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RESEARCH ARTICLE

Plesković et al: Adventitial mast cells in diabetes

Diabetes-induced redistribution of mast cells to the adventitia in ascending aortic aneurysms

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ABSTRACT

Mast cells (MCs) are inflammatory cells that reside mainly in the intima of healthy and early- atherosclerotic abdominal aortas but migrate to the adventitia in advanced atherosclerosis and abdominal aortic aneurysms. We compared MC infiltration in the intima, media, and adventitia of ascending aortic aneurysms from patients with diabetes mellitus (DM) or arterial hypertension (AH). Fifty-one patients (36–81 years) undergoing surgical repair were enrolled and allocated to a DM group without AH (n = 9) or an AH group without DM (n = 42). Aortic specimens were stained with hematoxylin–eosin and immunohistochemically labeled with anti-CD117 to detect MCs and anti-vWF to visualize blood vessels. Compared with the AH group, the DM group had fewer MCs in the intima and more in the adventitia (Mann–Whitney test, $p < 0.05$). In both groups, intact MCs outnumbered degranulated MCs in the adventitia, whereas no such difference was observed in the intima or media ($p < 0.05$). Medial vascular density did not differ between groups ($p < 0.05$). In the AH group, medial vascularization correlated positively with intact, degranulated, and total MC counts, whereas in the DM group it correlated only with degranulated MCs (Spearman, $p < 0.05$). These findings suggest that DM-associated aneurysms exhibit a distinct MC distribution and vascular response, indicating a pathogenesis that differs from that of AH-associated aneurysms.

Keywords: Mast cells, MCs, vascularity, type 2 diabetes, DM, arterial hypertension, AH, ascending aortic aneurysm.

INTRODUCTION

Although mast cells (MCs) are best known for their role in mediating IgE-dependent allergic reactions [1, 2], they also contribute to the regulation of a wide range of physiological processes. MCs play an important role in host defense by destroying bacteria and parasites, and they are actively involved in promoting vasodilation, angiogenesis, and modulating the activity of various immune and structural cells, including macrophages, T and B lymphocytes, eosinophils, endothelial and epithelial cells, and fibroblasts [1]. Their cytoplasmic granules contain numerous bioactive mediators, such as histamine, heparin, proteases, prostanoids, leukotrienes, cytokines, chemokines, and growth factors, which they release upon activation to influence tissue and organ function [3]. In addition to their role in hypersensitivity reactions, asthma, and anaphylaxis, MCs have been implicated in several pathological conditions, including gastrointestinal and cardiovascular diseases [4]. Notably, they contribute to vascular inflammation following hypoxia and ischemia/reperfusion injury, the progression of atherosclerosis, and the development of abdominal aortic aneurysms [2, 4-8].

MCs have been identified in the thoracic and abdominal aortic wall of healthy individuals [9, 10]. In the early stages of atherosclerosis, they are primarily located in the intima, while their presence in the adventitia is minimal. As atherosclerosis progresses, the number of MCs decreases in the intima and increases in the adventitia. In advanced atherosclerotic lesions and abdominal aortic aneurysms, MCs are scarce in the intima but become significantly more abundant in the adventitia [9, 11, 12]. In thoracic aortic aneurysm, mast cells were quantified in the adventitia and found to be more abundant in patients with aneurysms compared to non- aneurysmal controls [10]. Aortic aneurysms most commonly occur in the abdominal segment of the aorta, while thoracic aortic aneurysms are less frequent. Among the thoracic segments, the aortic root, ascending aorta, aortic arch, and descending aorta, the root and ascending aorta are the most frequently affected sites [13, 14].

The most well-known risk factors for the development of the ascending aorta are arterial hypertension (AH), age over 65, and male sex. Epidemiological data suggest that 4–8% of men and approximately 1.5% of women over the age of 60 are affected by aortic aneurysms [14-17].In patients with DM, aortic aneurysms are relatively rare and tend to progress more slowly[18-20].

