Structural features of the lower limb deep vein remodeling as a morphologic component in the pathogenesis of pulmonary thromboembolism in cancer patients

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Oncological patients are at high risk of developing thromboembolic complications, which is a manifestation of a complex set of symptoms - cancer. At the same time, the analysis of the literature shows that the question of the involvement of structural changes of the vascular wall in the pathogenesis of possible primary thrombus formation in cancer patients remains open. The aim of the study - to study the structural features of remodeling of the deep vein of the lower extremity as a morphological link in the pathogenesis of pulmonary embolism in cancer patients. Retrospective analysis of 54 protocols of autopsy of deaths from cardiopulmonary shock caused by pulmonary embolism in 2014-2018 was performed. In parallel, all patients were determined the number of free-circulating endothelial cells in the citrate blood by Hladovez J. method, in modification of Sivak V.V. (2007). Statistical processing of digital data was performed using the software "Excel" and "STATISTICA" 6.0. In retrospective analysis of autopsy protocols, the highest proportion of pulmonary embolism was reported in patients with cancer of the uterus and colon. Morphological changes of the deep vein of the lower extremities in cancer patients were manifested by endothelium desquamation and circular and focal muscular-fibrous hyperplasia of the intima, which caused disturbances of laminar flow of blood; muscular-fibrous atrophy with neovascularization of the middle membrane and sclerosis of vasa vasorum vessels of adventitia. The process of remodeling was also manifest by the inflammatory transformation of the vascular wall, the formation of obstructing and floating blood clots with their subsequent organization, vascularization and recanalization. The cause of intimal thickening, atrophy, and sclerosis with midbrain neovascularization is most likely a hypoxic mechanism of activation of transforming connective tissue growth factors that stimulate collagenogenesis and neoangiogenesis. Desquamation of endothelial cells can also be considered as a significant contributor to thrombus formation. Endothelial cells have a protective function aimed at eliminating damage to the vascular wall by thrombus formation and the development of fibrous intima hyperplasia. In addition, tumor cells are themselves capable of producing excess platelet growth factor, which causes intima proliferation. So, a component of pathomorphogenesis of pulmonary artery thromboembolism in cancer patients is a complex structural reconstruction of the wall of the deep vein of the lower extremity, which causes the development of its thrombosis. Deep vein remodeling in cancer patients is characterized by endothelial cell desquamation, intima and middle-membrane thickening and sclerosis in combination with vasa vasorum fibrous degeneration and perforant vein thrombosis. In response to hemodynamic disorders, compensatory remodeling develops: the combination of leiomyocyte atrophy with their hypertrophy and neoangiocerization of the middle membrane.

Keywords: deep thigh vein, cancer, pulmonary embolism.
in 35-40 people per 100 thousand population and is one of the leading causes of death in 15% of patients and in 43% of patients - a background for other fatal complications [6, 8, 10]. Among these patients, a significant percentage have patients with cancer, who are detected in 50% of cancer patients, which determines the course of the disease, requires dynamic monitoring and timely appointment of both pharmacological and mechanical means of preventing thrombus formation [7, 8, 13]. The high number of thromboembolic complications necessitates optimization of anticoagulant therapy and diagnostic methods [15, 16]. In most cancer patients, pulmonary embolism occurs through deep vein thrombosis of the lower extremities, or pelvis [4, 5, 11]. Among its most probable causes are: prolonged immobilization of patients, which causes impaired blood flow from the lower extremities, hypercoagulation caused by the procoagulative activity of tumor cells and the release of inflammatory cytokines and clotting factors; chemotherapy, hormone therapy, surgery and use of central venous catheter [1, 2, 5, 12]. At the same time, its morphogenesis at the specified pathology is not sufficiently elucidated. The analysis of the literature regarding remodeling of the deep vein of the lower extremities shows that there are no studies of the structural changes of the vascular wall in the pathogenesis of pulmonary artery thromboembolism in oncological patients, and the elucidation of its morphogenesis is of paramount importance in phlebology as a morphological basis of understanding.

The aim of this work is to find out structural features of remodeling of the deep vein of the lower extremity, as a morphological link of pathogenesis of pulmonary embolism in cancer patients.

