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Scanning Electron Microscopy of Palmar Fascia in Dupuytren's Disease of Advanced Stage

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Abstract

Background. Dupuytren's disease (palmar fascial fibromatosis) affects primarily palmar and digital fascia and results in progressive wrist deformity in many patients and often with bilateral involvement. Absence of corresponding data on the patients with severe wrist deformities along with their treatment issues is the ground for targeted research of pathomorphology of advanced disease stages. ***Purpose of the study*** — to identify features of ultrastructure of fibromatosis nodules and bands in palmar aponeurosis of patients with Dupuytren's disease of grade III–IV. ***Materials and Methods.*** The authors analyzed medical cards and surgical material of 20 patients aging 42–77 years. Segments from medial portion of pretendinous cord of IV digit were cut for examination under scanning electron microscope (JSM-840, Jeol, Japan). ***Results.*** Irrespective of disease history (from 1 to 20 years) fibrbous-fibrillar network and fine cylindrical collagen fibers prevailed in nodules of pretendinous cord which formed semicircular and circular end coils. Empty lacunae, functionally active fibroblasts and close cellular pairs were observed in nodules. Bands differed from nodules by lesser cellularity and less content of fine fibers, orientation of thick fibers mainly along one axis, straightening segments of undulated twisting and separate twisted and tightly interwoven fiber fragments. ***Conclusion.*** In Dupuytren's disease of grade III–IV nodules maintain the role of active contractile centers. Despite relatively small cellularity of pathologically altered tissues there is a potential for progressing, propagation and recurrence of fibromatosis.

Keywords: Dupuytren's disease, advanced stage, scanning electron microscopy.

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Background

Dupuytren's disease mainly affects palmar and digital fascia and in many patients results in progressing wrist deformity, often with bilateral involvement [1].

Etiology of the disease has not been yet disclosed, though it is generally agreed that the cause for pathology lies in genetic predisposition [2-4] usually combined with external and internal risk factors: chronic wrist lesions during manual work, alcohol consumption and smoking [5] and vibration [6]. Age and gender distribution of palmar fascial fibromatosis is determined by ethnic and geographical factors [7, 8], prevalence of male patient is typical. Disease usually onsets in the age range of 40 to 60 years [9]. At early stages when derma hardening and presence of fibromatous nodules is not accompanied by deficit of digits extension the patients can be treated conservatively, however, the efficiency of such procedures remain unproved [10].

Minimally invasive surgical procedures, like needle aponeurotomy and contracture enzyme injections are becoming popular [11], however their efficiency for long-term and severe contractures is disputable [12, 13] compared to open surgery. Thus in Europe majority of patients undergo aponeurotomy [14].

Metacarpophalangeal joint contracture over 30° and interphalangeal joint contracture of any degree are the general indications for surgical treatment [15]. However many patients are admitted for surgery already with severe wrist deformities [16] posing substantial difficulties for treatment and rehabilitation.

Study of surgical material allowed to distinguish three histological stages of the disease: proliferation stage — formation of nodule due to hyperproliferation of fibroblasts, and differentiation of myofibroblasts and deposits of collagen fibers of type I and II; involution stage — myofibroblasts orientation along strain lines; residual stage — hyperproduction of collagen and decreased cellularity due to myofibroblasts apoptosis [17, 18]. Excess amount of type III collagen is observed at early stages, while as the disease progresses the ratio of type III and type I collagen declines [19]. Nodules were rarely identified in patients with severe deformities [20] which contradicts clinical data on high recurrence rate in grades III-IV of the disease [16].

Ultrastructure of collagen fibers of palmar aponeurosis in Dupuytren's disease was studied using the material from patients with disease grade I-II or without indicate disease grade [21-23]. Lack of respective data on the patients with severe wrist deformities as well as on the issues in treatment of such pathology is the ground for targeted research of pathological morphology of advanced disease stages.