Our research group has previously analyzed ascending aortic aneurysms in patients with diabetes mellitus (DM) without AH, type 1 DM, or genetic mutations, and compared them with patients with AH without DM. We primarily focused on the infiltration of various types of inflammatory cells in the intima, media, and adventitia, and found that the intima and media were less infiltrated in DM patients. Specifically, the intima and media of DM patients contained fewer proinflammatory macrophages and B cells compared to AH patients, while the media and adventitia contained fewer plasma cells. No differences were observed in the number of Th cells, Tc cells, or anti-inflammatory macrophages [15, 16].

As mast cells were not analyzed in our previous studies, the aim of the present study was to investigate differences in MCs infiltration across all three layers of the ascending aortic aneurysm wall between patients with DM and those with AH.

MATERIALS AND METHODS

Patients

The study was conducted in accordance with the ethical guidelines of the Declaration of Helsinki from 1975. The purpose and course of the study were explained to each patient included in the study and written consent was obtained from them. The study with all procedures was approved by the National Committee for Medical Ethics (MEC 170/07/13, MEC 110/03/16).

We included 51 patients in the study who were indicated for surgical treatment of ascending aortic aneurysm. The patients were divided into a group of diabetic patients with type 2 diabetes (DM group; n=9) and a group of hypertensive patients (AH group; n=42). Patients who had DM and AH together, DM type 1 and/or gene mutations were excluded from the study.

Tissue samples

The aneurysmal expanded part of the aortas was surgically removed and fixed in 10% buffered formalin. Three samples were obtained transversely from the area exhibiting the greatest aneurysmal expansion,embedded in paraffin and cut into 4.5 μm thick stepped series of sections, the height of the step was 50 μm. The samples were stained with HE and immunohistochemically with anti-CD117 (clone: YR145, manufacturer: Cell Marque, catalogue number: 117R-16, antigen retrieval: CC1 (56 min), blocking: pre primary peroxidase inhibitor, titer: 1/70 (incubation 24 min), detection system:

optiView (Ventana, BM ULTRA), control: in our laboratory we use punches of liver, appendix, skin, tonsils, prostate, lung, kidney, melanoma, fixation times: 1-7 days to detect MCs and with anti-von Willebrand factor (vWf) (clone: rb, manufacturer: Dako, catalogue number: A0082, antigen retrieval: protease 1 (8 min), blocking: /, titer: 1/800 (incubation 32 min), detection system: ultraView (Ventana, BM ULTRA), control: in our laboratory we use punches of liver, appendix, skin, tonsils, prostate, lung, kidney, melanoma, fixation times: 1-7 days)to detect vascular endothelium according to the manufacturer's instructions.

Image analysis

Aortic aneurysm samples were imaged using a light microscope (Nikon Eclipse E400) equipped with a digital camera (Nikon Digital Sight DS-M5) and analyzed with the NIS- Elements software (version 3–D).Two independent investigators (AP and MZ), blinded to the experimental groups, manually analyzed the histological slides. Their measurements were consistent, with inter-observer differences of less than 10%. In cases of discrepancy, the evaluation of a third pathologist was used to reach a consensus. We measured the surface areas of the tunica intima, tunica media, and tunica adventitia [15, 16], and counted CD117- positive cells. The number of MCs was expressed as N/mm^2. CD-117 positive cells were divided into nonactivated–intact MCs (cytoplasm filled with granules, no granules in the surroundings) and activated-degranulated MCs (fewer granules in the cytoplasm, more granules in the surroundings) and all MCs (intact and degranulated).

Vascularization assessment of the tunica media was evaluated at 100x objective magnification, where 4 regions of interest - ROI (1.22 x 0.91 mm) were randomly selected. Under this magnification, in most cases the entire thickness of the media was covered. Vascularization was evaluated based on the number of ROIs containing blood vessels, using the following scoring system: 0 – no vessels in any of the four ROIs; 1 – vessels present in one ROI; 2 – vessels present in two ROIs; 3 – vessels present in three ROIs; 4 – vessels present in all four ROIs.

For each aneurysm we calculated the average value of the measured parameters on three samples, which were taken from the area of maximum expansion. The average values of the number of all MCs, intact and degranulated MCs, and vascularization categories in DM and AH groups were calculated and expressed as average ± SD.