Materials and methods

A retrospective analysis of 54 protocols of autopsy of deaths from cardiopulmonary shock caused by pulmonary embolism in 2014-2018 was conducted in the Ternopil Regional Clinical Oncology Center. In addition to necropsy data, morphological study of deep vein biopsies obtained from different topographic areas of the lower extremity was performed in 12 operations for acute ascending thrombophlebitis in cancer patients.

For the preparation of micropreparations standard protocols of sealing and dehydration of previously fixed in 10% solution of neutral formalin tissues were used, followed by pouring into paraffin and preparation of sections [3]. Deparaffin sections were stained with hematoxylin and eosin, Malory trichrome, resorcinol-fuchsin according to Weigert, alcin blue, and PAS reaction.

Submicroscopic examination was performed on biopsy material only. The biopsies of the veins were pre-fixed in a 2.5% solution of glutaraldehyde with an active reaction of medium pH 7.2-7.4 prepared on Millonig phosphate buffer. Postfixation was carried out with a 1% solution of osmium tetroxide on Millonig buffer for 60 minutes, after which the material was dehydrated in alcohols and acetone and poured into epoxy resins according to conventional methods [3]. Ultra-thin sections made on a UMTP-7 ultramicrotome were stained with a 1% aqueous solution of uranyl acetate, counterstained with lead citrate according to the Reynolds method, and studied in a PEM-125K electron microscope. Semi-thin sections were stained with methylene blue [3].

In all patients, the number of free-circulating endothelial cells in the citrate blood was determined according to the method of Hladovez J., in modification by Sivak V.V. [14].

In the work with histological preparations and semi-thin sections used microscopes SEOSCAN, Lumam R-8, MBI-15. Images from microscopes were displayed on a computer monitor using a VISION Color CCD Camera and Inter VideoWinDVR program.

Statistical processing of digital data was carried out using the software "Excel" ("Microsoft", USA) and "STATISTICA" 6.0 ("Statsoft", USA).

Results

In retrospective analysis of autopsy protocols, the highest proportion of pulmonary artery thromboembolism was reported in women (61.1% of observations versus 38.9% in men). The mean age of deceased women was 61.93±1.51 years and 62.44±2.61 for men. It is noteworthy that in all 54 deaths pulmonary embolism complicated 5-6 days postoperatively. It was most frequently observed in patients with malignant epithelial neoplasms of the uterus (22.2%), large intestine: 13.0% - rectal, 7.4% - colon, 5.6% - rectosigmoid and urinary bladder (9.3%). Patients with gastric cancer (7.4%), ovaries (7.4%), prostate (5.6%), thyroid (1.8%), and pancreas (1.8%) were slightly less likely. It is worth noting that a high percentage (18.5%) of thromboembolism is also reported in patients with bronchial cancer and lung cancer. This can be explained by the presence of chronic right ventricular heart failure with varicose veins of the lower extremities. In all these cases (n=54), autopsy revealed signs of phlebotrombosis of the deep veins of the lower leg and thigh with impaired blood flow caused by occlusion of blood clots of various manifestations of structural organization. Of these, in five cases, pathomorphologically, they corresponded to fresh red blood clots that closely connected with the intimate filaments of fibrin, and in five cases the red blood clots were freely placed in the lumen of the vein (floating blood clots). In these cases, all layers of the vein are swollen, with the branching of all its structures and diffuse neutrophilic infiltration (Fig. 1). The thrombotic masses were dominated by platelets, erythrocytes, fibrin filaments and leukocytes. We interpret the presence of neutrophils as the cause of possible lysis of the thrombus, as well as thromboembolic complications. Red blood clots were also detected in the lumen of the perforated veins (Fig. 2).

In the remaining 44 cases, occlusion of the lumen of the vein with organized blood clots with signs of fibrosis, recanalization and revascularization was registered.
The lumens of the newly formed canals are enlarged with the near wall layer of fibrin, which can be interpreted as a morphological manifestation of secondary thrombosis (Fig. 3). The lumps of newly formed blood clots have slit-like outlines and chaotic placement. The wall of the deep vein of the hip is sclerosed. Inner elastic membrane wavy, thickened, soldered to the structural components of the thrombus. The thickness of the inner layer of such veins was 14.93±1.82 μm, the average 65.74±14.31 μm and the outer 25.82±8.43 μm, which indicates the thickening of all its layers.

The endothelial cells had elongated outlines with a large hyperchromic nucleus. Their desquamation was registered histologically (Fig. 4).

