New Opportunities for Non-Invasive Assessment of Blood Microcirculation Parameters in Patients with Cardiac Pathology

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Abstract. Cardiovascular diseases such as hypertension (AH), coronary artery disease (CHD), and atrial fibrillation (AF) require precise diagnostic methods to assess the condition of the microvascular bed, which plays an important role in the etiology and progression of these socially significant diseases. In this article, we discuss new possibilities for assessing microcirculation parameters in healthy individuals and patients suffering from hypertension, CHD or AF using non-invasive digital optical capillaroscopy. Thanks to the new image processing program based on the principles of machine learning, it became possible not only to monitor the movement of blood in microvessels, but also to quantitatively assess the range of parameters important for the clinician and, above all, the speed of capillary blood flow, the number and size of erythrocyte aggregates. The results of our study showed a significant decrease in capillary blood flow velocity and an increase in erythrocyte aggregation in patients with hypertension, coronary artery disease, and AF compared to healthy people. These results suggest that the technique could potentially be used in future, dedicated studies to evaluate the effects of antiplatelet and anticoagulant therapies widely used in modern cardiology, assess optimal dosage, and identify early signs of increased bleeding in pericapillary tissues.

Keywords: microcirculation; erythrocyte aggregation; digital capillaroscopy; arterial hypertension; coronary heart disease; atrial fibrillation; capillary blood flow velocity.

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1 Introduction

Microrheological parameters reflect the fluidity of blood within capillaries and small vessels. These properties are largely determined by the characteristics of blood cells (such as their size and deformability) as well as by the interactions between formed elements, including erythrocyte and platelet aggregation, and the dynamic interplay between blood cells and the vascular endothelium.

The study of blood microcirculation is one of the most important topics in the assessment of pathophysiological processes occurring in the human body in cardiovascular diseases (CVD). Its relevance is due to the continuing spread of this pathology in the world. According to the World Health Organization, one of the most common cardiovascular pathologies is arterial hypertension (AH). Worldwide, it affects 1.28 billion adults aged 30–79 years [1]. The prevalence of hypertension among adults aged 30–79 years is 34% in men and 32% in women [2].

AH is caused by a persistent increase in blood pressure, the cause of which is dysregulation of vascular tone and at the same time is one of the main pathological links that have a negative impact on the microcirculatory vascular bed, disrupting its functioning [3]. Since these changes are systemic in nature, they eventually lead to

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functional and organic damage to the most important organs: the heart, kidneys, and the central nervous system AH [4].

The study of the anatomical and physiological features of precapillary arterioles in arterial hypertension unequivocally indicates an increase in the thickness of their muscular wall with a simultaneous narrowing of their lumen. This process is called remodeling and as shown in several studies, can manifest itself in patients with arterial hypertension even in the earliest stages [5].

Pathological changes in the microcirculation system may be associated not only with a decrease in the diameter of the blood-supplying microvessels, but also with rheological disorders of blood flow associated with intravascular aggregation of erythrocytes and local blood coagulation in microvessels [4].

Various physiological factors are involved in functional and structural changes in microcirculation in hypertension. Pathological processes such as activation of the renin-angiotensin-aldosterone system, increased growth of smooth muscle cells, remodeling of the extracellular matrix, increased deposition of collagen and fibronectin accompany the development of this disease [5, 6].

Most of the circulating cells in our blood interact with each other. Under physiological conditions, the processes of aggregation and disaggregation of erythrocytes are constantly in a state of relative equilibrium [7]. However, the mechanisms for maintaining the balance of erythrocyte aggregation become insufficient with age [8]. This is especially pronounced in patients suffering from coronary heart disease (CHD). This disease is also socially significant, often complicating the cause of hypertension and being one of the main causes of mortality, as well as temporary and permanent disability of the population in the developed countries of the world. Recent research has increasingly emphasized rheological and microcirculatory dysfunction in these diseases. Our preliminary studies have shown an increase in erythrocyte aggregation in patients with CHD [6]. Under pathological conditions, erythrocytes can form persistent aggregates, which can further interact with each other at a weak shear, forming larger and stronger aggregates.

