Pathogenetic peculiarities of sclerosing of blood vessels in hemangioma in minor aged children upon exposure by electromagnetic optical spectrum radiation

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Aim
Our aim was to study pathogenetically significant markers of sclerosing of hemangiomas in infants upon their exposure to optical radiation in the infrared region of the spectrum by analyzing thermography and morphology examination results.

Materials and methods
The basis for the thermographic study was our observation for 200 patients (birth to age 1), who received the hemangioma treatment in 2014–2016. We investigated thermograms of each vascular tumor in infants both before and after the treatment conducted with the use of the infrared thermography (IRT) method. An IRT session was carried out upon a calm, quiet staying of an undressed infant in a room at an ambient temperature of 22°C and humidity of 0.009–0.012 kg/m3. To record heat fields in correspondence with the actual heat state of the tumor areas under studies, we used our thermo-vision device RADUGA-6 manufactured by AOMZ. The IRT data were compared with those obtained in ultrasound examinations. Diagnostic indicators of sclerosing, upon testing blood serum specimens, taken from 5 practically healthy and 25 hemangioma-affected children, were analyzed with the use of the cuneiform dehydration technique. Microscopic examination of the sampled specimen structures was performed with the LEICA DM SL2 microscope supported with original software MORPHOTEST.

Results
It has been established that an alteration of the heat field structure in a vascular malformation depends on a treatment stage. When sclerosing the blood vessels in the malformation, the thermo-graphic indicators show a sharp decrease (from 39.90°C at a temperature gradient of +3.060°C to 36.00°C at a temperature gradient of +0.10°C) and approach the normal values. Structural changes in serum facies analyzed at each stage of the treatment reflected the respective alterations both in the infant organism and the malformation. Some criteria have been identified by us to indicate sclerosing of the malformation, which are as follows: wide radial cracks, incomplete concretions, Sierpinski-carpet and leaf-type structural patterns. The relevant ultrasound examination data have confirmed our suggestions that there is a reduction in hemangioma sizes, the absence of blood flow and a blood supplying blood vessel as well as regression of hemangiomas against the background of electromagnetic radiation in the optical infrared region of the spectrum.

Conclusion
It has been found that evidence data on temperature distribution in hemangiomas obtained with remote infrared thermography are in good correlation with the criteria of biological fluid morphology as well as the relevant ultrasound scanning data. The factual evidence makes possible to provide an objective assessment of the states and stages of development of the vascular malformations in order to select the most adequate therapy.

Keywords
Electromagnetic radiation of optical spectrum, Remote infrared thermography, Morphology of blood serum, Hemangioma
ing the proliferating skin or underskin hemangiomas complicated by ulcer, inflammation and bleeding [4]. Considering this factual material, the problem of effective treating hemangiomas in infants remains topical at the present time.

An application of the Photo-Chromotherapy Method (PCM) is based on the capability of the monochrome red light of inducing photo-bio-adaptive processes of repair and regenerative tissues in an infantile organism, with an increase in synthetic, phagocytic activity of neutrophils as well as an increase of indicators of the lymphoid subsystem of immunity that favors the stabilization of growth, so that regression of vascular malformations on the skin is promoted. Using PCM, we can induce vasodilation of the micro-blood vessels, activate the processes responsible for sclerosing of main blood supplying vessels within the tumor, and intensify humoral factors of the regulation of the local blood flow. The phototherapy with the monochrome red light results in an improvement of peroxidative and energetic metabolism, harmonization of the internal processes in the organism and elevation of non-specific resistance of the infant [4].

An adequate modern understanding of changes occurring in hemangioma during the treatment, including that with PCT, is not possible without assessments of prognostic criteria, which reflect the dynamics of the sclerosing processes in the mentioned vascular malformation type.

In this connection, undeniably, there should be fundamental research to be conducted in order to discover the essence of the light emitting diode emission effect [10, 12]. One of the key acceptors of such effect might be biological fluids, among them blood serum. The cuneiform dehydration technique implies some specific conditions onio-fluid the self-organization that results in obtaining a solid film (a facies) showing specific patterns which reflect certain individual biological parameters of homeostasis [3, 7, 8].

