
lek. Milena Michalska

**Wpływ wewnątrznaczyniowego leczenia tętniaków aorty
na zewnątrzkomórkowe sieci neutrofilowe i ich korelacja
z wybranymi parametrami stanu zapalnego i układu
krzepnięcia**

**Rozprawa na stopień doktora nauk medycznych i nauk o zdrowiu
w dyscyplinie nauki medyczne**

Promotor: prof. dr hab. n. med. Tadeusz Grochowicki

Katedra i Klinika Chirurgii Ogólnej, Naczyniowej, Endokrynologicznej
i Transplantacyjnej

Warszawskiego Uniwersytetu Medycznego



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STRESZCZENIE W JEZYKU ANGIELSKIM

Influence of endovascular treatment of aortic aneurysms on neutrophil extracellular traps and their correlation with selected parameters of inflammation and the coagulation system.

Introduction

Neutrophil extracellular networks (NETs) and the process of their creation, i.e., netosis (NETosis), is an important element in the pathogenesis of aortic aneurysm development. However, despite the importance of these processes, the role of netosis in patients with thoracoabdominal aortic aneurysm has not been studied so far, and the dynamics of this process in the perioperative period after endovascular treatment have not been determined. The perioperative period after endovascular intervention with the use of a stent graft is characterized by several changes in the coagulation system and the white blood cell system as well as in inflammatory parameters, which is related to the formation of a thrombus between the stent and the aneurysm. Stent graft implantation may lead to the activation of inflammatory processes, which may increase the risk of postoperative complications. One of the factors influencing this process is the activation of neutrophils and the release of extracellular neutrophil networks. Therefore, it is important to assess netosis, white blood cell count, inflammation, and coagulation in the perioperative period and their potential relationship with the risk of postoperative complications.

Principles and goals

In this paper, studies were conducted on netosis markers (NETs) and selected parameters of the white blood cell system, inflammation, and coagulation in patients with thoracoabdominal aortic aneurysm, before and after t-Branch stent graft implantation. The study aimed to determine the activity of NETs formation in patients before and after endovascular treatment of thoracoabdominal aortic aneurysms and their relationship with selected parameters of the inflammatory process, the coagulation system, and the white blood cell system. The role of ssDNA as a marker of NETs was investigated. All parameters were analyzed in terms of risk assessment of death and rehospitalization in patients with thoracoabdominal aortic aneurysms undergoing endovascular surgery.

Material and methods

A prospective, single-center, observational study was conducted in which 20 patients with indications for the treatment of thoracoabdominal aortic aneurysms with a t-Branch stent which were enrolled in the period from April 2021 to August 2021. The study included 85% of men and 15% of women, the mean age of patients was 70.65 years. The most reported comorbidities were hypertension and other cardiovascular diseases. All patients underwent successful endovascular surgery using a t-Branch stent with a quadruple vascular skeleton. The indication for stent graft implantation was a maximum aortic diameter ≥ 55 mm or a rapid increase in aneurysm diameter (≥ 10 mm in 12 months). The parameters of NETosis, protein blood cell system, inflammation, and coagulation system were examined in the preoperative period (within 24 hours before surgery) and the postoperative period (on the 3rd and 5th postoperative day). Free-circulating DNA (cfDNA) was isolated from the blood using the MagMAX™ Cell-Free DNA Extraction Kit. Double-stranded DNA (dsDNA) and single-stranded DNA (ssDNA) were then quantified using the Qubit dsDNA HS Assay Kit and the Qubit ssDNA Assay Kit. Cit-H3 concentration was determined by enzyme immunoassay ELISA (Cayman). The parameters of the white blood cell system (including neutrophils, lymphocytes, monocytes, basophils, and eosinophils), inflammation (CRP and procalcitonin), coagulation system (including platelet concentration and platelet parameters, prothrombin time, INR, fibrinogen and D- dimers). One year after the operation, a check-up was carried out to assess the health status of the examined patients. Statistical analysis was performed using non-parametric methods using the Statistica program. Cox hazard regression analysis identified independent factors contributing to the endpoint of death and readmission. Statistical significance was defined at $p < 0,05$.