Atrial fibrillation (AF) is a supraventricular arrhythmia with uncoordinated activation of the atria, leading to the loss of effective atrial contractility – a pathology associated with heart rhythm disorders, chaotic contraction of certain groups of atrial muscle fibers with a frequency of up to 300–500 per min. In AF, ordered cardiac activity is replaced by irregular contractions of the ventricles, which affects the rhythmic work of the entire circulatory system. A particular risk in AF is the high likelihood of a blood clot forming in the appendage of the left atrium, which can enter the brain with the blood flow and cause a stroke. This complication is the cause of 15–20% of all stroke cases [9]. Strokes occurring in patients with AF tend to be more severe, resulting in higher mortality rates [10]. Since AF primarily affects older adults (prevalence: 10–20% in people aged ≥ 85 years compared to 0.4–1.0% in people aged 55–60 years), the overall prevalence of AF is projected to more than double by 2050 due to increased life expectancy [11]. All this makes the study of this type of pathology and the need to control the state of aggregation processes against the background of anticoagulant therapy extremely relevant.

The main objective of the work was a noninvasive study of the parameters of blood microcirculation in the capillaries of the nail bed and, above all, the quantitative characteristics of erythrocyte aggregates circulating in the microvascular bed in patients with hypertension, coronary artery disease, and AF.

2 Materials and Methods

2.1 Patients

A total of 171 people were examined; of these, 19 were healthy volunteers of the control group. The remaining 152 people included in the study were patients of the cardiology department of the Medical Research and Education Center of Lomonosov Moscow State University. They were divided into 3 groups: 56 patients with AH, 46 patients with CHD, and 50 patients with AF (see Table 1). In the group with AH, there were 27 men and 29 women. In the CHD group, men prevailed – 34, and 12 women. In the group of patients with AF there were 24 men and 26 women. The control group consisted of 9 men and 10 women.

Table 1 Characteristics of the groups of patients suffering from arterial hypertension (AH), coronary artery disease (CHD), atrial fibrillation (AF), and the control group (Control).

Parameter	Control	AH	CHD	AF
Individuals	19	56	46	50
Males	9	27	34	24
Females	10	29	12	26
Mean age ± SD (years)	28 ± 12	60 ± 16	69 ± 10	72 ± 10

Patients with AF in the study took oral anticoagulants (mainly apixaban and rivaroxaban) according to the protocol adopted by the cardiology department. Patients with CHD received antiplatelet therapy, mainly Plavix (Clopidogrel).

The exclusion criteria were as follows: patients over 85 years of age, patients with oncological pathology, chronic liver and kidney diseases, type 2 diabetes mellitus, heart valve pathology, connective tissue, or central nervous system diseases.

2.2 Digital Optical Capillaroscopy

One of the methods for the intravital study of the microrheological properties of blood is digital capillaroscopy, a method of optical non-invasive microscopy that allows you to directly visualize the surface microvessels of the skin and the surrounding tissue *in vivo* [12].

Image processing software allows for non-invasive quantification of static and dynamic microcirculation parameters [13]. Capillaroscopy can be used to quantify important factors such as the degree of swelling of the perivascular tissue, the size of the capillary diameters, the measurement of the velocity of capillary blood flow, and the visualization of blood aggregates within the capillaries. Progress in the approach to the study of blood microcirculation is based on the use of software that allows not only to visually assess the presence of erythrocyte aggregates in the capillary bed, but also to quantify them. This is an important point, since it allows for personalized dosage of antiplatelet and oral anticoagulants, which have become widespread in clinical practice in recent years.

To visualize the capillaries of the human nail bed, a digital capillaroscope "Capillaroscan-1" (AET, Russia) was used, equipped with a high-speed CCD camera (1/3-inch monochrome IT CCD sensor with progressive scanning, resolution 640 × 480 pixels) and up to 200 frames per second, TM-6740GE (JAI, Japan)). The nail bed was illuminated using an LED lighting system. Two magnification options were used: 125× and 400×. 125-fold magnification was used to obtain panoramic images of the capillary bed. At the same time, 400-fold magnification made it possible to obtain more detailed images of the blood circulating in the capillaries, the presence or absence of erythrocyte aggregates, the static parameters of individual capillaries, their length and the size of the perivascular zone, and the capillary blood velocity (CBV).