Statistics

Analyses were performed using Microsoft Excel 2010 and Statistical Package for Social Sciences SPSS 20. The average number of CD117 positive cells/mm2 in tunica intima, media, and adventitia in the DM and AH groups were calculated and expressed as the average value
± SD [15, 16]. Levene test showed that variances were not equal. According to the Sharpiro- Wilk test, the data were not normally distributed. The samples were of different sizes (AH group n = 42, and DM group n = 9).To detect significant differences between testing groups, statistical analysis was performed using Mann-Whitney U test ($p< 0.05$). The associations between the numbers of intact, degranulated, and total MCs and the degree of vascularization in the media were evaluated using Spearman's rank-order correlation ($p< 0.05$).

RESULTS

Patients

Patients with aortic aneurysm were divided into two groups: the DM group (n = 9) and the AH group (n = 42). Both groups were predominantly composed of male patients and included mostly non-smokers or former smokers. No significant differences were observed between the groups in terms of age or plasma levels of triglycerides, total cholesterol, HDL, LDL, creatinine, urea, aortic diameter and type of aortic valve (tri or bicuspid).Systolic blood pressure was higher in the AH group, while diastolic pressure was higher in the DM group. Plasma glucose was higher in the DM group (Table 1).

Analysis of intact, degranulated and all MCs in the intima, media, and adventitia between AH and DM groups

In each patient, 3 samples of the aorta from the most expanded part of the aneurysm were examined and found MCs in the intima media and adventitia. Seven patients from the AH group and five from the DM group did not have CD117 positive cells in the intima. In the media, 13 patients from the AH group and only one from the DM group were without MCs, in the adventitia only 5 patients from the AH group were without MCs, while they were present in all aortas from the DM group.In the media, MCs were more numerous in the outer part and in the connective tissue next to the

blood vessels.

Table 2 shows the average number of intact, degranulated and all MCs/mm2 in the intima media and adventitia in the AH and DM groups.
Compared to the DM group, in the AH group were significantly higher number of intact, degranulated and all MCs in the intima and significantly smaller number in the adventitia (Figure 1 and Table 3).

Within the DM and AH group, there were significantly higher number of intact MCs than degranulated only in the adventitia, but not in intima and media (Figures 1, Table 4).

Assessment of vascularity in the media

The vessels were mostly present in the outer part of the tunica media in both investigated groups - AH and DM (Figure 1). We found no significant difference in vascularity in the media in the DM group (0.852 ± 0.747) than in the AH group (1.603 ± 1.204; Mann Whitney test (p = 0.073) (Table 5).

Analysis of vascularity and MCs in media

In the DM group, there were significant positive correlation (Spearmans rho) between vascularity and degranulated MCs (r = 0.815, p = 0.007), while in AH group was significant positive correlation between vascularity and intact (r = 0.519, p = 0.000), degranulated (r = 0.531, p = 0.000) and all MCs (r = 0.531, p = 0.000) (Figure 2).

DISCUSSION

MCs play a crucial role in host defense against pathogens and are therefore widely distributed throughout the loose connective tissue of the body. They are predominantly located at interfaces between the external environment and internal tissues, particularly in the gastrointestinal tract, respiratory tract, and skin, where they destroy antigens like bacteria, parasites and viruses [2, 21-23].
Progenitor cells for MCs originate from hemopoietic stem cells in the bone marrow, pass into the blood, migrate to tissues where they differentiate into mature MCs under the influence of various cytokines and stem cell factors [24]. In their cytoplasm MCs

contain granules with pro-inflammatory mediators, proteases (chymase, tryptase, carboxypeptidase), biogenic amins (histamine, serotonin, dopamine), lysosomal enzymes (beta-hexosaminidase) and glycosaminoglycane / proteoglycans (heparin, heparansulfate, chondroitin sulfate, serglycin), cytokines, and growth factors [8, 21, 25, 26]. Upon activation, these granules are released into the environment to destroy the antigen. The best-known mechanism of MCs activation is the binding of the antigen to IgE attached to FcεRI receptors on the cell surface [8, 25-27]. This interaction triggers the release (degranulation) of previously formed granules, which manifests as a type I allergic reaction. In addition to IgE-mediated activation, MCs can also be activated through Fc receptors for IgA and IgG, complement receptors (e.g., C3a), adenosine receptors, cytokines, chemokines, and various pathogen-associated molecular patterns (PAMPs) [2, 5, 28].