Purpose of the study — to identify features of three dimensional pattern of fibrous structure and cells of palmar aponeurosis in Dupuytren's disease of grade III-IV.

Material and Methods

The authors analyzed medical cards and tissue specimens — longitudinal bands of pathologically changed palmar aponeurosis of 20 patients (17 men and 2 women) aging 42-77 years suffering from Dupuytren's disease of grade III-IV according to R. Tubiana [24]. 17 patients had Dupuytren's contracture of grade III and three patients had deformity of grade IV. Disease history was reported from 1 to 20 years (1 year — 10%, 2 years — 20%, 4 years — 20%, 5 years — 20%, 7 years — 5 %, 8 years — 10%, 15 years — 10%, 20 years — 5%).

Segments from intermediate portion of pretendinous cord of IV digit were cut for examination under scanning electron microscope (JSM-840, Jeol, Japan). Formalin fixed tissue specimens were washed with distilled water, dehydrated in alcohol of 70–96%, soaked in camphene according to the original method (Patent RU No. 2008150910/12)

and air-dried. Dried specimens were fixed by conductive adhesive to highly polished clean aluminum discs, sprayed by silver in vacuum evaporator JEE-4 X/5B (Jeol, Japan) and in ion coater IB-6 (Eiko, Japan). Examination was carried out on scanning electronic microscope JSM-840 (Jeol, Japan). Classification of pattern of cell surface was used to describe the topography*.

Results

Tissue specimens of pathological palmar aponeurosis demonstrated scarring of tendinous structures with zones of fibromatosis and small segments of adipose tissue (Fig. 1).

Nodules demonstrated thick network of fine cylindrical collagen fibers (thickness less than 5 micron). Connection of fibers was supported by the system of separate collagen fibrils not engaged into the fibers. Thus fibrous scaffold could be described as collagenous and fibrous-filbrillar, while the quantity of thin fibers exceeded 50% of overall fibers. Orientation of collagen fibers was varying in different sections of nodules. As a rule fine fibers did not feature particular orientation and formed semicircular or circular end coils (Fig. 2). There were some nodule sections with uni- or multidirectional orientation of flattened thick fibers. Shape and size of nodule cells varied. There were cells of spherical, oval shape, flattened cells with relatively even microrelief and solitary end blisters, erythrocytes (Fig. 3).

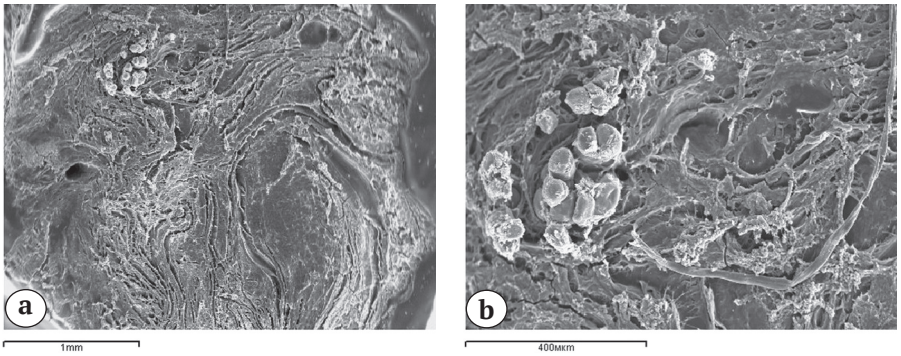

Fig. 1. Pathological palmar aponeurosis, ×33 (a); zones of fibromatosis and adipose tissue, ×110 (b)