The study was conducted after a 15- to 20-min rest in a sitting position between 10 and 12 a.m. in a temperature-controlled room ranging from 22 to 23.5 °C. The fingers of the left hand, on which the studies were usually conducted, were at the level of the heart. All participants were asked to refrain from smoking and caffeinated beverages the day before the survey.

Skin temperature was measured in the area of the dorsal middle phalanx of the tested finger of the left hand by precision medical thermometry; the mean skin temperature was 33.2 ± 1.7 °C with no significant differences in the study groups.

To determine the CBV after recording a video fragment, the program stabilizes the dynamic images of the capillaries and then processes the images in a given area of interest in an autonomous mode (Fig. 1). Traces of specific spots differ in the level of light intensity. The software detects different inclusions in the flow of moving capillary blood, marks them, and then recognizes them in the next frame. In this way, the average velocity along the axis of the capillary over 5-sec time intervals (500 frames) is determined. The CBV is evaluated in at least 6 capillaries and the results are averaged. We evaluated the CBVs only in the first-line capillaries, where the capillaries are in the same layer. Thus, the obtained CBV values are not affected by the movement of blood in the vessels lying above and below the capillary under study. A detailed procedure for measuring the CBV is described in our previous article [14].

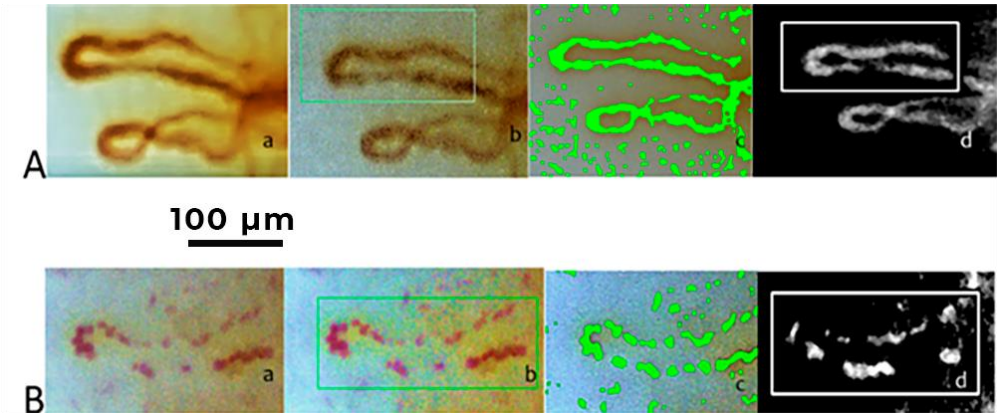

Fig. 1 Stages of software processing of capillary images on the nail bed. (A) Imaging of capillaries without erythrocyte aggregates, (a) before processing; (b) identification of the area of processing; (c) binarization (recognition of capillaries by a program); (d) processing of the selected image. (B) Imaging of capillaries with aggregates of erythrocytes, (a) before processing; (b) designation of a treatment area; (c) binarization (recognition of capillaries by a program); (d) processing of the selected image. Magnification 400×.

To analyze the obtained video recordings of blood flow in the capillary nail bed, an advanced computer program based on complex image analysis was used. The analysis algorithm included three stages: (1) stabilization of the video stream and suppression of noise in the image; (2) cumulative construction of the shape of the capillary and determination of its sections (arterial, transitional and venous sections); 3) straightening of the capillary image into a linear form, which makes it possible to calculate both the number of aggregates and their velocities. Only video recordings obtained with a magnification of 400× were processed with the help of the program. Each video had a duration of approximately 5 s, and 6–10 videos were recorded for each person, giving a total of 10–12 capillary images. In cases of stasis or pathology, the number of recorded video recordings, and, consequently, capillaries, increased. The following parameters characterizing the aggregation of erythrocytes were calculated: the number of blood aggregates per min, the size of blood aggregates (μm^2), and the CBV ($\mu m/s$).

2.3 Statistical Analysis

The data was processed, and the graphs were built using a program created by the authors in the Python programming language. In the "research results" Section, the figures show box plots, in which the lower and upper boundaries of the rectangle correspond to the first quartile (Q1) and the third quartile (Q3) with the median inside this rectangle. The whiskers in the drawings indicate standard deviations, the average values (white diamonds) are in the middle. Statistical significance between the experimental groups was determined using the Brunner-Müntzel test [15]. Two samples representing the experimental groups were considered statistically significantly different if the p-value was less than 0.05 (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$). The data in the tables are presented as averages ± standard deviations.