In addition to releasing preformed mediators, activated MCs synthesize and secrete both pro- and anti-inflammatory mediators, depending on the type of stimulus and type of tissue (the tissue context) [2, 8]. In humans, seven transcriptomically distinct MCs subtypes have been identified in various tissues, including the trachea, lungs, tongue, lymph nodes, small and large intestines, pancreas, skeletal muscle, skin, bladder, mammary glands, and vasculature [29, 30]. It is hypothesized that the local tissue microenvironment shapes MC polarization toward either protective or pathogenic phenotypes.MCs exert both beneficial and detrimental effects in cardiovascular diseases, such as hypertension, atherosclerosis, myocardial infarction, dilated cardiomyopathy, myocarditis, and thrombosis. One of the proposed beneficial roles of MCs in the cardiovascular system is the promotion of angiogenesis, mediated through the release of VEGF-A, tryptase, and the cysteinyl leukotrienes LTC_4 and LTD_4 [29].

Diabetes mellitus (DM) is a systemic, chronic inflammatory disease that is closely associated with atherosclerosis. The systemic inflammatory state in DM contributes to endothelial dysfunction, increased vascular permeability, the formation of multiple vascular thrombi, and hemodynamic disturbances [31].MCs, in turn, exacerbate DM-related complications such as atherosclerosis [32], kidney disease [33-36], and cardiomyopathy [37, 38]. Experimental studies have demonstrated that inhibition of MCs degranulation or MC deficiency in diabetic mice can prevent the progression of cardiomyopathy [39].

In damaged skin, activated MCs release a range of mediators, including tumor necrosis factor alpha (TNFα), histamine, vascular endothelial growth factor (VEGF), interleukin (IL)-6, IL-8, platelet-derived growth factor (PDGF), transforming growth factor beta (TGFβ), and nerve growth factor. These mediators play a critical role in promoting wound healing. Activated MCs contribute to the stabilization of the blood clot, enhance neoangiogenesis, fibrinogenesis, and epithelialization, increase vasodilation and endothelial permeability, facilitate the recruitment of neutrophils and monocytes to the injury site, and support the activation of keratinocytes and fibroblasts [40, 41].

Diabetic wounds are characterized by delayed closure, impaired angiogenesis, and prolonged inflammation. Experimental models have shown that intact skin from diabetic subjects contains a higher proportion of degranulated MCs compared to non-diabetic controls.

Furthermore, while MCs degranulation increases in response to injury in non-diabetics, this response is absent in diabetics. Notably, pharmacological inhibition of MC degranulation in diabetic models resulted in normalized wound healing. These findings suggest that in diabetes, intact MCs support proper wound repair, whereas MCs degranulation impairs the healing process. This indicates the presence of a functionally detrimental MCs phenotype in the diabetic state [41, 42].

MCs are known to reside in the intima of normal aortas and early atherosclerotic lesions such as fatty streaks; however, their numbers significantly decline in advanced atheromas and decrease even further in abdominal aortic aneurysms [9, 11, 43]. In contrast, the density of MCs in the media and adventitia increases with the progression of atherosclerosis and reaches its highest levels in the media and adventitia of abdominal aortic aneurysms [9, 11, 12].

Notably, significantly more degranulated MCs have been observed in the media and adventitia of abdominal aortic aneurysms compared to control and atherosclerotic aortas, where degranulated MCs were nearly absent. In the referenced study, 95% of patients had AH, while only 4% had DM [12].

