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Dysregulation of VEGF-dependent angiogenesis in cavernous lung tuberculosis

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ABSTRACT

Introduction: Tuberculosis (TB) remains one of the most dangerous infections with the high mortality in the whole world. The leading problems in the modern course of TB are both long-term therapy and appearance of multiplying and wide resistant forms, as well as the rise of comorbid pathology (e.g. AIDS-associated TB). Nonefficient antibiotic therapy forces the search of alternative ways of treatment. Prospective among existent ones is "target therapy" aimed on key links in a host organism. The aim of our study was to determine the morphofunctional characteristics of VEGF-A dependent angiogenesis in patients with fibrous-cavernous pulmonary tuberculosis, depending on the activity of dischargein order to justify the use of angiogenesis blockers.

Methods: Material for research were the fragments of the cavities' wall and pericavernous lung tissue of deceased or operated patients about fibro-cavernous tuberculosis with active bacillation (n = 89) and with clinical abacillation (n = 74). To assess the activity of VEGF-A-dependent angiogenesis, we used an immunohistochemical study with markers CD68, VEGF-A, CD34 and CollagenIV, followed by determination of the vascular perfusion index.

Results: Hyperactivation of VEGF-A dependent angiogenesis in the foci of specific inflammation leads to the dysregulation of the formation in functionally complete vessels and the creation of the most comfortable conditions for the persistence of *M. tuberculosis* in the form of sufficient aeration in the area of specific granulations, while reducing the vascular permeability of the fibrous layer vessels, providing inefficient recruitment of cells of the immune system and targeted delivery of drugs.

In the pericavernous zone and intact pulmonary tissue, hyperexpression of VEGF-A leads to aggravation of local pulmonary hypertension, increase of tissue hypoxia, fibrous remodeling of the aero-hematic barrier and the formation of the alveolar-capillary block, which is a morphofunctional indicator of respiratory failure.

Conclusions: Taking into account the absolute prevalence of proangiogenic factors in FCT in all patients, the use of targeted therapy aimed at VEGF-A blockade is pathogenetically justified.

1. Introduction

Tuberculosis (TB) remains one of the most dangerous infections with the high mortality in the whole world. The leading problems in the modern course of TB are both long-term therapy and appearance of multiplying and wide resistant forms, as well as the rise of comorbid pathology (e.g. AIDS-associated TB) [1–3]Nonefficient antibiotic therapy forces the search of alternative ways of treatment. Prospective among existent ones is "target therapy" aimed on key links in a host organism [4].The majority cases prove negative TB pathomorphosis caused by the combination of variability

Abbreviations: TB, tubeculosis; IP, index of perfusion; MBT, Mycobacteria tuberculosis; VEGF, vascular endothelial growth factor; FCT, fibrous cavernous tuberculosis; IHC, immunohistochemistry; IP, perfusion index.

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of Mycobacteria tuberculosis (MBT) and macroorganism's immune status. MBT is a unique bacterium abled not only to evolutionize through L-forms, but also to act on target cells for convenient milieu [5,6]. The number of independent scientists compare the named ability with tumor cells [7]. It was state that in the process of neoplastic transformation there is a blocking of programmed cell death – apoptosis, suppression of the immune system and activation of angiogenesis for sufficient oxygenation of genetically modified cells, their trophic, as well as dissemination throughout the organism [8–10]. The central regulator of angiogenesis and lymph angiogenesis is the family of endothelial vascular growth factors, which includes 5 types: VEGF-A, VEGF-B, VEGF-C, VEGF-D and placent growth factor (PIGF) [11].VEGF-A is a major participant in angiogenesis and lymph angiogenesis and is induced in response to tissue inflammation, hypoxia, and proinflammatory cytokine concentrations [12]. Consequently, VEGF-A can play a role in blood and lymph vessels regulating cell traffic, immune cell recruiting, and inflammation realization. Indeed, serum VEGF-A levels have been shown to be associated with systemic inflammation in inflammatory lung diseases [13], and it is postulated that VEGF - A can be a major component of neovascularization in TB granulomas. Moreover, recent data from models of mycobacterial infection in Danio fish and rabbits clearly demonstrate the important role of VEGF-A in the pathogenesis of mycobacterial infection [14,15]. Thus, the aim of our study was to determine the morphofunctional characteristics of VEGF-A dependent angiogenesis in patients with fibrous-cavernous pulmonary tuberculosis, depending on the activity of discharge.

2. Materials and methods

2.1. Group formation

Material for research were the fragments of the cavities' wall and pericavernous lung tissue of deceased or operated about fibrocavernous tuberculosis (FCT), lung (n = 163). All patients were divided into 2 main groups:

Group 1- FCT of lungs with active bacterial discharge – MBT+ – n = 84;

Group 2-pulmonary FCT without discharge (MBT-) – n = 79.

Fragments of 30 patients' lungs died of the diseases not associated with lung pathology (myocardial infarction, acute violation of cerebral circulation) have been used as a control group for comparison of morphological indicators. Criteria for inclusion of patients in the study were age from 18 to 65 years, negative clinical and laboratory data on the presence of comorbid pathology (viral hepatitis B, C and HIV) and exacerbation of chronic diseases of other organs and systems and informed consent.