The specific structural patterns composed by the contained molecules form the respective system and subsystem-related levels as well as the local structures at the macro-level which may be visually examined. At the present time, in blood serum in adults revealed have been a number of local mor-phological structured markers, indicating certain pathological processes, including that of sclerosing of blood vessels. So, when blood vessels are affected by sclerosing, the blood facies in adults demonstrate leaf-type structural patterns; and, as this takes place, it should be mentioned that the greater is the relation between the cross-sectional line and the longitudinal line size of the leaf pattern, the more pronounced are sclerotic alterations therein [3, 7]. As to infants with hemangiomas, regression of this sort of vascular tumors is determined by sclerosing of the major blood-supplying vessels in the hemangioma under treatment. In doing so, we should note that identifying markers of this process may bear witness to the efficacy of the applied therapy measures and detection of the reserves of the infant’s health in order to take the proper treatment measures aimed at strengthening of the reserves and prevention of the reserve depletion.

Among the existing methods of diagnostics, thermography of blood vessels occupies a highly important place because of due considerations of a correlation between the intensity and evidence of clinical manifestations of a disease and an actual temperature on the skin surface [9, 11]. Recently the infrared thermography (IRT) has been widely used as one of the methods of non-invasive diagnostics that is very topical for infantile diagnostics [5, 6, 8]. The temperature measured on the skin of an infant under the rest conditions, considering microclimate, excludes any changes in the body temperature and is said to be an integral indicator of the degree of functional activity of the vascular tumor or the tumor-projected-area organ.

The IRT method is based on recording and visualizing of temperature fields with the use of specific imaging equipment, namely, thermovision devices. The main element of the device is a sensitive receiver of the infra-red radiation; the receiver is responsible for conversion of the thermal radiation emitted by the human body in the infra-red wavelength range into electrical signals which are automatically processed and transformed into images of an area under examinations, which are called thermograms [9]. Thermograms obtained with the thermovision device exhibit temperature field distributions which correspond to the thermal state of the specified areas. The data delivered with the equipment represent both anatomical & topographical and functional alterations in the affected zone [1, 2, 9, 11]. However, it should be mentioned that the infrared thermography applied for the purpose of tumor growth diagnostics has limited applications both in Russia and abroad [9, 11].

Considering the above, we should state that searching for the proper criteria of sclerosing blood vessels
according to the morphological picture of dehydrated serum films in children aged under 1 with hemangiomas becomes topical at every stage of photo-chromotherapy (PCT).

Materials and methods
Our clinical studies are based on observing patients covering the cohort from newborns to in-fants aged under 1, who have been subjected to PCT of hemangiomas in the red spectral region in the period 2014–2016. When conducting the studies, we specified some applicable criteria for an evaluation of the efficacy of the carried-out therapy as follows: a reduction in sizes of malformations, the appearance of sharp tumor contours, a decrease in intensity of blood flow, changes in gradients of temperatures between the tumor and the enclosing tissue, since such type of dynamic monitoring of the hemangioma condition makes possible to obtain objective evidence data on therapy outcomes and facilitate individualization of the patient treatment tactics. The tumor tissue due to its intense metabolism demonstrate in more frequent cases a higher temperature as against the enclosing tissue, that is recorded in the relevant projection as an enhanced infrared radiation, and it is just this phenomenon that is used as the basic principle for the IRT diagnostics in clinical oncology. In pathogenesis of hemangiomas in children aged under 1, it is possible to detect alterations of the vascular bed within the pathological focus region, which may have manifestations in one form or another to a different degree. The IRT examinations in each child undressed were carried out upon expiration of 15 minutes of rest staying of an infant in a room at an ambient air temperature of 22°C and with an ambient air humidity of 0.009–0.012 kg/m³. To record the relevant thermal fields, which reflect the respective thermal condition of the tumor regions to be examined, utilized was thermovision device RADUGA-6 manufactured by AOMZ (Russia). The same patients were subjected to ultrasound scanning with the use of sonographic equipment as listed below: LOGIQ 400 MD, HDI-1500, HDI-400, ATL and USA with linear-type piezo-sensors at frequencies 7.0–12.0 and 9–15 MHz. Utilizing the Brightness-Mode, we assessed sizes, echogenicity, the presence or the absence of tumor encapsulation, contours of the malformation; in the color Doppler sonographic mapping (CDM) regime and in the color Doppler energy (CDE) mode we examined the character of blood flow and the presence or the absence of a blood-supplying vessel. The revealed alterations therein were recorded in the two mutually perpendicular projections that offered a possibility to produce 3D-images of the pathological focus regions.