3 Research Results

The results obtained are presented in Table 1. These data show that the appearance of aggregates in most cases is accompanied by a slowdown in the velocity of capillary blood flow.

These results are also visualized and displayed in Fig. 2–4. A significant difference was found between healthy people (control group) and patients (CHD, AH, and AF groups) in terms of indicators associated with the presence of erythrocyte aggregates in the bloodstream (Figs. 2–3). In the control group, there are practically no aggregates of erythrocytes, for example, the number of blood aggregates per minute is less than 1 (Table 2). Statistically significant differences are observed between the CHD and AF groups in the number of blood aggregates per minute ($p = 0.0007$) (Fig. 2) and in the velocity of capillary blood flow ($p = 0.035$) (Fig. 4). Statistically significant differences are also observed between the AH and AF groups in the number of blood aggregates per min ($p = 0.03$) (Fig. 2) and the size of blood aggregates ($p = 0.04$) (Fig. 4).

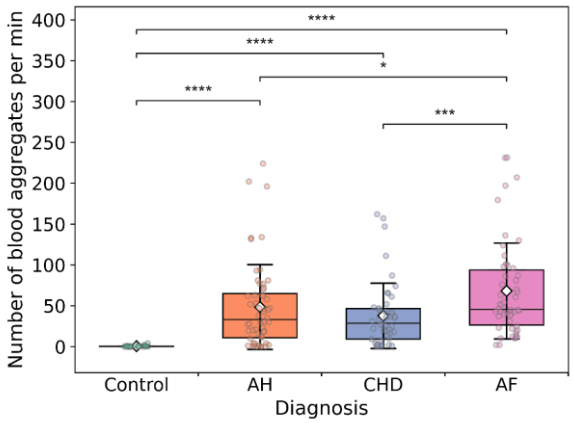

Fig. 2 Number of blood aggregates per minute for 4 groups: Control, AH, CHD and AF. Each point in the figure corresponds to the average for a single donor or patient. The lower and upper boundaries of the rectangle correspond to the first (Q1) and third quartile (Q3) with a median in the form of a horizontal line inside. Errors in the graph are standard deviations. The white rhombus in the center of the rectangle is the average value. * p <0.05; *** $p < 0.001$; **** $p < 0.0001$.

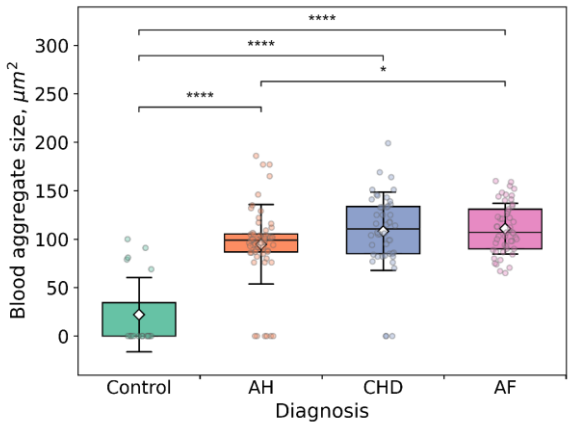

Fig. 3 The average size of the aggregates for the 4 groups: Control, AH, CHD, and AF. Each point in the figure corresponds to the average value for a single donor or patient. The lower and upper boundaries of the rectangle correspond to the first (Q1) and third quartile (Q3) with a median in the form of a horizontal line inside. Errors in the graph are standard deviations. The white rhombus in the center of the rectangle is the average value. * p <0.05; **** $p < 0.0001$.

Table 2 Microcirculation parameters in 4 patient groups: Control group, AH, CHD and AF. Average values and standard deviations of the data are presented.