2.2. Immunohistochemical study

An immunohistochemical (IHC) study was carried out according to a standardized procedure using serial paraffin sections 4 μm thick, placed on adhesive glasses coated with a polysyn (Menzel-Glaser, Germany) and DAKO reagents.

The IHC panel included the following antibodies: macrophage and histiocyte marker, 68 cluster of differentiation, macroscalin - CD68 (Monoclonal Mouse, CloneKP1, Dako Cytomation, Denmark Ready-to-Use), vascular endothelial growth factor A marker - VEGF-A (monoclonal mouse, cloneVG1, dent, DGT-D). Denmark, ata 1:50 dilution), collagenIV (monoclonal mouse, clone CIV 22, Dako Cytomation, Denmark, ata 1:25 solution), CD34 (monoclonal mouse, cloneVG1, Dako Cytomation, Denmark, ata 1:50 dilution). For staging the reaction with markers staining protocol recommended by the manufacturer was used [16]. The following equipment was used: a cutting station LEEC ltd (Leica, Germany), a hybrid histological processor LOGOS (Millestone, Italy), a modular center for pouring Leica EG 1150 (Leica, Germany), an automatic rotary microtome Leica RM 2255 (Leica, Germany), a laboratory Leica DM2000 microscope (Leica, Germany), Bond Max immunohisteal agent (Leica, Germany), Aperio CS2 digital drug scanner (Leica, Germany). A series of studies using positive and negative samples were used to control the method, which served as benchmarks.

2.3. Cell count and image analysis

The investigated images were obtained using an OLYMPUS C5050Z («Olympus», Japan) digital camera and a microscope OLYMPUS CX41 («Olympus», Japan) in magnification x200 (numerical aperture - 0.65; working distance - 0.6 mm). Morphometric analysis was performed in 10 random fields of view, by zone, depending on the distance from the source of specific inflammation. The absolute number of immunopositive cells was estimated manually using the NIH image J suite software (http://rsb.info.nih.gov/ij/). The perfusion index (IP) was defined as the ratio of the area of positively colored CD34 endothelium to the area of the vessel lumen.

$$IP = \frac{S_{Endotelium}}{S_{Lumen}}$$

Statistical data analysis was performed using Statistica for Microsoft Windows software package, version 10.0 (StatSoft Inc., USA). The probability value <0.05 were considered statistically significant.Data were reported as M ± SD, where M – arithmetic mean and SD – standard deviation. Statistical analysis included making of variation series of quantitative data, determination of distribution normality using the Kolmogorov-Smirnov test, calculating an arithmetic mean, standard deviation, mean error, coefficient of

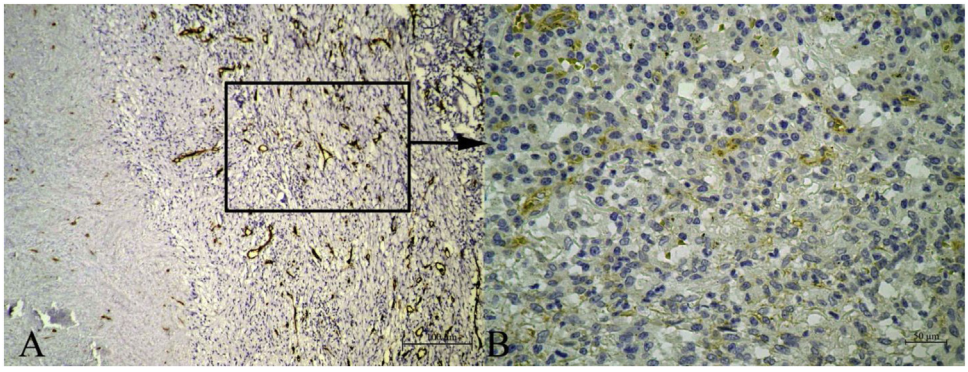

Fig. 1. FCT-MBT-. IGH expression CD34 (a), collagen IV (B). Capillary vessels without basal membrane (arrow), discontinuous contour of the basal membrane (thick arrow) in the zone of granulation tissue.

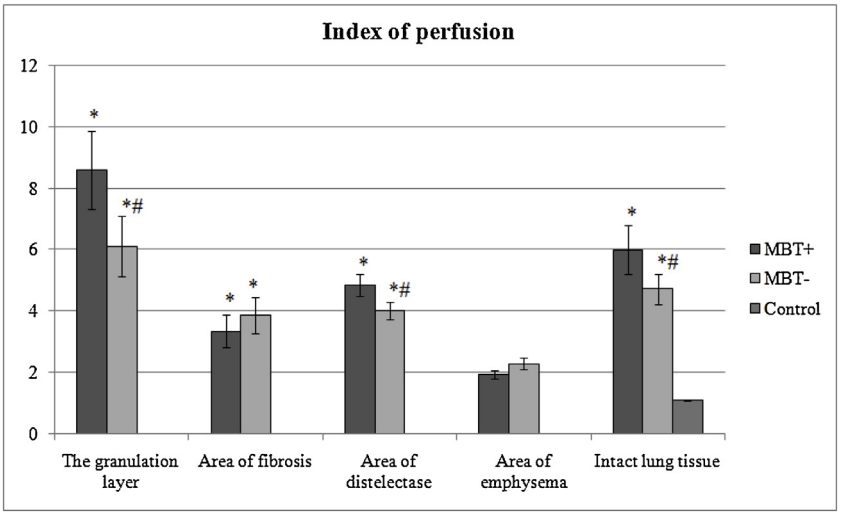

Fig. 2. Graph of vascular index of perfusion in fragments of pulmonary tissue of patients with FCT, depending on the bacteria excretion activity. *- p≤0,05 relative to the control group; # - p≤0,05 relative to MBT+.

variation and percentage deviation compared to the control. The significance of compared values differences was determined using the non-parametric Mann-Whitney U-test at a significance level $\alpha = 5\ \%$.To assess a statistical interrelationship Pearson's correlation coefficient was calculated.