In the present study, we compared the density of intact and degranulated MCs in the intima, media, and adventitia of ascending aortic aneurysms in patients with DM and

those with AH. AH is well established as one of the most common risk factors for aneurysm formation [14], whereas aneurysms are rare in patients with DM who do not also have AH [9, 15-18, 20].
Consequently, the DM group included only nine patients. In addition to the small sample size—particularly in the DM group—our study had several other limitations, including missing patient data such as HbA1c levels, duration of diabetes, medication use, and smoking history, the use of semi-quantitative vascular scoring, and data derived from a single centre.

Our findings showed that, compared to the AH group, the DM group had significantly fewer intact and degranulated MCs in the intima, and significantly more in the adventitia. Within the both groups, a significantly higher number of intact MCs compared to degranulated MCs was observed only in the adventitia, while no such difference was found in the intima or media.

Based on data from previous studies [9, 12, 41-43], it could be assumed that the ascending aortic aneurysms in the AH group would be less affected than those in the DM group in our study.

It is well established that in true aneurysms, all three layers of the vessel wall are affected, whereas in false aneurysms, only the media and adventitia are compromised. The thickest layer of the aorta is the tunica media, which, along with the adventitia, provides structural integrity and resistance to the vessel wall. Elastic lamellae within the media enable the aorta to expand during systole as it fills with blood and to recoil during diastole, thereby helping to stabilize and smooth arterial pressure in coordination with left ventricular function. Collagen fibers in the adventitia prevent excessive stretching of the aorta during systole [44]. The principal mechanical property of the aortic wall is its elasticity, primarily attributed to the tunica media. However, our previous studies demonstrated no significant differences in the composition of the aneurysmal wall, such as the volume density of elastic and collagen fibers, vascular smooth muscle cells, and ground substance, between the DM and AH groups [15, 16].These studies are consistent with our findings, which showed no statistically significant difference in aortic diameter between the AH and DM

groups. It is well established that MCs density correlates with the degree of vascularity in the tunica media of the abdominal aorta, in both atherosclerotic and aneurysmal conditions [2, 9, 11, 12]In our current study, we found just insignificantly greater vascularity in the tunica media of the AH group compared to the DM group. This trend of reduced vascularity in media in DM group aligns with previous findings indicating that diabetes inhibits angiogenesis and impairs wound healing. In the AH group, vascularity was positively correlated with the total number of MCs, including both intact and degranulated forms. In contrast, in the DM group, a significant positive correlation was observed only between vascularity and degranulated MCs.

Activated MCs secrete a wide array of mediators, including constitutive and inducible pro- angiogenic factors such as vascular endothelial growth factor (VEGF) [3, 45]. However, diabetes has been shown to suppress VEGF secretion. Experimental studies have demonstrated that an insufficient number of MCs, secrete reduced levels of VEGF, resulting in delayed wound healing in both diabetic and non-diabetic models. Moreover, in diabetic animals, VEGF expression and angiogenesis were significantly lower than in non-diabetics during the healing process. Pharmacological inhibition of MCs degranulation in diabetic models restored VEGF levels and angiogenesis to those seen in non-diabetics [41, 42].
Therefore, we expected a positive correlation between vascularity and intact, rather than degranulated, MCs in the DM group.

Despite the seemingly contradictory findings, we hypothesize that MCs may play a beneficial role in the aortic aneurysm wall. MCs are present in the intima of healthy and early atherosclerotic aortas but are rare in the adventitia. As atherosclerosis advances, MCs density decreases in the intima and increases in the adventitia. In advanced atherosclerosis and abdominal aortic aneurysms, MCs are scarce in the intima but abundant in the adventitia [9, 12]. Moreover, MCs are essential for normal wound healing in both healthy individuals and those with diabetes, as their deficiency has been associated with delayed repair [41, 42].

It is important to recognize that MCs are a highly pleomorphic population of inflammatory cells whose behavior is influenced by the local tissue environment.

Aneurysm formation is a chronic process, as is the progression of diabetes, and the microenvironments of the intima, media, and adventitia in an ascending aortic aneurysm differ substantially from those in experimentally induced skin wounds. Thus, further studies are needed to elucidate the role of MCs in the pathogenesis of ascending aortic aneurysms in diabetic patients.

