

# Rheological status (erythrocyte aggregation index, erythrocyte deformation index, plasma viscosity) in the Covid-19 patients with “Covid toes” and in patients with Raynaud’s phenomenon

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## Abstract

One of complications of Covid-19 infection is the so-called “Covid toes”. This is disease with non-studied pathophysiological mechanisms, which in some ways is like Raynaud’s phenomenon. Our goal was to research the rheological differences and similarities between these diagnoses. We studied rheological status in a group of patients with the so-called “Covid toes” and a group with Reynaud’s phenomenon as well as in a control group healthy individual. We measured simultaneously the erythrocyte aggregation index, the erythrocyte deformation index and the blood plasma viscosity. The methods for indices measurement were composed by our research group (Rheology and Diagnostic Analytical Services Laboratory in Ivane Beritashvili Center of Experimental Biomedicine). Based on our data, we can evaluate rheological changes observed in both groups examined. In the patients with Reynaud’s phenomenon, rheological changes were not associated with an increase in the erythrocyte aggregation index. Significantly elevated levels of the erythrocyte aggregation index have been obtained only in the group of COVID-19 patients with Covid toes. Our studies presented new scientific focus and research area, able to “transport” our experimental and analytical conclusions to the field of routine clinical practice - for successful management and treatment of “Covid toes” as one of complications of COVID-19.

**Keywords:** Erythrocytes, hemorheology, COVID-19 patients with “Covid toes”, Raynaud’s phenomenon, erythrocyte aggregation index, erythrocyte deformation index, blood plasma viscosity

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## 1. Introduction

The COVID-19 pandemic is a very difficult period in the World. Accompanying diseases are complicated and multiple organ failures developed leading to the high mortality.

In infected people may develop fever, pneumonia, cardiovascular disease, neurological disorders, insomnia, erythematous macules or papules, urticarial-, morbilliform-, varicella-form, papulo-squamous petechial eruptions, purpuric lesions, acro-ischemic lesions, etc. [1,2]. The timing of eruptions and redness in the disease course and potential associations between morphologic subtypes with different COVID-19 associated syndromes and/or outcomes remain unclear [3].

Expert guidance in recognizing these signs represents an opportunity to improve diagnosis and management. The dermatologists at Harvard Medical School and Massachusetts General Hospital created the COVID-19 Dermatology Registry and, in collaboration with the American Academy of Dermatology (AAD) and International League of Dermatologic Societies (ILDS), invited health care workers globally to submit

data for possible cutaneous manifestations of confirmed or suspected COVID-19. Our research group was member of this study.

We are delighted to participate in such research, which is supported by the Global pandemic prevention organization.

An international closed registry to collect cases of COVID-19 cutaneous manifestations was established. The construction of registry bases on strategy of patients personification, which was made in the frame of law about relationships between patients-doctors-researchers.

The registry has collected COVID-19 diagnosis types demographics, co-morbidities, dermatologic condition details, timing of symptoms, skin biopsy results, COVID-19 symptoms and outcomes, including hospitalization, oxygen and ventilator requirements, and deaths. Diagnosis so-called "Covid toes" has special space in the register. Our interest and specific goal were to analyze of rheological properties of patients with so-called "Covid toes".

What is "Covid toes"? Why in this work we are compared the rheological status of patients with "Covid toes" with the rheological status of patients with Raynaud's phenomenon?

Reports suggest that a rash on COVID-19 patient's toes typically lasts between 2 and 12 days, with most people having a rash for 8 days. One or more toes may swell and turn pink, red, or a purplish color. Others may see a small amount of pus under the skin. "Covid toes" is an unexplored disease, but symptomatically is very similar to the Raynaud's phenomenon.

Raynaud's phenomenon is a special disease: on the one hand is associated with microcirculation disorders, but on the another hand some rheological parameters in patients remain unchanged [4]. Raynaud's disease causes "toes". In Raynaud's disease, smaller arteries that supply blood to the skin become narrow, limiting blood flow to the affected areas - due to the vasospasm.

In frame of our research we investigated the rheological status of patients (rheological status is the system of the body - together with coagulation- / anticoagulation- and vascular factors). The main determinants of rheological status of patients are erythrocyte aggregation index, erythrocyte deformability index and blood plasma viscosity.

The purpose of our study was to investigate the rheological status of COVID-19 patients - with "Covid toes" and to compare the results obtained with similar data for patients with Raynaud's phenomenon as well as with data for the control group of healthy individuals.

## **2. Methods**

We investigated 56 COVID-19 patients with "Covid toes" (all patients were with the positive PSR tests). 25 patients with Raynaud's phenomenon and 25 healthy individuals were also examined. Mean age of each group was about 28 years. We have measured rheological parameters applying the next hemorheological methods:

### *2.1. Method of erythrocyte aggregation index*

The method of erythrocyte aggregation index count was called by us "Georgian Method".

Erythrocyte aggregation index count is the area of aggregated erythrocytes divided by the full area of the erythrocytes (in volume unit). The procedure can be performed with the aggregometer (Tas-Plus, Leitz, Germany). This new innovative method is famous in all over the world as direct, numerical and exact.