The study was carried within the framework supported by the federal state autonomous educational institution of higher education «V.I. Vernadsky Crimean Federal University» grant number VG03/2018 and held in the Center for collective use of scientific equipment «Molecular Biology» of Medical Academy named after S.I. Georgievsky (Academic Unit) of V.I. Vernadsky Crimean Federal University with the support of the CFU development program.

3. Results

Because of the study, the presence of persistent diffuse positive expression of VEGF-A independently on the localization in the cavernous focus was established. However, significant differences in the prevalence of processes of angiogenesis and vascularization were determined depending on the discharge activity in conventionally segregated areas of specific inflammation: pyogenic area, a layer of specific granulation tissue, fibrous layer, pericavernous area and the intact lung tissue.

3.1. Pyogenic area

In pyogenic layer, the maximum degree the severity of the reaction VEGF-Awas fixed. It was also determined focal weakly expressed cytoplasmic presence of marker CD34 in the form of the foci of endothelial cells'clusters, separated from each other by necrotizedepithelioid and connective tissue cells. In some cases, immunopositive CD34 s̈hadowsöf vessels were visualized in the area of tissue detritus. CollagenIV demonstrated negative reaction.

3.2. Specific granulation tissue

The zone of specific granulation tissue was histologically characterized by the proliferation of a large number of capillary vessels separated by chaotically intertwining thin collagen fibers and pronounced infiltration by CD68+ macrophages. During the IGH reaction with VEGF-A marker, it was found that some of these macrophages are highly active in relation to the expression of vascular growth factor A: 33,50 ± 1,03 (MBT+) and 43,12 ± 1,12 (MBT -) (Table 1).

Evaluating the expression of the CD34 marker in comparison with the pyogenic zone, a statistically significant increase in the number of positively colored elements forming tubular structures in the form of a moderately expressed vascular network were determined ($p < 0.05$) (Fig. 1A).

The number of CD34 + vessels was 12.87 ± 1.84, which lower in the group without discharge. In this case, regardless of the discharge capillaries had no basement membrane, or characterized by discontinuity of its contour (Fig. 1B). The thickness of the basal membrane visualized by collagen IV in the granulation tissue zone in patients with MBT + was 1.65 ± 0.24 μm, and with MBT - 2.01 ± 0.08 μm. Structural inferiority of newly formed vessels was accompanied by a statistically significant increase in vascular permeability in relation to the control group, which was confirmed by an increase in IP in the groups with FCT (Table 2) (Fig. 2).

Correlation analysis revealed a weak feedback between the expression of CD34 and collagen IV in the specific area of granulation tissue (R=-0,50) and moderate direct relationship between the number of CD34+ vessels in this zone with the number of positive VEGF+/CD68+ (R = 0.51) and collagenIV (R = 0,65) in pericavernous area.

3.3. The fibrous layer

The fibrous layer of the cavern under the conditions of FCT-MBT + was characterized by the fibrosis of the coarse-fiber connective tissue, the presence of necrosis foci and bundles of small CD34+ vessels similar to those in the granulation layer.

In the rest of the MBT + fibrous walls and in all studied samples of lung tissue of patients with MBT, the reduction of CD34+ elements was due to differentiation of vessels into arterial and venous types (9.11 ± 1.01). VEGF + cells were recorded in the form of diffuse scattered infiltration of mature connective tissue and near lymphoid aggregates formed during the transition to pericavernous zone accordingly to $CD68^+$ of weak activity.

In IHC reaction with collagenIV the basement membrane were clearly visualized in the form of thick continuous lines thickened to 9.12 ± 0,81 μm, circularly covering the perimeter of the vessel. In larger vessels with the phenomena of sclerosis and focal hyalinosis, defined in a routine histological study, the distribution of collagen IV occurred in the form of a double contour of the vessel: from the

Table 1
Distribution of VEGF-A + macrophages in the CD68+ macrophage population.