CONCLUSION

Our findings show that, in diabetic patients, MCs are less abundant in the intima and more abundant in the adventitia compared to patients with AH. This altered distribution pattern suggests that diabetic patients may have a distinct pathogenesis of aneurysm progression compared to those with AH.

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Conflicts of interest: Authors declare no conflicts of interest.

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Data availability: Data supporting the findings of this study are available from the corresponding author upon reasonable request.

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TABLES AND FIGURES WITH LEGENDS

Table 1. Characteristics for all patients in the AH and DM group (average ± SD).

Variable	DM group	AH group	*p* value Mann-Whitney test	R
	n = 9	*n* = 42		
Age (year)	62.56 ± 11.92	57.59 ± 8.33	0.42	0.114
Sex	7 male, two female	35 male, 7 female	0.45	0.106
Smoking	yes 1; no 8 (never smoked 5 stopped smoking 3)	yes 7; no 35 (never smoked 26, stopped smoking 9)		
Total cholesterol (mmol/l)	5.31 ± 1.06	5.01 ± 0.55	0.78	0.040
LDL (mmol/l)	3.72 ± 1.24	3.27 ± 0.68	0.52	0.091
HDL (mmol/l)	1.30 ± 0.45	1.41 ± 0.53	0.79	0.036
Triglyceride (mmol/l)	2.29 ± 1.04	1.71 ± 0.77	0.14	0.208
Creatinine (µmol/l)	82.86 ± 11.85	82.73 ± 11.75	0.94	0.011
Urea (mmol/l)	7.07 ± 1.62	6.77 ± 1.50	0.61	0.071
Aortic diameter (cm)	5.94 ± 0.73	5.54 ± 0.55	0.141	0.206
Aortic valve	tricuspid = 9 bicuspid = 0	tricuspid = 29, bicuspid = 13	0.053	0.271
Systolic blood pressure (mmHg)*	127.22 ± 6.67	138.34 ± 14.21	0.030	0.302
Diastolic blood pressure	79.73 ± 12.61	73.89 ± 8.94	0.050	0.274

(mmHg)*				
Glucose (mmol/l)*	8.88 ± 0.06	5.51 ± 0.28	0.000	0.656

*Significantly different amount of the component within the DM and AH group; Mann-Whitney test (p <0.05).

Table 2. Density of MCs (N/mm^2 ± SD) in intima, media and adventitia in AH and DM group.

AH (n = 42)	**Intima**	**Media**	**Adventitia**
Intact	8.583/ mm^2± 11.940	0.244/mm^2 ± 0.359	6.497/mm^2 ± 7,875
Degran.	3.015/mm^2 ± 3.970	0.116/mm^2 ± 0.196	2.454/mm^2 ± 3.866
All	11.598/mm^2 ± 15.160	0,359/mm^2 ± 0.527	8.951/mm^2 ± 11.539
DM (n = 9)	**Intima**	**Media**	**Adventitia**
Intact	0.659/mm^2 ± 1.290	0.039/mm^2 ± 0.036	13.638/mm^2 ± 5.136
Degran.	0.520/mm^2 ± 1.286	0.022/mm^2 ± 0.033	5.327/mm^2 ± 3.524
All	1.179/mm^2 ± 2.556	0.061/mm^2 ± 0.060	18.965/mm^2 ± 7.688

Table 3. The number of MCs/mm^2 in the intima, media, and adventitia of the ascending aortic aneurysm aortic wall in patients with AH and DM

	DM (*n*=9)	**AH (*n*=42)**				
Variable	**Mean rank (MCs/mm^2)**	**Mean rank (MCs/mm^2)**	***p***	**U**	**Z**	**r**
Intima						
Intact*	16.56	28.02	0.032	104.000	-2.140	0.300
Degran.*	17.67	27.79	0.050	114.000	-1.932	0.271
All*	16.44	28.05	0.030	103.000	-2.165	0.303
Media						
Intact	22.83	26.68	0.471	160.500	-0.720	0.100
Degran.	21.50	26.96	0.290	148.500	-1.057	0.150
All	24.50	26.32	0.735	175.500	-0.339	0.050
Advnentitia						
Intact*	39.56	23.10	0.003	67.000	-3.016	0.422
Degran.*	38.67	23.29	0.005	75.000	-2.819	0.395
All*	40.00	23.00	0.002	63.000	-3.115	0.436

*Significantly different, Mann-Whitney test ($p< 0.05$).