Parameter	Group (number of individuals)			
	Control (*N* = 19)	AH (*N* = 56)	CHD (*N* = 46)	AF (*N* = 50)
Number of blood aggregates per min	0.5 ± 1	48 ± 52	38 ± 40	68 ± 59
Blood aggregate size, μm^2	22 ± 38	95 ± 41	108 ± 40	111 ± 26
Capillary blood velocity, μm/s	1501 ± 378	1220 ± 588	1398 ± 737	1098 ± 626

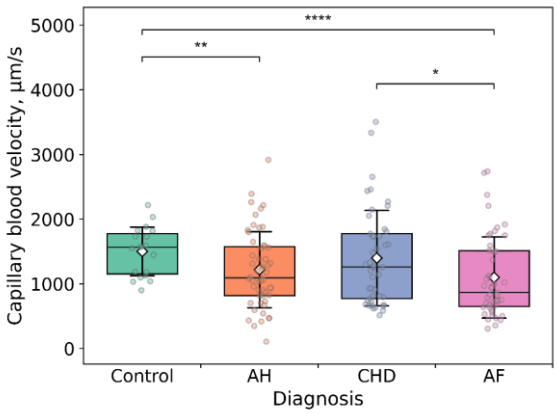

Fig. 4 Capillary blood flow velocity for 4 groups: Control, AH, CHD and AF. Each point in the figure corresponds to the average value for one donor or patient. The lower and upper boundaries of the rectangle correspond to the first (Q1) and third quartile (Q3) with a median in the form of a horizontal line inside. Errors in the graph are standard deviations. The white rhombus in the center of the rectangle is the average value. * p <0.05; ** p <0.01; **** p < 0.0001.

4 Discussion

The data obtained during the study show that the appearance of aggregates in most cases is accompanied by a slowdown in the velocity of capillary blood flow. The reason for this is probably that in hypertension there is an increase in vasoconstriction and/or weakening of vasodilation, as a reaction of precapillary smooth muscle cells, associated with the activation of the sympathoadrenal system. At the same time, the ratio of the wall thickness of the precapillary vessels, mainly arterioles relative to the lumen of the vessel, increases, which leads to a decrease in tissue perfusion, which is especially important for vital organs – the heart, brain, kidneys [4].

As shown in Table 2, the capillary blood flow rate in patients with hypertension was reduced – 1220 ± 588 μm/s, while in the control group of patients without cardiac pathology this indicator was 1501 ± 378 μm/s. In hypertension, the number of aggregates circulating in the capillary network significantly exceeded the same indicator in the group in the group of healthy volunteers, although significantly less than in patients with CHD and AF. It should be noted that 12 out of 56 patients in this group took aspirin.

However, patients with AF are most at risk of impaired rheological properties of the blood. In this group, the lowest capillary blood flow velocity was found – 1098 ± 626 μm/s, the largest number of aggregates per minute – 68 ± 59, as well as the highest size of aggregates compared to patients in the other group, even though almost all patients with AF were taking oral anticoagulants (rivaroxaban, apixaban, dabigatran). Higher doses of anticoagulants could improve the situation but could also increase the risk of bleeding. We consider it expedient to conduct studies on a larger number of patients with this pathology.

Detection of early signs of microcirculation disorders makes it possible to implement a novel approach to the diagnosis and treatment of hypertension patients and, as a result, reduce the risk of complications [16, 17]. In patients with CHD, the average blood flow velocity was 1398 ± 737 μm/s, which is significantly lower than in healthy controls (1501 ± 378) and higher than in AF patients (1098 ± 626) (see Table 2). For the CHD group, the number of aggregates per minute was 38 ± 40, which was the lowest in the presented groups of patients. This is apparently because almost all patients with CHD were taking antiplatelet drugs (aspirin, clopidogrel), and some (three people) were taking a combination of both drugs.

The study showed that erythrocyte aggregates may not necessarily appear in all the capillaries observed. This is clearly seen in Fig. 5A. The arrows indicate aggregates in the bed of two capillaries, while in the other capillaries no aggregates are found. The cause of this phenomenon is not fully understood. This may be due to the unstable activity of the smooth muscle sphincters of the overlying arterioles, caused by a chaotic heart rhythm.

Thus, a non-invasive study of blood microcirculation using capillaroscopy of the nail bed can serve not only scientific purposes but also find wide application in clinical practice for the optimal dosage of antiplatelet agents and anticoagulants. Early detection of the presence of microhemorrhages (Fig. 5B) can allow the doctor to more accurately monitor other possible signs of bleeding and, if necessary, reduce the dose of the anticoagulant in a timely manner, and if there is a high risk of bleeding, cancel it.