Quantitative analysis of free-circulating blood endothelial cells revealed that in acute thrombosis their level was 9.42±0.51 x10⁴/l, and in cases of organized thrombus - 6.53±0.20 x10⁴/l (p<0.001). Microscopically desquamated endothelial cells are polymorphic. Cells with pyknosis and rexis of the nucleus, as well as with karyolysis, cytoplasm edema and partial fragmentation were identified.

Electron microscopy revealed that the elongated endothelial cells were adjacent to a wide, swollen, area of collagen fiber accumulation. In the swollen, enlightened cytoplasm, there are numerous organelles that are destructively altered. The tubules of the granular endoplasmic reticulum are expanded and deformed, with the formation of irregular cavities. Damage to the mitochondria is accompanied by significant matrix enlightenment and destruction of the cristae. Primary and secondary lysosomes are presented and are freely located both in the cytoplasm and near the Golgi complex. The nuclei are star-shaped, with irregular outlines, due to the deep torsion of the karyolemma. In the center of the nucleoplasm there is an electron-transparent karyoplasm. Wide areas of condensed osmiophilic karyoplasm located below the karyoplasm (Fig. 5).

We recorded fibrotic remodeling of the intima in all cases of observation, both in the prethrombotic and...
postthrombotic segment of the investigated vein, which indicates the diffuse manifestation of the pathological process. Preferably, the thickening of the fibrous intima was circular or local in nature and combined with a thickening at the border of the middle layer and subendothelial space, in which noted a large number of activated fibroblasts with elongated hyperchromic nuclei that produce excessive amount of extracellular matrix, as evidenced by the increased positive response to the presence of non-sulfated and sulfated glycosaminoglycans when stained with alcian blue and PAS reaction.

Medium layer remodeling in pre- and post-thrombotic vein segments was manifested by severe sclerosis with leiomyocyte atrophy, in combination with their secondary hypertrophy. A characteristic feature of the middle layer is the presence of a neovascularization process (Fig. 6). The lumps of the arterioles are full-blooded, the endothelium with an enlarged, hyperchromic nucleus.

Changes in the outer layer of the venous wall were manifested by severe sclerosis. Particular attention is paid in all cases the presence of dilatation lumen vasa vasorum, plethora and walls sclerosis (Fig. 7).

Discussion
Pulmonary embolism, as a complication of thrombosis of the lower extremities and pelvis, is recorded in 35-40 people per 100 thousand population and is one of the main causes of death in 15% of patients and in 43% of patients - a background for other fatal complications [6, 8, 10]. The analysis of the literature regarding remodeling of the deep vein of the lower extremities shows that there are no studies of the structural changes of the vascular wall in the pathogenesis of pulmonary embolism in oncological patients.

We have found that the degree of endothelial cell desquamation in the process of postthrombophlebitic syndrome depended on the process prescription. Damaged endotheliocytes underwent conjugation, exposed intima, circulated with blood flow, which can be considered the structural basis of thrombus formation. According to Okhotnikova O.M. et al. [9], it is the endothelial cells that have a protective function aimed at eliminating damage to the vascular wall by thrombus formation and the development of fibrous hyperplasia of the intima, and Rozanov I.D. et al. [11] state that tumor cells are themselves capable of producing excess platelet-derived growth factor in excess, which causes intima proliferation.

We do not exclude that the structural changes of the deep vein wall outside the thrombus segment have a significant role in the development of postthrombophlebitic syndrome. This is evidenced by the remodeling of all layers of the vascular wall in the pre- and post-thrombotic segments of the vein: internal, middle and external.

The morphological changes of the proximal and distal segments with respect to deep vein thrombosis in patients

Fig. 5. Submicroscopic organization of the intima of the deep vein of a patient with colon cancer. Destructively altered endothelial cell (a), sclerosis (b). x9000.

Fig. 6. Intramural capillaries in the middle layer of the deep vein of the thigh (a). Semi-thin section. Methylene blue stain. x200.

Fig. 7. Dilatation of lumen and wall sclerosis of vasa vasorum (a). Histological section of deep vein. Staining with hematoxylin and eosin. x120.
with cancer are manifest by circular and focal muscular-fibrous intima hyperplasia with neovascularization of the middle layer. Morphogenetically, it can be assumed that the process of vein remodeling begins with thrombophlebitis with the subsequent development of thrombus organization and recanalization and vascularization.