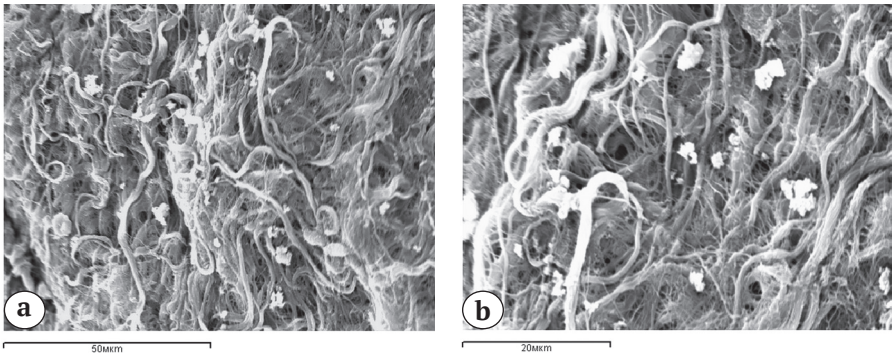

Fig. 2. Section of nodule in palmar aponeurosis, Dupuytren's disease, ×1000 (a); fine fibers forming semicircular and circular end coils, ×1600 (b)

* Atlas of scanning electronic microscopy of cells, tissues and organs / edited by O.V. Volkova, V.A. Shakhlamov, A.A.Mironov. M. : Meditsina, 1987. — p. 464.

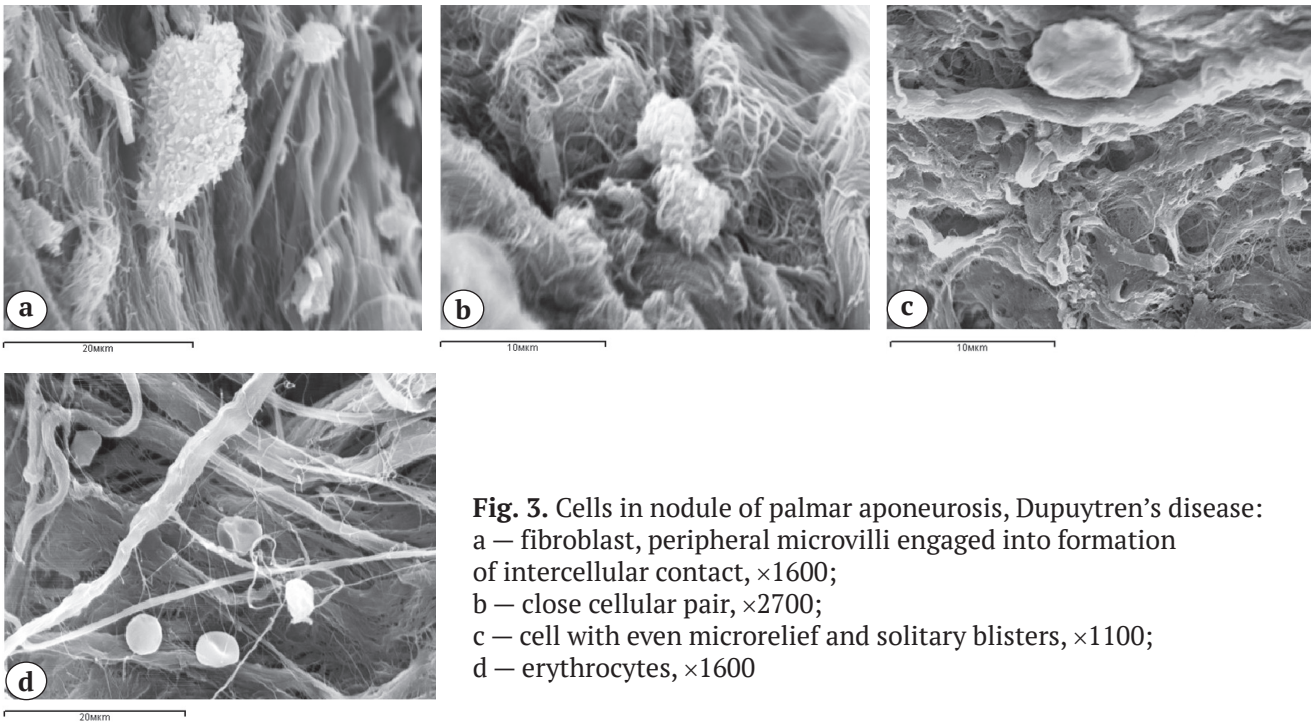

Fig. 3. Cells in nodule of palmar aponeurosis, Dupuytren's disease:
a — fibroblast, peripheral microvilli engaged into formation of intercellular contact, ×1600;
b — close cellular pair, ×2700;
c — cell with even microrelief and solitary blisters, ×1100;
d — erythrocytes, ×1600