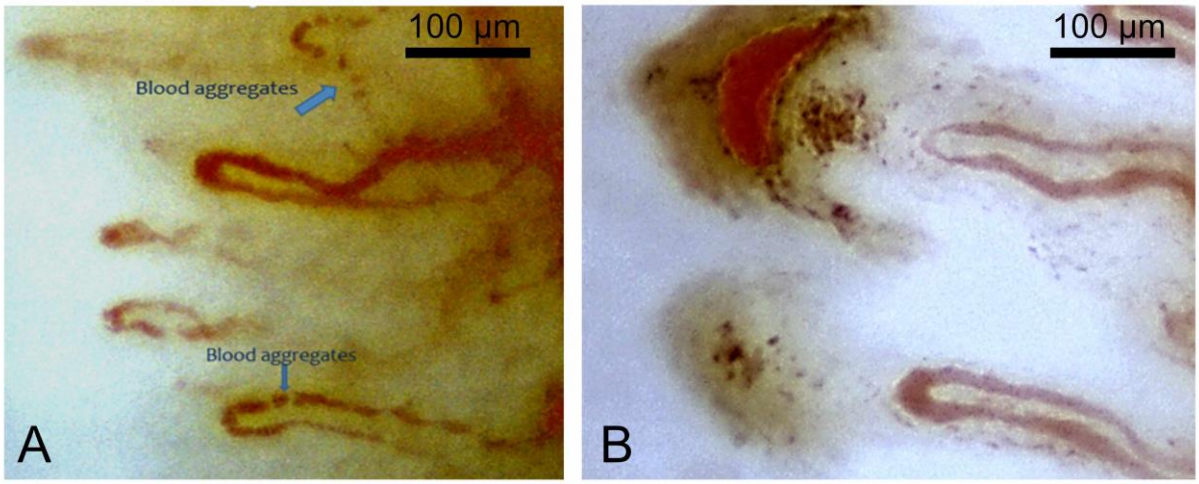

Fig. 5 (A) Nail bed capillaries. The arrows indicate the erythrocyte aggregates in two capillaries. Magnification 400×. (B) Nail bed capillaries with microhemorrhages around them in a patient with AF taking rivaroxaban. Magnification 400×.

This study has some limitations. One of them is the relatively small size of the control group, due to strict volunteer selection criteria. Another limitation is the lack of differentiation of healthy volunteers by age, while age may affect microcirculation indices to a certain extent. In general, it should be noted that capillaroscopy allows one to examine mainly the nail bed capillaries and surrounding tissues. At the same time, this method is non-invasive and does not cause any inconvenience to the patient. Capillaroscopy has already entered medical practice and is widely used in rheumatology [18], for the diagnosis of connective tissue diseases [19–21]. Apparently, the non-invasive method of optical digital capillaroscopy will eventually find application in cardiac pathology.

5 Conclusions

New approaches to the detection and quantification of circulating aggregates in the capillary channel using digital capillaroscopy could pave the way for novel diagnostic and therapeutic strategies in cardiac pathology. In a clinical context, this might help mitigate the risk of complications related to oral anticoagulant and antiplatelet therapy. An important aspect is the relative simplicity and non-invasiveness of the method of digital optical capillaroscopy, which allows it to be used repeatedly to assess the capillary bed and tissues adjacent to the capillaries in dynamics.

Compliance with Ethical Standards

The Ethics Committee of the Medical Research and Education Center of Lomonosov Moscow State University (No11/22 of 05.12.2022) approved the study design. Patients and healthy volunteers participating in the study were informed of the purpose of the study and gave written informed consent in accordance with the Declaration of Helsinki.

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Conflict of Interest

The authors declare that there is no financial or commercial conflict of interest.

Contribution of the Authors

The idea of the work and the design of the experiment (Y.I.G., A.V.P., L.I.D.), data collection (Y.I.G.), data processing (E.N.S., A.A.R.), writing a video image processing program (A.A.Y.), writing and editing a manuscript (Y.I.G., P.B.E., A.V.P., A.E.L.).

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