Our experimental studies implied tests of blood samples taken from 25 infants, aged under 1, diagnosed with skin hemangiomas, at every stage of photo-chromotherapy, as against blood specimens taken from the practically healthy infants. Blood sampling was performed before the experimental PCT, subsequently upon completion of PCT course 1, 2 and 3 as well as 1 year after health restoration; at the same time blood tests were carried out in the cohort of the healthy children; in each case a blood sample volume was 0.5 cm³. All collected blood specimens were centrifugically processed at a centrifuge speed of 1000 RPM, so that serum samples were separated and tested with the use of cuneiform dehydration [3]. A drop of the biological fluid amounting to 15–20 μL (5–7 mm in diameter) was placed onto a degreased microscope glass slide with a lecithine substrate, using a micro-dropper with a dosing device. Thereupon, the drop was dried at an ambient air temperature 20–25°C, avoiding air draft, under an ambient air relative humidity 65–70 %. The drop to be dried should be completely immovable, and the microscope glass should be located in a strictly horizontal position. The time required for drying (till the time of the structural pattern analysis) should be 18–24 hours. The microscopic examination in our case was performed with microscope instrumentation LEICA DM SL2, with an application of original Software Morphotest. Morpho-logical analysis was carried out with utilization of optical light, dark field and polarization micro-copy with a magnification of x5, x10, x40 and x100, respectively [3].

The facies type reflecting the pattern of symmetry, the number and shapes of major system- and subsystem-related criteria, comprising cracks, isolated areas and concretions, might be identified to be either of physiology nature being represented by the radial and (or) partially radial types, or of pathology character being composed by the irradiial, circular, amorph, double-facies types, as well as to be of stable or non-stable types. In order to determine the facies and their structural type, namely, stable or not stable type, pathologically stable or nor stable type, we also conducted an additional drop testing of the serum sample taken from the same child.
Results and discussion

Upon thermometry of hemangiomas with different locations, we have obtained evidence as indicated below. The obtained thermal profile differed in colors of the elements on mapping images that made possible to identify separating lines of individual temperature regions in different parts in the child body and the vascular malformation. It was established that on thermograms abnormally cold zones were blue colored; hot areas were black, red or orange; isothermal regions were represented in yellow and green. According to such color map of the tumor, we were capable of visually identifying its extension and sizes, location and activity of the processes occurring therein. The smallest size of the tumor focus for hemangiomas detected with RIT was 1 mm in diameter. The conducted thermometry allowed reliably revealing an increase in temperature in the tumor region under examination. A local increase in a temperature in hemangiomas was contoured as a hyper-thermic focus area with a gradient of temperatures from + 3,0°C to + 0,2°C. The maximum temperature in the tumor was recorded to be 39,0°C. Upon assessing the patterns of the thermal map we classified the following types of the tumors: hyper-
thermal, hypothermal and isothermal malformations. During the growth and the stabilization of the process, the contours for the majority of the tumors were found to be clear-cut on the thermograms, while during the tumor regression we noted diffuse contour lines. As to the children upon successful treatment of hemangiomas, the indicators of the thermal field between the symmetrical areas showed practically no difference from the normal ones, and the temperature distributions were recorded to be from 35,9 °C to 36,0 °C, with a gradient under 0,1 °C. The thermograms of such children were isothermal. Heat fields were represented in yellow and green. Figure 1 exhibits an example of various thermographic maps of cavernous hemangiomas in the chest in children aged under 1.

A hemangioma in Figure 1a demonstrates sharply cut boundaries with the distinct maximum of light effect that is dark-color-marked as an extensive area of hyperthermy. The structure of the thermal field is homogenous. It should be noted there is a disorder in the thermal symmetry. Determined can be a hyperthermal focus with a temperature gradient of + 2,25 °C. Disorders in symmetry with a high gradient of temperatures can be used as one of the criteria for thermo-vision diagnostics to indicate that there is an active growth process of the vascular malformation [1, 2, 6]. Clinically, in our case, the hemangioma is characterized by an active proliferative growth.