The authors observed few large fibroblasts (d = 10-20 micron) of oval-oblong shape, mixed type of microrelief consisting of blisters and microvilli, peripheral microvilli which were engaged into providing contact with intercellular substance. There were many cells with destruction signs, having abnormal shape and smaller size. Empty lacunae as signs of cell loss were not infrequent.

Bands were characterized by prevalence of collagen fibers of 10-15 microns with few fine fibers (Fig. 4). Cylindrical collagen fibers of undulated twisted shape were oriented mainly along one axis, however, in some segments the fibers had no specific orientation. Fibers connection was ensured by the network of fine fibers. Fibers demonstrated compact placement. Many segments demonstrated straightening of twisted collagen bundles. The authors observed also tightly interwoven helical fibers and segments with flattened thick undulated twisted collagen fibers alternating with straightened fibers. There were solitary cells in the bands undetected over greater extension.

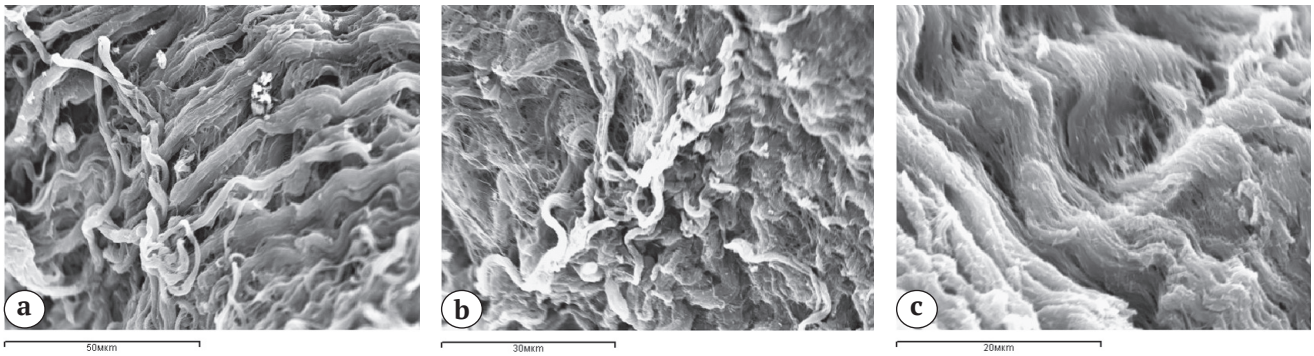

Fig. 4. Sections of palmar aponeurosis band, Dupuytren's disease, ×1100 (a); tightly interwoven helical fibers, ×1400 (b); cylindrical collagen fibers of undulated twisted shape, ×1600 (c)

Discussion

Normal palmar aponeurosis consists of well organized, flattened thick collagen fibers with longitudinal orientation (type I) supported by thin network of fibrils which do not merge with fibers [21]. Dupuytren's contracture is characterized by less content of adipose tissue, segments of collagen disruption and increased number of fine fibers [23]. J.A.A. Hunter, C. Ogdon and L. Józsa et al did not observe variances in collagen scaffold of nodules and bands in pathological aponeurosis [21, 23] though such were identified at ultrasound examination [26].

J.W. Legge et al reported that Dupuytren's disease features appearance of helices and shortening of twisted collagen waves that are considered as the contraction mechanism [22]. H. Millesi believes that the key specifics of collagen fibers in Dupuytren's disease is the loss of corrugated structure [26].