Localization	MBT+		MBT-		Control group	
	VEGF-A (M ± SD)	CD68 (M±SD)	VEGF-A (M±SD)	CD68 (M±SD)	VEGF-A (M±SD)	CD68 (M±SD)
Pyogenic layer	+++	+++	+/-	+/-	10,02 ±0,19	23,67 ±1,30
The granulation layer	33,50 ±1,03*	143,45 ±7,01*	43,12 ±1,12*	117,31 ±7,08*#	10,02 ±0,19	23,67 ±1,30
Area of fibrosis	38,01 ±1,15*	122,11 ±5,79*	29,41 ±0,83*	75,71 ±4,17*#	10,02 ±0,19	23,67 ±1,30
Area of draining bronchus	35,54 ±1,87*	103,05 ±4,69*	84,69 ±3,51*#	84,69 ±4,75*#	10,02 ±0,19	23,67 ±1,30
Area of distelectase	42,12 ±1,01	86,74 ±3,19*	47,24 ±1,82*	64,92 ±2,71*#	10,02 ±0,19	23,67 ±1,30
Area of emphysema	8,01 ±0,82	9,16 ±0,45*	7,30 ±0,38	9,10 ±0,74*	10,02 ±0,19	23,67 ±1,30
Intact lung tissue	42,51 ±1,49*	51,24 ±2,63*	29,75 ±1,01*#	30,19 ±1,30#	10,02 ±0,19	23,67 ±1,30

*- p≤0,05 relative to the control group;# - p≤0,05 MBT- relative to MBT+.

Table 2
Morphometric characteristics of the functional capability of the vascular bed in fragments of pulmonary tissue of patients with FCT depending on discharge of patients and in the control group.

Zone	MBT+ (n = 89)		MBT- (n = 74)		Control group (n = 30)	
	collagen IV M ± SD	CD34 M ± SD	collagen IV M ± SD	CD34 M ± SD	collagen IV M ± SD	CD34 M ± SD
Pyogenic layer	–	0,34 ±0,01*	–	0,13 ±0,02*		
The granulation layer	1,65 ±0,24*	19,48 ±0,81*	2,01 ±0,08*	12,87 ±1,84#	3,36± 0,01	12,04± 0,01
Area of fibrosis	5,03 ±0,86*	7,01 ±0,93*	9,12 ±0,81*#	9,11 ±1,01		
Area of distelectase	4,87 ±0,57	16,31 ±1,82*	3,98 ±0,25	15,33 ±2,87		
Area of emphysema	3,12± 0,34	4,14 ±0,64*	2,99 ±0,26*	3,55 ±0,72*		
Intact lung tissue	5,77 ±0,59*	28,00 ±1,94*	4,08 ±0,27	21,39 ±3,18 *#		

*- p≤0,05 relative to the control group; # - p≤0,05 relative to MBT+.

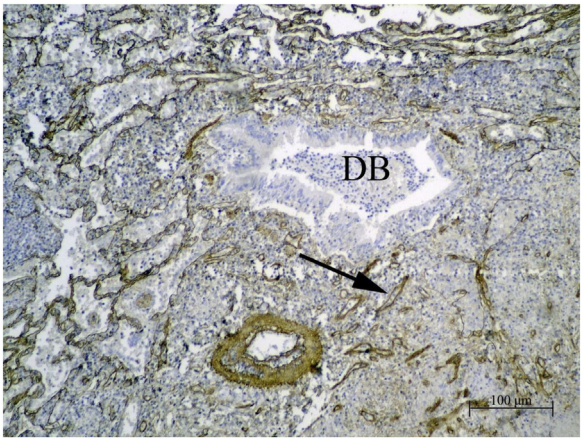

Fig. 3. FCT-MBT+. Expression of the collagenIV marker. Basal membranes of the formed vessels of the fibrous layer in the cavity (arrow), the region of the draining bronchus (DB). HC. × 100.

intima and the outer contour of smooth muscle cells with diffuse uniform staining tunica media (Fig. 3).

It should be noted that in patients with MBT - due to the thickening of the fibrous layer in comparison with the MBT + group, these phenomena have a greater severity and prevalence. However, the indices of vascular IP in the group without discharge (3.87 ± 0.59) and with active bacterial excretion (3.35 ± 0.54) showed no statistically significant differences between the groups, but were significantly increased in relation to the control group (1.10 ± 0.01, p < 0.05).

3.4. Pericavernous area

In pericavernous area, regardless of active discharge, formed of alternating sections of distelectase, atelectasis and emphysema. The intensity of cytoplasmic expression of the endothelial marker varied significantly in all these areas. Thus, the maximum number of CD34 + elements was determined in the areas of dis-and atelectasis, which is due, in our opinion, to the uneven decline of the alveoli per unit area: 16,31 ± 1,82 – MBT + and 15,33 ± 2,87 – MBT-.

Analysis of collagen IV expression indicated heterogeneity of changes in the basal membranes of CD34 + vessels. In patients with FCT-MBT + the destructurization of the blood vessels and increase of blood, vessels were recorded mainly at the foci of pneumonia. In clinical abacillation, the basal membranes are thickened due to the growth of hard-to-degrade type 4 collagen and the formation of a clearly defined double vascular contour of the inner and outer surfaces. Parallel histochemical reaction with pikrofuksin by van Gieson allowed identifying the thickening of the vessel walls due to the replacement of elastic fibers of collagen. The latter type of vas-