Table 4. The density of MCs (N/mm^2) in the intima, media, and adventitia of the ascending aortic aneurysm aortic wall within patients with AH and DM

	Intact	**Degran.**				
Variable	**Mean rank (MCs/mm^2)**	**Mean rank (MCs/mm^2)**	***p***	**U**	**Z**	**r**
AH (*n*=42)						
Intima	47.26	37.74	0.068	682.000	-1.823	0.200
Media	45.89	39.11	0.187	739.500	-1.319	0.144
Adventitia*	49.35	35.65	0.010	594.500	-2.575	0.281
DM (*n*=9)						
Intima	10.11	8.89	0.580	35.000	-0.553	0.130
Media	10.56	8.44	0.380	31.000	-0.878	0.207
Adventitia*	13.33	5.67	0.002	6.000	-3.046	0.718

*Significantly different, Mann-Whitney test ($p < 0.05$).

Table 5. Vascularity score in tunica media in AH and DM group. Mann-Whitney test.

	DM (*n*=9)	**AH (*n*=42)**				
Variable	**Mean rank (MCs/mm^2)**	**Mean rank (MCs/mm^2)**	**p**	**U**	**Z**	**r**
Vascularity in media	18	27.71	0.073	117.000	-1.791	0.251

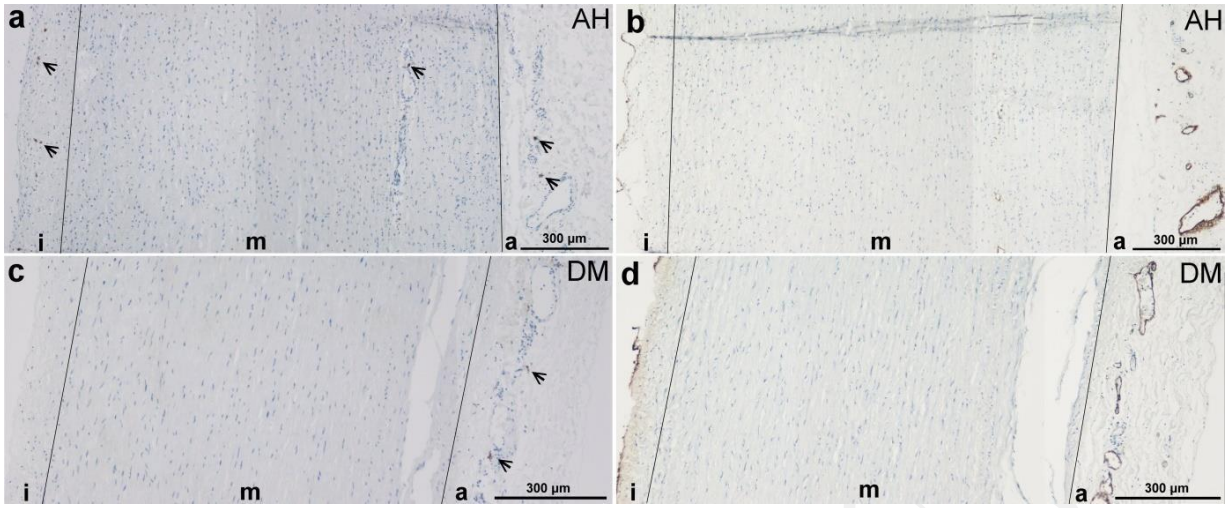

Figure 1. a,b: CD117 positive cells in the aortic wall (arrows); c,d: vWf positive cells in the aortic wall of patients with AH and DM group; i: intima; m: media; a: adventitia. Note: figures a, b, c and d were stitched to provide a panoramic view of all layers of the vascular wall.