The absence of dystrophic-inflammatory processes in the inner and middle layers of the peritrombotic segments of the vein allows us to consider intima thickening and neovascularization as independent processes. Basically, their fibrotic degeneration have probably a hypoxic mechanism, which is confirmed by sclerosis vasa vasorum, and neovascularization should be considered a compensatory process.

Conclusions

1. The component of pathomorphogenesis of pulmonary artery thromboembolism in cancer patients is a complex structural reconstruction of the wall of the deep vein of the lower extremity, which causes the development of its thrombosis.

2. Deep vein remodeling in cancer patients is characterized by endothelial cell desquamation, intima and middle layer thickening and sclerosis in combination with vasa vasorum fibrous degeneration and perforant vein thrombosis.

3. In response to hemodynamic disorders, compensatory remodeling develops: the combination of leiomyocyte atrophy with their hypertrophy and neovascularization of the middle layer.

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Зареєстрована у хворих на рак матки та ободової кишки. Морфологічні зміни глубокої вени нижніх кінцівок у хворих на рак проявлялись десквамацією ендотелію з циркулярною та вогнищевою м'язово-фіброзною гіперплазією інтри, що викликало порушення ламінарності току крові: м'язово-фіброзною атрофією з неоваскуляризацією середньої оболонки і склерозом судин вазутор адвентиції. Процес ремоделювання проявляється також запального трансформацією судинної стінки, утворенням обтуруючих і флотуючих тромбів із подальшою їх організацією, васкуляризацією та реканалізацією. Причиною потовщення інтри, атрофії та склерозу з неоваскуляризацією середньої оболонки, найімовірніше, є гіперплазичний механізм активації трансформуючих факторів росту сполучної тканини, який стимулює колагеноз- та неоканігіогенез. Десквамацію ендотеліоцитів також можна вважати суттєвим фактором сприяння тромбутворення. Саме ендотеліоцитам належить захисна функція, сприяючи усуненню пошкодження судинної стінки шляхом тромбутворення та розвитку фіброзної гіперплазії інтри. Окрім цього, пухлинні клітини самі по собі здатні продукувати в надлишку фактори росту, що стимулюють проліферацію інтрі. Таким чином, складною ланкою патоморфогенезу тромбоемболії легеневої артерії у хворих на рак є структурна перебудова стінки глубокої вени нижніх кінцівок, що сприяє розвитку тромбу. Ремоделювання глубоких вен у хворих на рак характеризується десквамацією ендотеліоцитів, потовщенням і склерозом інтрі та середньої оболонки у поєднанні із фіброзною дегенерацією вазутор та тромбозом перфорантних вен. У відповідь на гемодинамічні порушення розвивається компенсаторне ремоделювання: поєднання атрофії пеймоцитів з їх гіпертрофією і неоваскуляризацією середньої оболонки.

Ключові слова: глубока вена стегна, рак, тромбозболія легеневої артерії.

Структурні особливості ремоделювання глубокої вени нижнєї кінцічності, як морфологічного звезда тромбоемболії легеневої артерії більшого раком Боднар П.Я.

Онкологічне хворіння належать до групи високого риску розвиття тромбозболічних осложнений, які являються проявленнями складного синдрому комплекса - ракової болезні. Відносити до такої групи, аналізований в місцевому статистичному плануванні, є фактор гемодинамічних порушень, який сприяє розвитку тромбозку вен нижніх кінцівок. Цей процес характеризується склерозом судинної стінки, вогнищевою м'язово-фіброзною гіперплазією інтри, що викликає нарушения ламінарності току крові. Причинаючи до розвитку тромбозу, ремоделювання глубокої вени нижніх кінцівок сприяє розвитку тромбозу вен нижніх кінцівок у хворих на рак.

Ремоделювання глубокої вени нижніх кінцівок у хворих на рак характеризується десквамацією ендотеліоцитів, потовщенням і склерозом інтрі та середньої оболонки у поєднанні із фіброзною дегенерацією вазутор та тромбозом перфорантних вен. У відповідь на гемодинамічні порушення розвивається компенсаторне ремоделювання: поєднання атрофії пеймоцитів з їх гіпертрофією і неоваскуляризацією середньої оболонки.

Ключові слова: глубока вена стегна, рак, тромбозболія легеневої артерії.