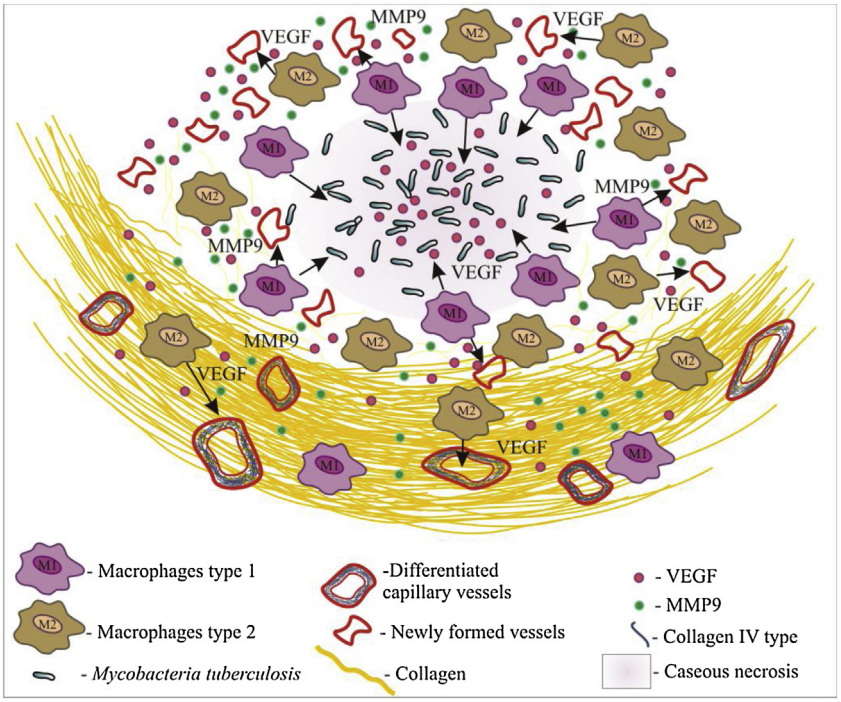

Fig. 4. Scheme of induction of neoangiogenesis in the cavity wall by macrophages of 1st and 2nd types.

cular wall change was also characterized by a significant increase in IP in comparison with the control group of patients without bronchopulmonary pathology.

The number and types of VEGF + macrophages also varied depending on the severity of alterative-exudative processes. In cases of progressive caseous pneumonia, extensive clusters of hyperactivated CD68+/VEGF + macrophages were determined. In patients with FCT-MBT the population of CD68+ cells was represented mainly by weakly activated tissue VEGF + macrophages, the highly activated ones were in the form of small groups of explicit alveolar macrophages in focal collabated alveoli. The correlation analysis revealed a direct strong correlation (R = 0.68) between the number of VEGF + cells in the zone of the lymphoid follicles and the number of CD68+ macrophages localized in pericavernous area that supports the theory of macrophages'polarization by the cells of the lymphoid series.

3.5. *Intact lung tissue*

Analyzing IGH reaction result regardless of the activity of bacterial excretion, it was found the rise of vessels area of with increased IP and progressive thickening of the walls due tothe proliferation and disintegration of collagen IV.

4. Discussion

Immunophenotypic features of the macrophages' population, angiogenesis processes in the focus of specific inflammation, and surrounding intact pulmonary tissue indicated that activated CD68+ macrophages have the ability to influence on each phase of the angiogenic process [17,18]:

1 Changes in the local extracellular matrix,
2 Induction of endothelial cells to migration or proliferation,
3 Inhibition of vascular growth with formation of differentiated capillaries.

The detailing of macrophage pool functional activity with the using the VEGF-A IGH marker allowed to determine two immunophenotypes of CD68+/VEGF - and CD68+/VEGF + macrophages [19,20], which were statistically different in quantity depending on the activity of discharge with the ratio of VEGF+:VEGF - cells 1:2in the progression of the process, and 1:1.5.in clinical abacillation.

Taking into account the degree of macrocyalin expression in given macrophages, in vitro studies' data about the direct induction of VEGF-A synthesis by anaerobic M. tuberculosis for the comfortablemilieu, (e.g. tissue oxygenation), can be considered confirmed [21,22]. Proof of neoangiogenesis' activity are the maximum rate of CD34 in the wall of the cavity (mainly due to the specific granulation tissue) and in areas of dis - and atelectasis pericavernous zone.

However, despite the identity of the IGH reaction, in our opinion, different mechanisms lead to its severity. Therefore, in granulation tissue, the process of neoangiogenesis with the formation of a large number of thin-walled defectivecapillaries induced on the one hand by progressive necrobiotic changes in pulmonary tissue, and on the other by the absolute prevalence of hyperactivated CD68+/VEGF + macrophages in this zone. In this case, the number of newly formed vessels correlates back to the area of expression of collagen IV, which is the result of a high rate of neoangiogenesis due to massive stimulation – as well as the presence of proinflammatory macrophages type 1, producing including MMP9 (metalloproteinase 9) [23,24]. The latter is necessary for depolymerization of basal membranes and the formation of the so-called fibrin gel, which is both a reactivator and a structural platform for dynamically increasing endothelial cells (Fig. 4).

With the transition of the granulation layer into the fibrous one, there is a depression of the number of CD34 + vessels with simultaneous intensification of collagen formation of type 4 and an increase in the IP of vessels, which indicates a sharp decrease in vascularization with a pathological increase in vascular permeability [25].