The authors of the present study present for the first time the results of scanning electronic microscopy of pretendinous cord of palmar aponeurosis in patients with Dupuytren's contracture of grades III-IV. Pretendinous cord is result of transformation of the normal fascial pretendinous band of palmar fascial complex [27].

Pathological pretendinous cord contains nodules and tendon-like bands causing thickening, higher density and shortening of palmar aponeurosis and contracture of metacarpophalangeal joint, which during progression involves structures of digital fascia causing contractures of interphalangeal joints.

It was found that irrespective of disease history (from 1 to 20 years) nodules of pretendinout cord of all patients did not contain mature collagen fibers, and fine cylindrical and cylindrical collagen fibers prevailed which formed semicircular and circular end coils. Evidently coils may indicate the process of fibrous scaffold contracture, and visualization of fiber ends — dismaturity of scaffold while fibers ends in normal situation are visualized only at fetal stage [28].

Despite signs of cellular loss (empty lacunae) the nodules features functionally active fibroblasts and even close cell pairs. Bands demonstrated less cellularity and less content of fine fibers, orientation of thick fibers mainly along one axis, presence of straightening segments of undulated twisting and separate twisted and tightly interwoven fiber fragments — possible rupture sites.

According to clinical and pathomorphological classification of H.F. Chiu and R.M. McFarlane, the Dupuytren's disease over 3 years corresponds to the advanced stage and histologically manifested by low cellularity and mature collagen fibers [29]. The authors of the present paper in their study material observed prevalence of fine collage fibers with thickness less than 5 micron in all patients irrespective of disease history. Thus, the present study material may be qualified as fibrocellular type under classification of J.J. Rombouts et al who consider recurrence risk for such type as medium and proliferation risk as indefinite [30].

In patients at advanced stages of contracture the authors of the present study observed cells with ultrastructural signs of enhanced biosynthetic and proliferous activity despite decreased cellularity of nodules and bands and signs of cell loss. Such cells can be considered as disease container triggering its vicious circle [31].

Due to the above the tactics of postponing surgical treatment until grades III-IV of the disease is not justified not only from standpoint of severe secondary changes in neurovascular bundles, muscles, tendons and joints [32], but also of persisting risk of disease recurrence and proliferation.

Three dimensional pattern of fibrous scaffold and cells of palmar aponeurosis in Dupuyren's disease of grades III-IV indicates that fibromatous nodules keep the role of active contractile centers. Despite trace

amounts of cells in pathological tissues there is a potential for progression, proliferation and recurrence of fibromatosis, thus delay of surgery until grades III-IV of the disease is not justified.

Publication ethics: All patients provided informed consent for the research. The study was approved by ethics committee of the institution (minutes No. 2 (57) dated 19.03.2018) and conducted in accordance with standards of Helsinki declaration of 1975 in revision of 2013.

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Authors' contribution

N.A. Shchudlo – study concept and design, analysis and interpretation of the data, the writing of the manuscript.

T.A. Stupina – study concept and design, acquisition and analysis of study data, writing and editing of the manuscript.

M.M. Shchudlo – study concept and design, analysis of the data, editing of the manuscript.