Results

After endovascular surgery of a thoracoabdominal aortic aneurysm, a significant increase in NETs parameters was observed. A significant increase in all three NETs markers was found on the 3rd postoperative day, compared to the period before the operation. For double-stranded DNA (dsDNA), single-stranded DNA (ssDNA), and citrullinated H3 histones (Cit-H3), the increase was $1347,93\text{ng/ml} \pm 1551,65$ to $5289,75\text{ng/ml} \pm 2815,52$, respectively ($p < 0,05$); $6551,00\text{ng/mL} \pm 7987,49$ to $26151,75\text{ng/mL} \pm 14182,07$ ($p < 0,05$);

16,20ng/ml \pm 9,92 to 66,57ng/ml \pm 54,10 ($p < 0,05$). Histone levels before and on the 5th postoperative day did not differ significantly and amounted to 16,20ng/ml \pm 9,92 and 26,97ng/ml \pm 17,48 (ns), respectively. Concentrations of dsDNA and ssDNA on the 5th postoperative day were significantly higher than before surgery and amounted to 1347,93ng/ml \pm 1551,6 and 5340,50ng/ml \pm 3767,26, respectively ($p < 0,05$); and 6551,00ng/mL \pm 7987,49 and 27426,75ng/mL \pm 18367,58 ($p < 0,05$). Based on the Spearman rank correlation results, it can be concluded that there was a strong correlation between ssDNA and dsDNA. In the preoperative period, it was $r = 0,975555$ ($p < 0,05$), on the 3rd postoperative day $r = 0,908271$ ($p < 0,05$), and on the 5th day $r = 0,863158$ ($p < 0,05$). No correlation was observed between the parameters of netosis and the parameters of inflammation, the coagulation system, and the white blood cell system.

Significant changes in the fraction of the white blood cell system were observed in the perioperative period. There was a significant increase in the number of neutrophils from $4,31 \times 10^3 / \mu\text{l} \pm 1,63$ before surgery to $7,84 \times 10^3 / \mu\text{l} \pm 3,06$ on the 3rd day ($p < 0,05$) and $6,91 \times 10^3 / \mu\text{l} \pm 6,91$ on 5th day ($p < 0,05$) postoperatively. In the pre- and post-operative period, it was found using indicators determining the dynamics of NETs secretion from neutrophils that the increase in netosis was not associated with an increase in the number of neutrophils. A decrease in the number of lymphocytes was observed from $2,48 \times 10^3 / \mu\text{l} \pm 1,42$ before surgery to $1,64 \times 10^3 / \mu\text{l} \pm 1,64$ on the 3rd day ($p < 0,05$) and $1,98 \times 10^3 / \mu\text{l} \pm 1,42$ on the 5th postoperative day ($p < 0,05$). The calculated ratio of neutrophils to lymphocytes before surgery on the 3rd and 5th postoperative day was $2,15 \pm 1,49$, respectively; $6,89 \pm 4,92$; $4,92 \pm 5,14$ (Day I vs. III $p < 0,05$; Day I vs. V $p < 0,05$).

A significant increase in procalcitonin and CRP inflammatory parameters was observed. The values of procalcitonin for the period before the operation, on the 3rd and 5th day of operation were, respectively: $0,05\text{ng/ml} \pm 0,03$; $1,03\text{ng/mL} \pm 3,51$; $0,45\text{ng/ml} \pm 1,08$ (I vs. III-day $p < 0,05$; I vs V day $p < 0,05$). The CRP values for the period before the operation, on the 3rd and 5th day of operation, were, respectively: $21,86 \text{ mg/l} \pm 31,19$; $143,58 \text{ mg/l} \pm 54,35$; $141,28\text{mg/l} \pm 52,07$ (day I vs III $p < 0,05$; day I vs V $p < 0,05$).

The observation time of the patients was $314,68 \text{ days} \pm 30,24$ after surgery. Two patients died as a result of postoperative complications. The first patient died as a result of

hemorrhagic stroke; the second patient died due to sudden cardiac arrest. Five patients were also hospitalized during one year of follow-up.

Risk factors for the occurrence of the endpoint were: concentration of dsDNA and ssDNA on 3rd day (HR=1,000259 [CI 1,000029-1,000488] p<0,05; HR=1,000060 [CI 1,00009- 1,000111] p<0,05); neutrophil concentration on the 3rd and 5th postoperative day (HR=1,327054 [CI 1,030406-1,709107] p<0,05; HR=1,259972 [CI 1,005217- 1,579291] p<0,05); NLR value on the 3rd and 5th postoperative day (HR=1,225678 [CI 1,043126-1,440176] p<0,05; HR=1,178757 [CI 1,031318-1,347274] p<0,05).

Conclusions

After endovascular treatment of thoracoabdominal aortic aneurysms, neutrophils are activated, and NETs are released. The increase in NET formation in the postoperative period was independent of the increase in the number of neutrophils. Single-stranded DNA (ssDNA) is one of the markers of NETs and correlates with dsDNA. Cox hazard analysis showed that the risk factors for the endpoint were dsDNA and ssDNA, neutrophils, and NLR.