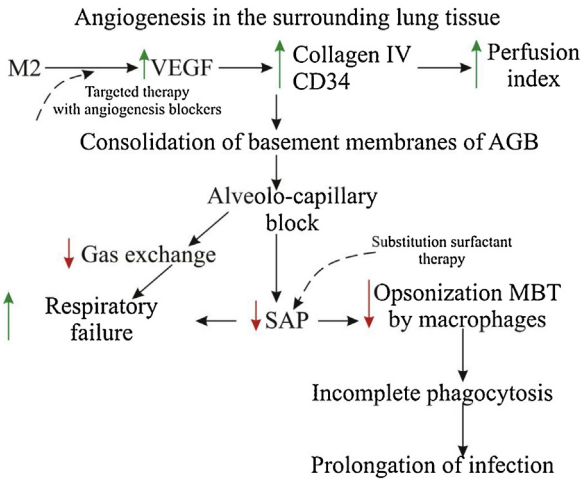

Fig. 5. Scheme of pathological angiogenesis and its significance for pulmonary tissue remodeling in FCT.

Found functional characteristics of pathological angiogenesis, in our opinion, are one of the main links that complicates the targeted delivery of drugs to the focus of specific inflammation and may lead to the formation of drug-resistant forms of tuberculosis. Comparable in intensity indicators of CD34 expression visualized either in pericavernous area. However, this reaction is associated with ununiformed alveolar collapse, or in the case of progressive alterative-exudative reactions – with diffuse infiltration of interalveolar septa by weakly activated macrophages and large clusters of highly activated CD68+ cells [26–31].

Progressive dysfunction of the generation of blood vessels both in pericavernous area and in the surrounding intact lung tissue causes the damage of the vascular wall, disruption of the processes of filtration and reabsorption, increase of tissue hypoxia due to abrupt swelling of the intercellular structures, which compress the vascular walls and, as a consequence, lead to the deficiency of nonspecific and specific cellular components of the immune system [32]. The final result of pathological angiogenesis and perfusion disorders is the prolongation of the inflammatory process, activation of fibroblasts, the growth of coarse-fiber connective tissue, including the interalveolar septa, what closes the v̈icious circleöf increasing respiratory failure by forming an alveolar-capillary block (Fig. 5) [33].

Despite the heterogeneity in morphological and functional parameters of VEGF-A-dependent angiogenesis in FCT, its value for the organism is fatal: the effectiveness of drugs is minimized due to their difficult delivery as well as aggravatedtissue hypoxia, leading to fibrous remodeling of the aerogematic barrier and progression of respiratory failure [34–37]. Therefore, inhibition of vascular endothelial growth factor A is a potential type of alternative targeted therapy [38–41].

5. Conclusion

Hyperactivation of VEGF-A dependent angiogenesis in the foci of specific inflammation leads to the dysregulation of the formation in functionally complete vessels and the creation of the most comfortable conditions for the persistence of *M. tuberculosis* in the form of sufficient aeration in the area of specific granulations, while reducing the vascular permeability of the fibrous layer vessels, providing inefficient recruitment of cells of the immune system and targeted delivery of drugs.

In the pericavernous zone and intact pulmonary tissue, hyperexpression of VEGF-A leads to aggravation of local pulmonary hypertension, increase of tissue hypoxia, fibrous remodeling of the aero-hematic barrier and the formation of the alveolar-capillary block, which is a morphofunctional indicator of respiratory failure.

Taking into account the absolute prevalence of proangiogenic factors in FCT in all patients, the use of targeted therapy aimed at VEGF-A blockade is pathogenetically justified.

