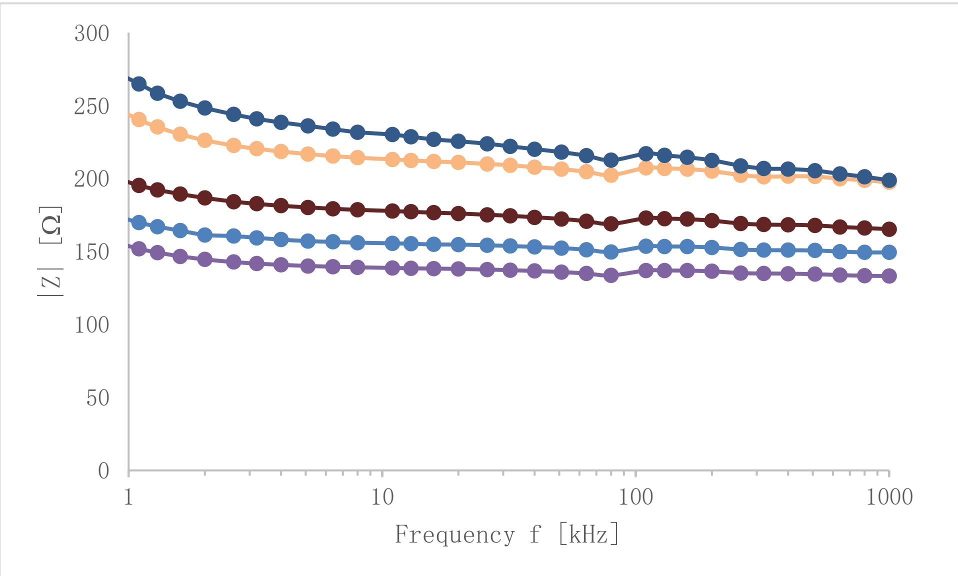

Fig. 2. Bode Plot of serum (β dispersion region)

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It is evident from Fig. 1 and 2 that the impedance magnitude |Z| decreases with the increase of frequency. The decrease is significant for α dispersion region and impedance flattens at around 10^3 Hz.

Based on Kruskal-Wallis nonparametric test, the statistically significant differences between impedance |Z| of serum of certain patients were investigated. When the impedance was measured in the frequency range from 10 Hz to 1 MHz, only little serum samples (8,7%) were significantly different according the value of impedance |Z|. Therefore, the frequency range was limited to 11 kHz – 1 MHz. In the result, the number of samples significantly different according the value of impedance |Z| increased to 34,4%.

For investigation of dependencies between biochemical and electrical serum parameters, the single frequency was chosen from the range 11 kHz – 1 MHz. The chosen frequency was 64 kHz and linear and nonlinear correlation coefficients were calculated between the impedance magnitude |Z| measured at the frequency 64 kHz and biochemical serum parameters. The statistically significant correlations were found between |Z| and platelet counts (PLT), mean platelet volume (MPV) and chloride ions (Cl-). The values of Pearson's correlation coefficients are detailed in table 2.

Table 2. Pearson's correlation coefficients between biochemical parameters and the impedance magnitude |Z| of serum

	PLT	MPV	Cl-
|Z|	0,213	-0,207	0,196

The data presented in table 2 show that correlations are statistically significant, however correlation coefficients are very low. No statistically significant correlations were found between the impedance magnitude |Z| of serum and parameters of the immune system. The Mann–Whitney U test was used to determine if there are the differences between children with and without deficiency in parameters of the immune system according to impedance magnitude |Z| of serum measured in frequency range 11 kHz – 1 MHz. The statistically significant differences were found ($p < 0.001$). Values of |Z| are higher in the case of children without deficiency in parameters of the immune system.

4. Discussion

To the best of the authors' knowledge, no research on electrical properties has been carried out of serum in children with deficiency in parameters of the immune system, and in similar electrode system. In our research it was observed that impedance magnitude |Z| decreases with the increase of frequency. Similar results were reported by Rangadhar et al., who also explained the nature of interaction between serum as rich source of electrolytes and electrode [13]. The differences in serum impedance values between those presented in our work and reported by Rangadhar et al. can be explained by differences in electrode system.

In some scientific reports, biosensors for immunoglobulin detection were presented. Ohno et al. described the sensor for human immunoglobulin A detection based on electrochemical

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impedance spectroscopy (EIS) [9]. EIS was also used as detection technique in biosensor for the determination of human immunoglobulin G presented by Qi et al. [8].
The method of impedance measurements presented in our work is significantly easier then biosensors presented in articles cited above. Taking into account our results, it can be stated that this method is promising for fast and easy screening detection of immunological disorders.

5. Conclusions

There remains a wide range of other serological tests that can be considered in the context of the bioelectric properties of serum. In the opinion of the authors, this topic of research in children is still open and requires intensive work and research as it promises further positive results.

Author Contributions: this study was designed by G.P. and D.Ł.; preparation of samples M.K-N.; bioelectric measurements D.Ł.; data collection and analysis was performed by G.P. and K.P.; statistical analysis K.P.: A.L-U. assisted in revised the manuscript.; visualization, K.P.; supervision, G.P. and K.P; project administration, G.P.; G.P. and K.P. prepared the final manuscript which was approved by all authors.

Funding: Uniwersytet Medyczny im. Piastów Slaskich we Wroclawiu, grant number ST.A240.16.092.

Acknowledgments: The author would like to thank doctor Katarzyna Gul from Department of Paediatric Bone Marrow Transplantation, Oncology and Hematology, Wroclaw Medical University for help in selection of children for study group.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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