DOI: 10.4149/BLL_2023_021

*Bratisl Med J 2023; 124 (2)*
133 – 142

EXPERIMENTAL STUDY

# Depletion of vascular adaptive mechanisms in hypertension-induced injury of the heart and kidney

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**ABSTRACT**

BACKGROUND: Vascular endothelial growth factor (VEGF) is a signalling protein of critical importance for angiogenesis. In an effort to better understand its significance in hypertension-induced injury of the heart and kidney we aimed at studying the changes in its expression in an experimental model and correlated it with capillary density in the myocardium and the renal parenchyma.

METHODS: We used two age groups of spontaneously hypertensive rats (6- and 12-month-old), indicative of early and advanced hypertension. VEGF expression was assessed and a semi-quantitative analysis of its immunoreactivity was conducted. Changes in capillary density in the myocardium and kidney were assessed for statistical significance and correlations with VEGF expression were established.

RESULTS: We reported stronger VEGF expression in animals with early compared to advanced hypertension in all examined structures. Capillary density decreased significantly at age 12 months compared to 6 months and was significant in all examined structures. A positive correlation was established between capillary density and the expression of VEGF.

CONCLUSION: These findings underscore the key significance of VEGF for compensatory angiogenesis and suggest that a statistically significant depletion of these vascular adaptive mechanisms is a major aspect in the cascade of hypertension-induced injury of the heart and kidney *(Tab. 3, Fig. 8, Ref. 47)*. Text in PDF *www.elis.sk*

KEY WORDS: vascular endothelial growth factor (VEGF); capillary density (CD); myocardium; kidney; hypertension.

## Introduction

Hypertension is a leading cause of morbidity worldwide and poses a significant health and economic threat to aging societies (1, 2). It represents a major risk factor for cardiovascular and renal disease since both the heart and kidneys are target organs of hypertensive injury (3). Cardiac hypertrophy initially constitutes an adaptive response of the heart to increased stress, however, in the long term, it forms the prerequisite for heart failure (4). As cardiac hypertrophy develops, an upregulated cardiac angiogenesis is activated as a compensatory mechanism in the early adaptive phase, which may initially be sufficient to reduce the disparity between the increased size of the cardiac muscle cells and capillary density (CD) (5). In particular, vascular endothelial growth factor (VEGF) is a signalling protein of critical importance in the compensatory hypertrophic response to myocardial stress initiated by hypertension (6).

In humans, the VEGF family of proteins comprises five members – VEGF-A, VEGF-B and placental growth factor, which are mainly responsible for the formation of blood vessels, as well as VEGF-C and VEGF-D, which regulate lymphangiogenesis. Furthermore, VEGF-A (usually referred to simply as VEGF in a more narrow sense) is the main driver of angiogenesis, which represents the formation of new blood vessels from previously existing vasculature. Cardiomyocytes can both produce and be targeted by VEGF-A (7). It induces the chemotaxis of endothelial cells (EC), promotes their survival and mitosis and thus participates in the growth and remodelling of blood vessels (8). In turn, VEGF-A exerts its effects on cardiomyocytes via VEGF receptor 1 (VEGFR1) and 2 (VEGFR2), which are both present on their cellular membrane. Thus, the balance of VEGF action on VEGFR1, which counters the development of cardiac hypertrophy and VEGFR2, which has a pro-hypertrophic impact, is a key aspect of myocardial remodelling (9). As part of the heart's adaptive

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**Acknowledgements:** This research is supported by the Bulgarian Ministry of Education and Science under the National Program 'Young Scientists and Postdoctoral Students – 2'.

mechanisms in various injury patterns, the activation of cardiac muscle cells by VEGF-A promotes their contractility and survival, enhances cardiac stem cell recruitment and, as mentioned above, cardiac angiogenesis (7). On the contrary, in the maladaptive stage, VEGF-A expression is reduced (10, 11). This leads to insufficient angiogenesis, which is considered the first step in the transition of cardiac hypertrophy to heart failure (12).

In the kidney, VEGF-A is vital for the formation of glomerular capillary tufts, maintenance of the fenestrated endothelium of glomerular capillaries and glomerular repair in response to injury (13). While it mediates the proliferative and antiapoptotic effects on tubular epithelial cells, VEGF-A may also contribute to the development of renal interstitial alterations (14). Expression of renal VEGF is mostly confined to visceral epithelial cells of Bowman's capsule – podocytes, distal tubular segments, collecting ducts and to a relatively lesser degree – proximal tubules (15, 16). VEGFR-2 is the predominant receptor type found in the kidney and is present on the membranes of preglomerular and glomerular endothelial cells, peritubular endothelial cells in proximal and distal tubules and collecting ducts, as well as some non-endothelial cells, such as mesangial cells, cortical fibroblasts and interstitial cells in the medulla (13, 15, 16, 17).