Figure 1b gives a thermogram of a cavernous hemangioma which is represented by some regions, differing in degrees of the expressiveness of the light effect, where the pattern of the thermal field is inhomogenous. The central zone of hyperthermy is clear-cut (dark-colored); the border line of the hyperthermal field is well-marked and is bounded by a a less hot (red color) zone with a sharp transition into a zone of iso- and hypthermy (yellow and green colored on the thermogram). There is a disorder in the thermal symmetry available. Identified can be a hyperthermal focus with a temperature gradient of + 0,8 °C in the centre and + 0,5 °C along the periphery of the vascular malformation. Such alterations in the thermogram can bear witness to the fact that there is a stabilization of the tumor processes. The thermographic mapping pattern of the cavernous hemangioma in Figure 1c is not sharp; the contours are not symmetrical; structurally, the thermal field shows non-uniformity. The foci of the hyperthermal fields are bounded by some areas of hypo-and isothermy. The contour-related asymmetry is enhanced, and the hyperthermal focus shows a temperature gradient from + 0,3 ° to + 0,2 °C. Clinically, the given hemangioma can be classified as regressing. In Figure 1, examining the hemangioma area, we can find some insignificant regions with a pronounced minimum of brightness as a hypothermy zone; the malformation depicted herein is mainly of isothermal pattern. The boundary lines are not clear, and the contours are indistinctly seen. The structure of the thermal field is homogenous; no temperature gradients are found; the malformation is isothermal with reference to the enclosing tissues. It might be viewed as an indicator of involution of the vascular malformation, i.e. hemangioma.

Informativity and reliability of the data delivered by remote IRT in PCT of hemangioms in infants are about 100 %. But taking into account the fact that remote IRT is a non-invasive, safe and rather simple technique, which may be applied as many times as it is required for the same infantile individual, this new technology can be successfully recommended for individual administration of power exposure dosing under the specified PCT parameters depending on the IRT initial data.

By this means, based on the IRT initial data on actual temperature distributions in hemangiomas and enclosing tissues, we are capable of objectively assessing the condition and the stage of progression of the vascular malformation in order to select the most adequate therapy.

Our analysis of the serum facies in children has demonstrated distinct differences in the groups under studies. When identifying the facies of serum in infants with complicated hemangiomas before PHT, it
was established that in the common group the facies with extremely low level of structuring were dominating: the texture of the facies were found to be either of amorphical or irra-dial type; it was identified as pathologically stable (see Figure 2 herein).

Upon microscopic examination of the solid films of serum in infants after completion of PCT course 1, there was another morphotype of the facies identified (see Figure 3a, b). Most of children under examination demonstrated a partially radial type of the systemic organization of the facies of blood serum, and only in one case the radial type of the facies was found. Some local pathological formations as markers of hypoxic and inflammatory processes (wide radial cracks, the Sierpinski carpets, leaf-and bundle-type structures and incompleted concretions) appeared. The mentioned alterations in the bio-fluid indicated that there were changes in the homeostatic processes in the child organism targeted at sclerosing the vessels in the hemangioma that was evidenced by the signal markers of sclerosing appeared in most children: they were leaf-type structures appeared therein. But it should be noted that at that stage of PCT the alterations were of physiologically unstable nature.

The visualized representation of hemangiomas in infants demonstrated that there were small-scaled bright spots on its surface that was an indication of the beginning of the sclerosing processes.

The structure of the facies of blood serum in children with hemangiomas upon course two of PCT at-

Fig. 3. Facies of serum of an infant upon completion of course one of PCT. The initial signs of the systemic features: partially radial symmetry of cracks; the local signs: a) formation of the facies of small-structures of leaf-type in an intermediary zone; b) formation of larger structures of leaf-type in an edge zone. Magnification x40.

Fig. 4. Facies of blood serum in child upon completion of course two of PCT: a) Radial type of symmetry of cracks of facies, formation of isolated regions and concretions; b) Radial type of the facies of blood serum, prolongation of cracks, formation of isolated regions and concretions, merger of leaf-type structures. Magnification x40.
tested that there was a restoration of the normal types of the morphostructure as a reflection of the appearing anti-tumor and bio-adaptive effects of PCT (see Figure 4a, b herein).

The architectural structure of the facies changed: the type of symmetry became radial; formation of isolated regions and concretions took place that was an indication of the normalization of the first and second levels of the self-organization. In some infants we revealed some blood serum facies showing some cracks atypical in sizes: the cracks were either shortened to one-half of the facies radius, or they were longer in their length intersecting the facies circumference.

Visual imaging of hemangiomas detected that there were some largesized bright sclerotic fields appeared throughout its entire surface, and the tumor demonstrated shrinkage; in palpation it was found that the tissue was soft.

Upon completion of course three of PCT, the structure of the blood serum facies approached the normal type recorded for conventionally healthy infants without hemangiomas. The sclerosing markers were absent (see Figures 5–7 herein).

Examinations of the facies of blood serum in infants carried out 1 year after the recovery confirmed that there were normal types of the facies with clearcut radial structure of the cracks identical to those found in healthy children. No markers of pathological processes like inflammation, hypoxia or sclerosing were detected. According to the visual images, at the location of hemangioma a soft connective tissue scar was identified.