References

[1] S.S. Shin, et al., Isoniazid therapy for Mycobacterium tuberculosis infection in HIV clinics, Int. J. Tuberculosis Lung Dis. 20 (7) (2016) 961–966, http://dx.doi.org/10.5588/ijtld.15.0988.
[2] K.A. Wilkinson, et al., HIV-associated tuberculosis, Clin. Dev. Immunol. (2012), 950964, http://dx.doi.org/10.1155/2012/950964.
[3] C.R. Diedrich, J.L. Flynn, HIV-1/Mycobacterium tuberculosiscoinfection immunology: how does HIV-1 exacerbate tuberculosis? Infect. Immun. 79 (2011) 1407–1417, http://dx.doi.org/10.1128/IAI.01126-10.
[4] D.J. Frank, D.J. Horne, N.K. Dutta, M.T. Shaku, R. Madensein, T.R. Hawn, A.J.C. Steyn, P.C. Karakousis, B.D. Kana, G. Meintjes, B. Laughon, Z. Tanvir, Remembering the host in tuberculosis drug development, J. Infect. Dis. 712 (2018), http://dx.doi.org/10.1093/infdis/jiy712.
[5] G. Slavchev, L. Michailova, N. Markova, L-form transformation phenomenon in Mycobacterium tuberculosis associated with drug tolerance to ethambutol, Int. J. Mycobacteriol. 5 (4) (2016) 454–459, http://dx.doi.org/10.1016/j.ijmyco.2016.06.011.
[6] N. Markova, G. Slavchev, L. Michailova, Unique biological properties of Mycobacterium tuberculosis L-form variants: impact for survival under stress, Int. Microbiol. 15 (2) (2012) 61–68.
[7] H. Polena, et al., Mycobacterium tuberculosis exploits the formation of new blood vessels for its dissemination, Sci. Rep. 6 (2016), e33162, http://dx.doi.org/10.1038/srep33162.
[8] K. Jin, T. Li, H. van Dam, F. Zhou, L. Zhang, Molecular insights into tumour metastasis: tracing the dominant events, J. Pathol. 241 (5) (2017) 567–577, http://dx.doi.org/10.1002/path.4871.
[9] M. Mohme, S. Riethdorf, K. Pantel, Circulating and disseminated tumour cells - mechanisms of immune surveillance and escape, Nat. Rev. Clin. Oncol. 14 (3) (2017) 155–167, http://dx.doi.org/10.1038/nrclinonc.2016.144.
[10] Jianrong Lu, et al., The Warburg effect in tumor progression: mitochondrial oxidative metabolism as an anti-metastasis mechanism, Cancer Lett. 356 (2 Pt A) (2014) 156–164, http://dx.doi.org/10.1016/j.canlet.2014.04.001.
[11] M. Lohela, M. Bry, T. Tammela, K. Alitalo, VEGFs and receptors involved in angiogenesis versus lymphangiogenesis, Curr. Opin. Cell Biol. 21 (2009) 154–165, http://dx.doi.org/10.1016/j.ceb.2008.12.012.
[12] S. Koch, S. Tugues, X. Li, L. Gualandi, L. Claesson-Welsh, Signal transduction by vascular endothelial growth factor receptors, Biochem. J. 437 (2011) 169–183, http://dx.doi.org/10.1042/BJ20110301.
[13] H.Y. Park, C.R. Hahm, K. Jeon, W.J. Koh, G.Y. Suh, M.P. Chung, et al., Serum vascular endothelial growth factor and angiopoietin-2 are associated with the severity of systemic inflammation rather than the presence of hemoptysis in patients with inflammatory lung disease, Yonsei Med. J. 53 (2012) 369–376, http://dx.doi.org/10.3349/ymj.2012.53.2.369.
[14] M. Datta, L.E. Via, W.S. Kamoun, C. Liu, W. Chen, G. Sieno, et al., Anti-vascular endothelial growth factor treatment normalizes tuberculosis granuloma vasculature and improves small molecule delivery, Proc. Natl. Acad. Sci. U. S. A. 112 (2015) 1827–1832, http://dx.doi.org/10.1073/pnas.1424563112.
[15] S.H. Oehlers, M.R. Cronan, N.R. Scott, M.I. Thomas, K.S. Okuda, E.M. Walton, et al., Interception of host angiogenic signalling limits mycobacterial growth, Nature 17 (2015) 612–615, http://dx.doi.org/10.1038/nature13967.
[16] N.S. Ahmad, S.G. Martin, S.J. Storr, Immunohistochemical assessment of leukocyte involvement in Angiogenesis, Angiogenesis Protoc. (2016) 49–57, http://dx.doi.org/10.1007/978-1-4939-3628-1_3.
[17] G. Musumeci, C. Loreto, S. Giunta, V. Rapisarda, M.A. Szychlinska, R. Imbesi, A. Castorina, T. Annese, S. Castorina, P. Castrogiovanni, D. Ribatti, Angiogenesis correlates with macrophage and mast cell infiltration in lung tissue of animals exposed to fluoro-edenite fibers, Exp. Cell Res. 346 (1) (2016) 91–98, http://dx.doi.org/10.1016/j.yexcr.2016.06.017.
[18] E.P. Golubinskaya, T.G. Filonenko, A.V. Kubyshkin, et al., Immunophenotype of the macrophage population in fibrous, cavernous pulmonary tuberculosis, Bull. Sib. Med. (2019).
[19] A. Khan, V.K. Singh, R.L. Hunter, C. Jagannath, Macrophage heterogeneity and plasticity in tuberculosis, J. Leukoc. Biol. (2019), http://dx.doi.org/10.1002/jlb.mr0318-095rr.
[20] M.A. Kalfa, E.P. Golubinskaya, T.G. Filonenko, et al., Proliferative activity of stromal cell elements at fibrous-cavernous pulmonary tuberculosis depending on macrophageal immunophenotype, New Armenian Med. J. (2019).
[21] F.C.R. Zucchi, A.M.C. Tsanaclis, Q. Moura-Dias, C.L. Silva, A. Pelegrini-da-Silva, L. Neder, O.M. Takayanagui, Modulation of angiogenic factor VEGF by DNA-hsp65 vaccination in a murine CNS tuberculosis model, Tuberculosis 93 (3) (2013) 373–380, http://dx.doi.org/10.1016/j.tube.2013.02.002.
[22] H. Polena, F. Boudou, S. Tilleul, et al., Mycobacterium tuberculosis exploits the formation of new blood vessels for its dissemination, Sci. Rep. 6 (2016), e33162, http://dx.doi.org/10.1038/srep33162.