References

1. Engstrand C., Krevers B., Nylander G., Kvist J. Hand function and quality of life before and after fasciectomy for Dupuytren contracture. *J Hand Surg Am.* 2014;(39)7:1333-1343. DOI: org/10.1016/j.jhsa.2014.04.029.
2. Michou L., Lermusiaux J.L., Teyssedou J.P., Bardin T., Beaudreuil J., Petit-Teixeira E. Genetics of Dupuytren's disease. *Joint Bone Spine.* 2012;79(1):7-12. DOI: 10.1016/j.jbspin.2011.05.027.
3. Becker K., Tinschert S., Lienert A., Bleuler P.E., Staub F., Meinel A. et al. The importance of genetic susceptibility in Dupuytren's disease. *Clin Genet.* 2015;87(5):483-487. DOI: 10.1111/cge.12410.
4. Larsen S., Krogsgaard D.G., Aagaard Larsen L., Iachina M., Skytthe A., Frederiksen H. Genetic and environmental influences in Dupuytren's disease: a study of 30,330 Danish twin pairs. *J Hand Surg Eur Vol.* 2015;40(2):171-176. DOI: 10.1177/1753193414535720.
5. DiBenedetti D.B., Nguyen D., Zografos L., Ziemiecki R., Zhou X. Prevalence, incidence, and treatments of Dupuytren's disease in the United States: results from a population-based study. *Hand (NY).* 2011;6(2):149-158. DOI: 10.1007/s11552-010-9306-4.
6. Palmer K.T., D'Angelo S., Syddall H., Griffin M.J., Cooper C., Coggon D. Dupuytren's contracture and occupational exposure to hand-transmitted vibration. *Occup Environ Med.* 2014; 71(4):241-245. DOI: 10.1136/oemed-2013-101981.
7. Lanting R., Broekstra D.C., Werker P.M., van den Heuvel E.R. A systematic review and meta-analysis on the prevalence of Dupuytren disease in the general population of Western countries. *Plast Reconstr Surg.* 2014;133(3):593-603. DOI: 10.1097/01.prs.0000438455.37604.0f.
8. DiBenedetti D.B., Nguyen D., Zografos L., Ziemiecki R., Zhou X. Prevalence, incidence, and treatments of Dupuytren's disease in the United States: results from a population-based study et al. Epidemiological profile of Dupuytren's disease in Taiwan (Ethnic Chinese): a nationwide population-based study. *BMC Musculoskelet Disord.* 2015;16:20. DOI: 10.1186/s12891-015-0476-7.
9. Holzer L.A., de Parades V., Holzer G. Guillaume Dupuytren: his life and surgical contributions. *Hand Surg Am.* 2013;38(10):1994-1998. DOI: 10.1016/j.jhsa.2013.07.012.
10. Ball C., Izadi D., Verjee L.S., Chan J., Nanchahal J. Systematic review of non-surgical treatments for early dupuytren's disease. *BMC Musculoskelet Disord.* 2016;17(1):345. DOI: 10.1186/s12891-016-1200-y.
11. Kníže J., Miletín J., Nejedlý A., Chorvát M., Novotná K., Tichá P., Fibír A., Sukop A., Knížetová A. Current treatment options of dupuytren´s disease. *Acta Chir Plast.* 2018;59(3-4):142-148.
12. Leclère F.M., Kohl S., Varonier C., Unglaub F., Vögelin E. Range of motion, postoperative rehabilitation and patient satisfaction in MCP and PIP joints affected by Dupuytren Tubiana stage 1-3: collagenase enzymatic fasciotomy or limited fasciectomy? A clinical study in 52 patients. *Arch Orthop Trauma Surg.* 2018;138(11):1623-1631. DOI: 10.1007/s00402-018-3034-6.
13. Brazzelli M., Cruickshank M., Tassie E., McNamee P., Robertson C., Elders A. et al. Collagenase clostridium histolyticum for the treatment of Dupuytren's contracture: systematic review and economic evaluation. *Health Technol Assess.* 2015;19(90):1-202. DOI: 10.3310/hta19900.
14. Bainbridge C., Dahlin L.B., Szczypa P.P., Cappelleri J.C., Guérin D., Gerber R.A. Current trends in the surgical management of Dupuytren's disease in Europe: an analysis of patient charts. *Eur Orthop Traumatol.* 2012;3(1):31-41. DOI: 10.1007/s12570-012-0092-z.
15. Reilly R.M., Stern P.J., Goldfarb C.A. A retrospective review of the management of Dupuytren's nodules. J Hand Surg. 2005;30(5):1014-1018. DOI: 10,1016/ j.jhsa.2005.03.005.
16. Tolstik A.N., Deikalo V.P. [Technology for the rehabilitation of patients with severe flexion deformities and recurrence of contractures of the fingers of the hand in Dupuytren's disease]. *Novosti khirurgii* [Surgery News]. 2006;14(3):33-41. (In Russian).
17. Luck J.V. Dupuytren's contracture: a new concept of the pathogenesis correlated with surgical management. *J Bone Joint Surg Am.* 1959;41(4):635-664.
18. Wilutzky B., Berndt A., Katenkamp D., Koshmehl H. Programmed cell death in nodular palmar fibromato-