Blood samples were centrifuged and about 0.1 ml blood was diluted 1:200 in own plasma, in the Thoma pipettes (preliminary rinsed with 5% sodium citrate solution - without addition of any other anticoagulants to the blood under study). Following standard mixing the diluted blood was placed into a glass chamber, 0.1 mm high. The quantitative index of erythrocyte aggregation, which was assessed with a special program at the Texture Analysis System (TAS-plus, "Leitz, Germany), represented itself the relationship of the aggregated and unaggregated red cells [5,7,8].

## *2.2. Method of erythrocyte deformation index*

Evaluation of erythrocyte deformability index was performed by the aid of the nucleophore membrane filter. The quantitative method is based on assessing velocity of the erythrocytes passage through the very small pores (5µm, which is a diameter of the smallest capillary) of the filter, at constant pressure (10 cm of water column) and temperature (37°C). Erythrocyte population was obtained by centrifuging the blood samples at 3000 tourn over/m., for 15 min. The plasma was aspirated with micropipette and the remaining red blood cells - added with bovine serum albumin (0.2 mg per 5 ml), were dissolved in the phosphate buffer. Then the blood was centrifuged (second time), at tourn over/m., for 15 min. The precipitated erythrocytes, as well as a thin layer of leukocytes and thrombocytes, were separated in the phosphate buffer. This procedure was repeated three times. Purified erythrocytes were diluted (in the phosphate buffer), at 10% hematocrit. Evaluation of the deformability erythrocyte index - measuring a velocity of the erythrocyte passage through the filter (mm/min), was recorded. The high quality polycarbonate filters (with 5µm diameter pores) were used in measuring procedures.

## *2.3. Blood plasma viscosity evaluation*

Blood plasma viscosity was examined in the capillary viscometer (at 37°C), where the movement of plasma in the capillaries is induced by the force of gravity [6,8].

## *2.4. Low, statistics and administrate investigations*

All information consents between investigators and patients were signed. Information consents are significant for both - patients and investigators. The analysis of the data was carried out by statistical programs Origin 4.1 (Microsoft Software. Inc) and Microsoft Excel. All studies with human participation were carried out in accordance with the Helsinki Declaration.

## **3. Results**

We investigated the erythrocyte aggregation index, erythrocyte deformation index and blood plasma viscosity in COVID-19 patients - with “Covid toes”, comparing these data with similar results obtained for patients with Raynaud’s phenomenon as well as with data for the control group (healthy persons).

We could point out that the levels of erythrocyte deformation index and of plasma viscosity have been identical in the groups of COVID-19 patients - with “Covid toes”, and in cases with Raynaud’s phenomenon. The quantitative characteristics obtained of these two parameters were worse than the same parameters in the control group (healthy individuals) and were not in the “referent values” (intervals of existing normal clinical values).

With regard to the erythrocyte aggregation index in the COVID-19 patients - with “Covid toes” and in the patients with Raynaud’s phenomenon, the differences between these two groups were very large. In patients with “Covid toes”, the erythrocyte aggregation index increased by 300% compared to the norm and 2.5 times - compared to the clinical standards.

It should be noted that the results of our studies on the rheological parameters of healthy individuals (control group) well corresponded to the clinical standards.( See table 1).

### 3.1. Table

Table 1

Characteristic of rheological status (erythrocyte aggregation index, erythrocyte deformation index and blood plasma viscosity) in COVID-patients - with “Covid toes”, in patients with Raynaud’s phenomenon and in the control group (healthy persons); (M±m) and intervals of clinical norms characterizing human rheological status, were also presented

<b>Description of COVID -19 patients – with “COVID toes”, patients with Raynaud’s phenomenon and healthy persons</b>	<b>Erythrocyte aggregation index, %</b>	<b>Erythrocyte deformation index, %</b>	<b>Blood plasma viscosity, mPa.s</b>
patients with Raynaud’ phenomenon	10,1±5,0	2,66±0,05	1,57±0,5
COVID-19 patients - with “COVID toes”	45,0±3,1	2,69±0,05	1,60±0,4
clinical norms (healthy controls- at a similar age)	20,4±3,5	2,50±0,05	1,50±0,3
intervals of clinical norms	15-30	<2,50	<1,5

## 4. Discussion

Erythrocyte aggregation index is always disordered in the COVID-19 patients. Any invasion of the virus can be determined by a combination of different types of movements, precisely because there are violations of the velocity vector of erythrocytes, their impulses and angular moments. This happens due to a collision with the virus, or the fact that erythrocyte must accelerate and move along the “path broken by the virus”. This causes an unexpected collision of erythrocyte in the plasma, which in turn, moves with glides at different times near the endothelial layers of the blood vessels. This contributes to the bonding, monetization (“rouleaux formation”) and aggregation of erythrocyte. Thus, the presence of a new Corona virus promotes the formation of aggregates not only immediately during erythropoiesis, not only in the microvasculature, as usual, but also in the macrocirculation.

All these rheological changes cause an “explosion” of rheological disturbances in COVID-19 infection.

Georgia is the leader of an international project of the World Pandemic Prevention Organization. This experimental work is a part of an international project involving over 25 institutions from 17 countries. In our global international cooperation, we have set ourselves the goal of drawing the attention of the scientific generation to those aspects that need further research.

The so-called "Covid toes" is a very difficult problem. The clinical picture of "Covid toes" is multifactorial. Covid toes is now an "open question" that needs further research.

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