[23] L.S. Angelo, R. Kurzrock, Vascular endothelial growth factor and its relationship to inflammatory mediators, Clin. Cancer Res. 13 (10) (2007) 2825–2830, http://dx.doi.org/10.1158/1078-0432.ccr-06-2416.
[24] N.P. Kumar, B. Velayutham, D. Nair, S. Babu, Angiopoietins as biomarkers of disease severity and bacterial burden in pulmonary tuberculosis, Int. J. Tuberc. Lung Dis. 21 (1) (2017) 93–99, http://dx.doi.org/10.5588/ijtld.16.0565.
[25] N. Yi, B.G. Jung, X. Wang, R. Vankayalapati, B. Samten, The early secreted antigenic target of 6 kD of Mycobacterium tuberculosis inhibits the proliferation and differentiation of human peripheral blood CD34 + cells, Tuberculosis 101 (2016) S28–S34, http://dx.doi.org/10.1016/j.tube.2016.09.018.
[26] Y. Zhou, S. Yoshida, Y. Kubo, T. Yoshimura, Y. Kobayashi, T. Nakama, M. Yamaguchi, K. Ishikawa, Y. Oshima, T. Ishibashi, Different distributions of M1 and M2 macrophages in a mouse model of laser-induced choroidal neovascularization, Mol. Med. Rep. 15 (6) (2017) 3949–3956, http://dx.doi.org/10.3892/mmr.2017.6491.
[27] M. Falleni, F. Savi, D. Tosi, E. Agape, A. Cerri, L. Moneghini, G.P. Bulfamante, M1and M2macrophages' clinicopathological significance in cutaneous melanoma, Melanoma Res. 27 (3) (2017) 200–210, http://dx.doi.org/10.1097/CMR.0000000000000352.
[28] G.J. Kotwal, S. Chien, Macrophage differentiation in normal and accelerated wound healing, Results Probl. Cell Differ. 62 (2017) 353–364, http://dx.doi.org/10.1007/978-3-319-54090-0_14.
[29] C.D. Mills, Anatomy of a discovery:M1 and M2 macrophages, Front. Immunol. 6 (2015) 212, http://dx.doi.org/10.3389/fimmu.2015.00212.
[30] F.O. Martinez, S. Gordon. The M1 and M2 paradigm of macrophage activation: time for reassessment, F1000Prime Rep. 6 (2014) 13, http://dx.doi.org/10.12703/P6-13.
[31] M. Genin, F. Clement, A. Fattaccioli, M. Raes, C. Michiels, M1 and M2 macrophages derived from THP-1 cells differentially modulate the response of cancer cells to etoposide, BMC Cancer 15 (2015) 577, http://dx.doi.org/10.1186/s12885-015-1546-9.
[32] A. Szade, A. Grochot-Przeczek, U. Florczyk, A. Jozkowicz, J. Dulak, Cellular and molecular mechanisms of inflammation-induced angiogenesis, J. IUBMB Life 67 (3) (2015) 145–159, http://dx.doi.org/10.1002/iub.1358.
[33] D.H. Kong, M.R. Kim, J.H. Jang, H.J. Na, S. Lee, A review of anti-angiogenic targets for monoclonal antibody cancer therapy, Int. J. Mol. Sci. 18 (8) (2017), http://dx.doi.org/10.3390/ijms18081786, pii: E1786.
[34] P. Desikan, A. Rangnekar, Host-targeted therapy for tuberculosis: time to revisit the concept, Indian J. Med. Res. 147 (3) (2018) 233–238, http://dx.doi.org/10.4103/ijmr.IJMR_652_17.
[35] K. Patil, S. Bagade, S. Bonde, S. Sharma, G. Saraogi, Recent therapeutic approaches for the management of tuberculosis: challenges and opportunities, Biomed. Pharmacother. 99 (2018) 735–745, http://dx.doi.org/10.1016/j.biopha.2018.01.115.
[36] A. Shehzad, G. Rehman, M. Ul-Islam, W.A. Khattak, Y.S. Lee, Challenges in the development of drugs for the treatment of tuberculosis, Braz. J. Infect. Dis. 17 (1) (2013) 74–81, http://dx.doi.org/10.1016/j.bjid.2012.10.009.
[37] M. Marimani, A. Ahmad, A. Duse, The role of epigenetics, bacterial and host factors in progression of Mycobacterium tuberculosis infection, Tuberculosis (Edinb) 113 (2018) 200–214, http://dx.doi.org/10.1016/j.tube.2018.10.009.
[38] D. Kiran, B.K. Podell, M. Chambers, R.J. Basaraba, Host-directed therapy targeting the Mycobacterium tuberculosis granuloma: a review, Semin. Immunopathol. 38 (2) (2015) 167–183, http://dx.doi.org/10.1007/s00281-015-0537-x.
[39] A. Koch, H. Cox, V. Mizrahi, Drug-resistant tuberculosis: challenges and opportunities for diagnosis and treatment, Curr. Opin. Pharmacol. 42 (2018) 7–15, http://dx.doi.org/10.1016/j.coph.2018.05.013.
[40] C. Stek, B. Allwood, N.F. Walker, R.J. Wilkinson, L. Lynen, G. Meintjes, The immune mechanisms of lung parenchymal damage in tuberculosis and the role of host-directed therapy, Front. Microbiol. 30 (9) (2018) 2603, http://dx.doi.org/10.3389/fmicb.2018.02603.
[41] Y. Abe, M. Nakamura, Y. Oshika, H. Hatanaka, T. Tokunaga, Y. Ohkubo, T. Hashizume, K. Suzuki, T. Fujino, Serum levels of vascular endothelial growth factor and cavity formation in active pulmonary tuberculosis, Respiration 68 (5) (2001) 496–500.