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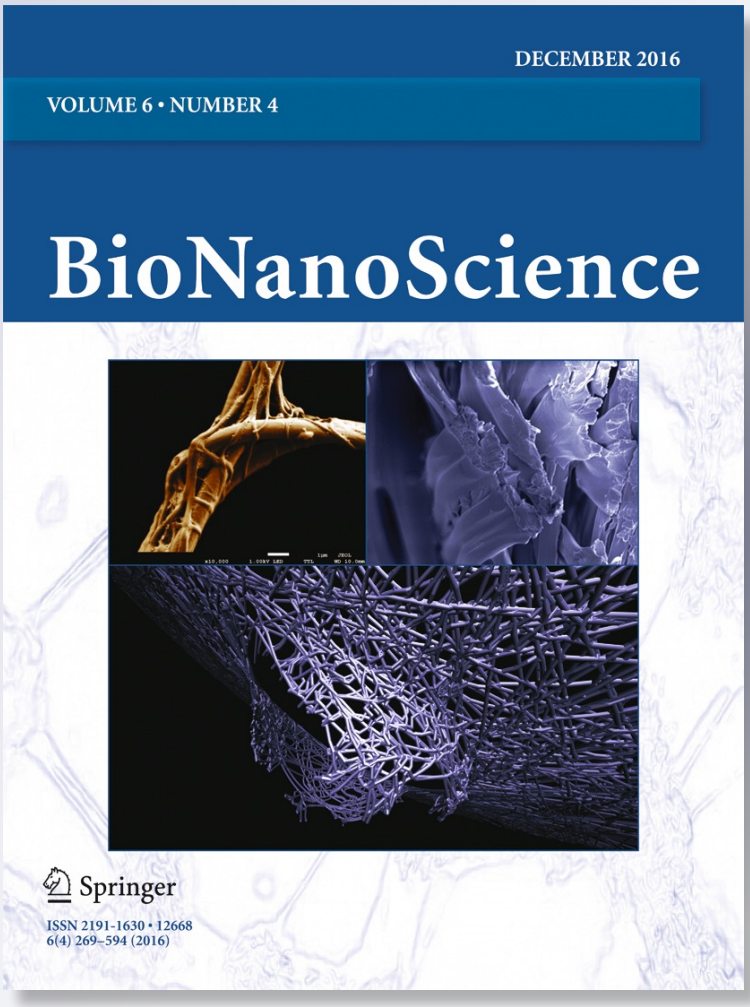

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Contraction of Blood Clots Is Impaired in Deep Vein Thrombosis

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Abstract The goal of this study was to investigate the connection of blood clot contraction (retraction) with deep vein thrombosis. In the blood of deep vein thrombosis patients, the extent and rate of clot contraction is dramatically reduced compared to healthy subjects, especially in deep vein thrombosis complicated with pulmonary embolism. Clinical and laboratory correlations suggest that impaired contraction of blood clots and thrombi is an important pathogenic mechanism that affects the course and outcomes of deep vein thrombosis.

Keywords Blood clotting · Clot contraction · Deep vein thrombosis · Pulmonary embolism

1 Introduction

Deep venous thrombosis (DVT) is one of the most common cardiovascular diseases and occurs at a rate of about 1 case per 1000 adults [1]. DVT is the formation of a blood clot or thrombus within a deep vein, most often in the lower extremities [2]. When a deep vein thrombus breaks off, its pieces can be carried by the blood flow downstream through the hearth into the lung arteries, causing a life-threatening complication called pulmonary embolism (PE).

DVT is diagnosed using ultrasound or venography as well as coagulation tests, such as a D-dimer test. DVT can be treated with anticoagulants as blood thinners; however, the results of DVT treatment are often unsatisfactory. Therefore, knowing the cellular and molecular mechanisms of the disease could provide information to find new and efficient therapeutic modalities. Blood clots are known to undergo volume shrinkage, driven by the platelet contractile proteins, which is called clot contraction or retraction. [3]. Despite biological and clinical importance, the mechanical remodeling of blood clot and thrombi has been underestimated and understudied [4].

In this work, we aim at revealing the pathogenic role of clot contraction in DVT, using a novel method developed in our laboratory to follow the dynamics of this process in vitro [4].

2 Materials and Methods

Twenty-five patients with DVT of the lower extremities were enrolled in the study, including 9 (36 %) patients with clinically and radiologically documented PE. Twenty-one (84 %) patients were studied within 21 days from the onset of symptoms, 4 patients were studied between 21 and 40 days after the clinical onset. In 11 (44 %) patients, a thrombus was localized in the proximal region of the common femoral vein, 7 (28 %) had thrombosis of the femoral vein, 3 (12 %) had a thrombus at the proximal border of the external iliac vein, 2 (8 %) in the common iliac vein, and 2 (8 %) in the popliteal vein. Fifteen (60 %) patients had venous thrombectomy. Only patients who were not treated with anticoagulants or antiplatelet drugs by the time of examination were included in the study.

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Blood of the DVT patients and donors was withdrawn using a standard protocol approved by the Ethical Committee of the Interregional Clinical Diagnostic Center (ICDC, Kazan, Russia). Blood samples from 25 healthy subjects were used as a control. The groups of DVT patients and control donors were comparable by the age (65 ± 11 vs. 58 ± 12 years, respectively) and sex (males comprised 56 vs. 60 %, respectively). All the DVT patients underwent routine cell count, including platelets, and coagulation tests (fibrinogen, D-dimer, activated partial thromboplastin time, prothrombin time, thrombin time, and ADP-induced platelet aggregation).