sis (Morbus Dupuytren). *Histol Histopathol*. 1998;13(1): 67-72. DOI: 10.14670/HH-13.67.
19. Lam W.L., Rawlins J.M., Karoo R.O., Naylor I., Sharpe D.T. Re-visiting Luck's classification: a histological analysis of Dupuytren's disease. *J Hand Surg Eur*. 2010;35(4):312-317. DOI: 10.1177/1753193410362848.
20. Verjee L.S., Midwood K., Davidson D., Essex D., Sandison A., Nanchahal J. Myofibroblast distribution in Dupuytren's cords: correlation with digital contracture. *J Hand Surg Am*. 2009;34(10):1785-1794. DOI: 10.1016/j.jhsa.2009.08.005.
21. Hunter J.A.A., Ogdon C. Dupuytren's contracture II-scanning electron microscopic observations. *Br J Plastic Surg*. 1975;28:19-25.
22. Legge J.W., Finlay J.B., McFarlane R.M. A study of Dupuytren's tissue with the scanning electron microscope. *J Hand Surg Am*. 1981;6(5):482-492.
23. Józsa L., Salamon A., Réffy A., Renner A., Demel S., Donhöffer A., Pintér T., Thöring J. Fine structural alterations of the palmar aponeurosis in Dupuytren's contracture. A combined scanning and transmission electronmicroscopic examination. *Zentralbl Allg Pathol*. 1988;134(1):15-25.
24. Tubiana R. Dupuytren's disease of the radial side of the hand. *Hand Clin*. 1999;15(1):149-159.
25. Vanek P., Strömberg J., Fridén J., Aurell Y. Morphological patterns of the pretendinous cord in Dupuytren's disease: a predictor of clinical outcome? *J Plast Surg Hand Surg*. 2018;52(4):240-244. DOI: 10.1080/2000656X.2018.1470521.
26. Millesi H. Dupuytren's Contracture. In: European Instructional Lectures. Berlin, Heidelberg: Springer Berlin Heidelberg, 2009. pp. 137-152.
27. Baitinger V.F. [Clinical anatomy of palm aponeurosis]. *Voprosy rekonstruktivnoi i plasticheskoi khirurgii* [Issues of Reconstructive and Plastic Surgery].2012;1(40):22-33. (In Russian).
28. Provenzano P.P., Vanderby R. Jr. Collagen fibril morphology and organization: Implications for force transmission in ligament and tendon. *Matrix Biology*. 2006;25:71-84. DOI: 10.1016/j.matbio.2005.09.005.
29. Chiu H.F., McFarlane R.M. Pathogenesis of Dupuytren's contracture: a correlative clinical-pathological study. *J Hand Surg Am*. 1978;3(1):1-10.
30. Rombouts J.J., Noel H., Legrain Y., Munting E. Prediction of recurrence in the treatment of Dupuytren's disease: evaluation of a histologic classification. *J Hand Surg Am*. 1989;14(4):644-652.
31. Musumeci M., Vadalà G., Russo F., Pelacchi F., Lanotte A., Denaro V. Dupuytren's disease therapy: targeting the vicious cycle of myofibroblasts? *Expert Opin Ther Targets*. 2015;19(12):1677-1687. DOI: 10.1517/14728222.2015.1068758.
32. Dumitrescu-Ionescu D. A new therapeutic approach to dupuytren's contracture / disease (DD). *Adv Plast Reconstr Surg*. 2017;1(5):129-136.

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