By this means upon performing the analysis of the criteria of crystallization, an identification of system-, sub-system-related and local signs of self-organization of the bio-fluids as well as markers of pathological processes in blood serum in the infants at every stage of PCT, it has been established that a restoration of level I and II of self-organization takes place (the system-related concentrated waves, radial symmetry of cracks, appearance of isolated regions and concretions). Already upon completion of course two of PCT, we have been able to clearly identify that the criteria of sclerosing of blood vessels in hemangioma in the morphotype of blood serum have been met; the occurrence rate of pathological process markers like inflammations, hypoxia and intoxication has decreased. Upon completion of the PCT courses, the morphotype of the facies do not show any differences from that found in healthy children.

Fig. 5. Facies of blood serum in a child upon completion of course three of PCT. Radial type of facies, extended cracks, formation of isolated regions and concretions. Magnification x40.

Fig. 6. Facies of an infant upon recovery after successful hemangioma PCT: a) Radial type of cracks, formation of individual sectors, isolated regions and concretions. b) Facies of a child in 1 year after the recovery upon successful completion of hemangioma PCT. Radial type of cracks, isolated areas and concretions. No markers of pathological processes are found. Magnification x40.
When analyzing the data obtained with ultrasound scanning, the following factual evidence was produced: before therapy, in 57.8% of the cases recorded were hemangiomas with a higher echogenicity; in 77.7% of the cases the contours thereof were not clear, and in 100% of the cases encapsulation was not found. The type of blood flow was detected to be either central in 38.8% of the cases or mixed in 27.8% of the cases; in 70.2% of the cases detected was an arterial blood supplying vessel. In dopplerography recorded were high values of blood flow velocity (V_max ≥ 16s/s). That should be treated as factual evidence for the presence of proliferation of the angiomatosis process. Upon reaching the required clinical effect, at the first stages, there were clear-cut boundaries of the malformation identified, a decrease in echo-density and a significant reduction in volumes and intensity of blood flow. During the hemangioma regression, the malformation was not detectable. The clinical picture for the children was characterized by the following: after PCT course 1, regression of hemangioma was recorded nearly for a half of the group under studies; in case of cavernous heman-giomas, regression was recorded only for 15% of the infants. According to the ultrasound scanning data, we detected a reduction in sizes of the tumor by 46–71%, a decrease in blood flow velocity by 11–22%, and encapsulation was recorded in 40% of the cases. Upon PCT course 3 and 4, the percentage of the children showing regression of the tumor was increasing. We recorded in those in-fants a reduction in sizes of the malformation by 98%; we identified a weak periphery blood flow or the absence thereof in 96% of the studied cases; clear boundaries or encapsulation were found in 98%, and the disappearance of the blood supplying vessel was recorded in 80% of the cases. Upon PCT course 4, the rest of the children with simple hemangiomas demonstrated regression of the hemangiomas, and for the other children showing other forms of hemangiomas recorded were percent-ages of stabilization and regression. In cases complicated by hemangioma ulcers, in PCT obtained were data on some local effects of monochromic low intensity red-light emission on pyoinflammat-o-ry processes within the affected wound area: sclerosing of the main blood supplying vessel took place, activation of connective tissue elements (fibroblasts, histiocytes, macrophages, lymphocytes, building-up of bactericidal action of neutrophiles) resulted in an accelerated cleansing of the wound with removal of necrotic mass, a reduc-tion by more than two times in duration of exudative phase of the inflammation process with synchronous developing of granulation tissue, so that epithelializa-tion of the wound, on the average, was completed.
on day 5 from the date of the beginning of the light therapy that was considerably shorter than the respective reference parameters: it became shorter by three or five times (see Figure 8a and b herein).

Conclusion
Electromagnetic influence provided by the optical range in the spectral red light region initiates regression of the vascular malformations in children aged under one. The clinical effect is provided due to inducing the sclerosing process in blood vessels in hemangiomas. The data obtained with the use of remote IRT on actual temperature distributions in hemangiomas, the criteria for morphology of bio-fluids and the data produced with ultrasound scanning are in good correlation; they make it possible to objectively assess the condition of the vascular bed in the tumor. Some markers of sclerosing blood vessels identified according to the blood serum morphology data and IRT evidence can be useful for an assessment of efficacy of the treatment carried out.

Statement on ethical issues
Research involving people and/or animals is in full compliance with current national and international ethical standards.

Conflict of interest
None declared.

Author contributions
The authors read the ICMJE criteria for authorship and approved the final manuscript.

References