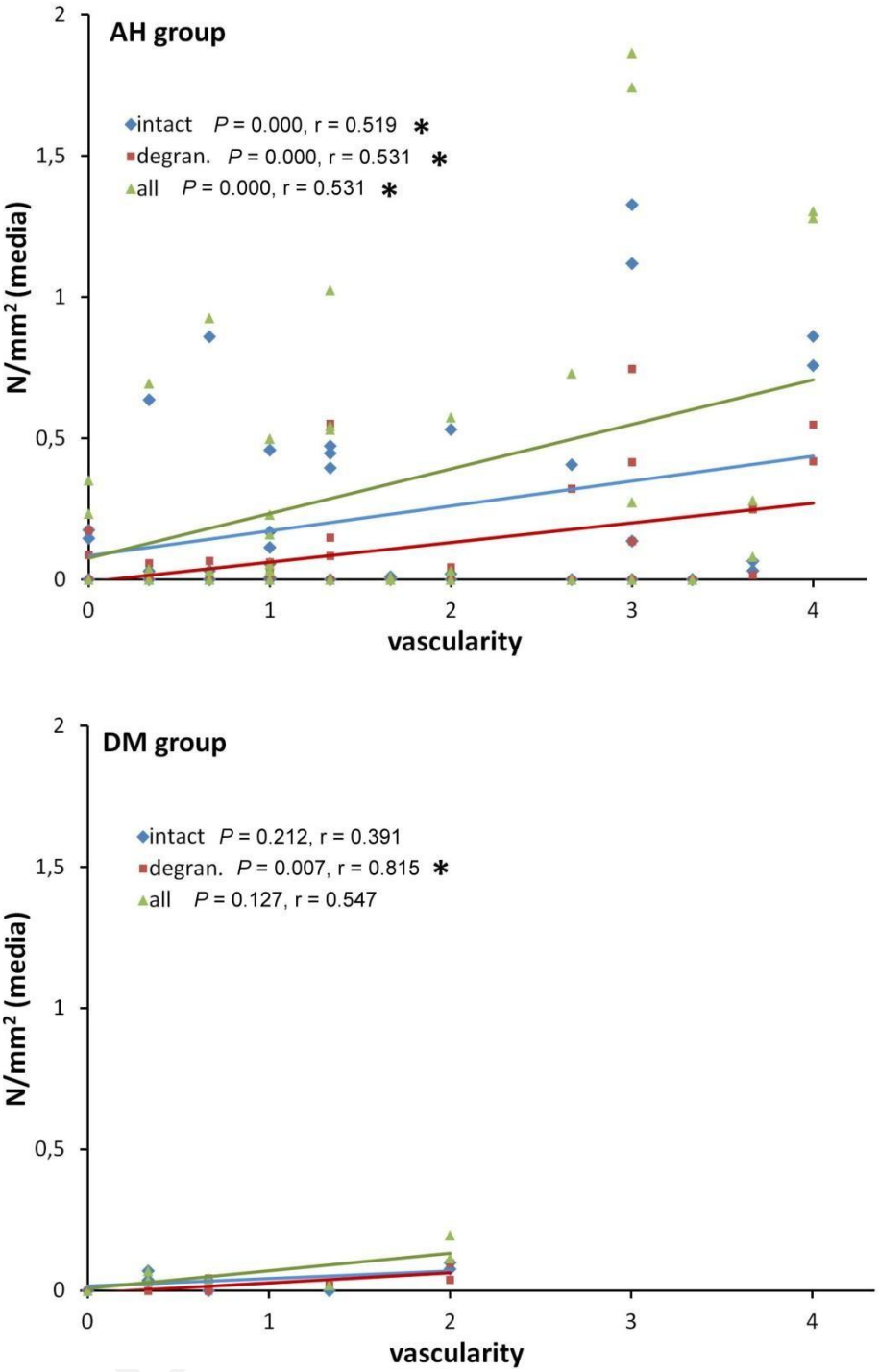

Figure 2. Spearman's correlation between vascularity assessment and number of intact, degranulated and all MCs (N/mm^2) in tunica media of AH and DM group (*significant correlation (p< 0.05)).