Clot contraction was studied using an optical tracking method. Citrated whole blood was activated by adding 1 U/ml thrombin and 2 mM $CaCl_2$ and then immediately transferred to a $12 \times 7 \times 1$ mm plastic cuvette, which was pre-coated with 4 % Triton X-100 in phosphate-buffered saline to prevent fibrin attachment to the walls and allow for the unconstrained clot shrinkage. Cuvettes with the activated blood samples were quickly transferred to the Thrombodynamics Analyzer System (HemaCore, Moscow, Russia) to automatically record clot-induced light scattering. The changes in clot size were captured by the system every 15 s for 20 min. The collected clot images were analyzed computationally to extract the following parameters of clot contraction: extent of contraction (percentage of the initial clot size), lag time, and average contraction velocity.

3 Result and Discussion

We found that contraction of clots formed in the blood of DVT patients was substantially reduced compared to the blood from healthy subjects. The average degree of clot contraction in DVT blood samples was reduced by approximately one third from 51.5 ± 0.6 % in control down to 33.3 ± 2.2 % in DVT. The average velocity of clot contraction in DVT was also much lower than that of healthy donors and comprised $(27.0 \pm 0.2)*10^{-3}$ %/s vs $(41.0 \pm 0.5)*10^{-3}$ %/s, respectively. The lag period in DVT was more than two-fold longer than in healthy donors (198 ± 17 and 74 ± 5 s, respectively). Importantly, in the blood of patients with PE, the extent of clot contraction was significantly lower than that in the patients with DVT without PE (30 ± 4 and 44 ± 5 %, respectively, $P < 0.05$). This remarkable finding strongly suggests that impaired clot contraction is a pathogenic factor that determines integrity of a thrombus and the likelihood of its rupture and embolization (Fig. 1).

The reduced extend and rate of clot contraction and an increase of lag time in DVT correlated with the lower platelet count and suppressed platelet aggregation as well as with higher levels of fibrinogen and D-dimer. Regardless of the underlying mechanism of reduced platelet count and platelet dysfunction in DVT, these changes can contribute to a reduction in the extent of clot contraction via weakening of the cellular contractile activity. An increased fibrinogen level, which is known to accompany DVT and confirmed in our study, likely suppresses clot contraction by increasing the number of fibrin fibers that are not associated with activated platelets, which comprise a mechanically passive portion of the blood clot, thus reducing the extent and rate of clot contraction. A higher hematocrit and hemoglobin content, as well as leukocytosis, also correlate with the parameters of reduced clot contraction. These changes in molecular and cellular blood composition may directly or indirectly affect platelet function and clot mechanical properties, resulting in the impaired clot contraction in DVT.

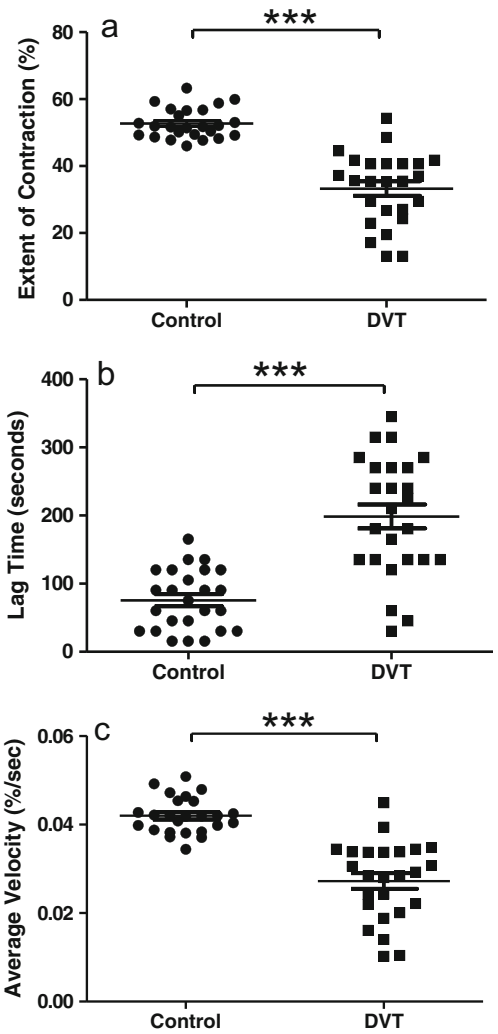

Fig 1 Parameters of clot contraction in the blood of DVT patients vs. healthy subjects. Optical tracking was used to assess the differences in (**a**) extent of clot contraction, (**b**) lag time, and (**c**) velocity of contraction. The values were compared using an unpaired, two-tailed Student's *t* test. ***$P < 0.001$

4 Conclusion

Our results reveal that impaired contraction of blood clots and thrombi may comprise a novel pathogenic mechanism in DVT and PE. Further studies of the clinical importance of clot contraction can improve the diagnosis, prognosis, and treatments in thrombotic conditions, such as non-complicated and complicated DVT.

Compliance with Ethical Standards Blood of the DVT patients and donors was withdrawn using a standard protocol approved by the Ethical Committee of the Interregional Clinical Diagnostic Center (ICDC, Kazan, Russia).

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