The integrity and maintenance of renal vasculature depends on the interaction and precise balance between proangiogenic and antiangiogenic factors (18). The role of VEGF in renal physiology and pathology has been studied in various experimental models, yet results are controversial. While it has been suggested that the inhibition of VEFG in the adult kidney is not associated with prominent changes in the glomerular filtration barrier, thus limiting its significance (19), contrasting data have pointed to its potential renoprotective effects under pathological conditions (20). In particular, VEGF may be important in maintaining glomerular integrity in the hypertensive setting (21), however its role in hypertension-induced kidney injury has been widely overlooked.

In an effort to better understand the significance of VEGF in hypertension-induced injury of the heart and kidney, we aimed at studying the changes in its expression during different stages of hypertension in an experimental model and correlated VEGF expression with CD in the myocardium of the left and right ventricle (LV and RV, respectively), as well as the renal cortex (RC) and renal medulla (RM).

**Tab. 1. Mean systolic (mmHg) and mean diastolic blood pressure (mmHg) of 6- and 12-month-old spontaneously hypertensive rats. Each group consisted of six animals (n = 6). SHR – spontaneously hypertensive rats; SD – standard deviation.**

| Age group | Mean systolic blood pressure (mmHg)±SD | Mean diastolic blood pressure (mmHg)±SD |
|---|---|---|
| 6-month-old SHR | 182.6±3.5 | 114.7±5.0 |
| 12-month-old SHR | 195.3±6.6 | 121.6±4.5 |

## Material and methods

### *Experimental animals*

Two age groups of SHR were used for the purposes of the present study – 6-month-old (established hypertension) and 12-month old (advanced or late stage hypertension) (22). Each group comprised six male rats randomly selected from a large population of SHR in the Laboratory of the Department of Anatomy, Histology and Embryology at the Medical University of Sofia, Bulgaria. All animal procedures were conducted in the manner previously described (23). We measured systolic and diastolic arterial blood pressure using the tail-cuff method on a Model MK-2000ST (Muromachi Kikai Co., Ltd., Tokyo, Japan) and recorded the obtained values in all age groups of SHR as previously described (23).

### *Tissue preparation*

#### *Heart*

Three randomly selected rats from each group were sacrificed for myocardial specimens according to the previously described standardised procedure (23).

#### *Kidney*

Three randomly selected rats from each group were sacrificed and their kidneys were obtained for analysis according to the previously described standardised procedure (24).

### *Light microscopy*

Slides from the heart and kidney were processed for routine light microscopic study in the manner previously described (23, 24).

**Tab. 2. Semi-quantitative analysis of the intensity of immunohistochemical staining for vascular endothelial growth factor in the myocardium of the left and right ventricle and in the renal cortex and medulla in 6- and 12-month-old spontaneously hypertensive rats. The percentage of each score indicates the percentage of visual fields that the IHC Profiler assigned this score to.**

| VEGF | LV | RV | RC | RM |
|---|---|---|---|---|
| 6-month-old SHR | High-positive (3+) (28%)<br>Positive (2+) (41%)<br>Low-positive (1+) (17%)<br>Negative (0) (14%) | High-positive (3+) (29%)<br>Positive (2+) (38%)<br>Low-positive (1+) (30%)<br>Negative (0) (3%) | High-positive (3+) (23%)<br>Positive (2+) (41%)<br>Low-positive (1+) (35%)<br>Negative (0) (1%) | High-positive (3+) (35%)<br>Positive (2+) (38%)<br>Low-positive (1+) (20%)<br>Negative (0) (7%) |
| 12-month-old SHR | High-positive (3+) (1%)<br>Positive (2+) (7%)<br>Low-positive (1+) (55%)<br>Negative (0) (37%) | High-positive (3+) (0%)<br>Positive (2+) (5%)<br>Low-positive (1+) (49%)<br>Negative (0) (46%) | High-positive (3+) (0%)<br>Positive (2+) (12%)<br>Low-positive (1+) (47%)<br>Negative (0) (41%) | High-positive (3+)(2%)<br>Positive (2+) (6%)<br>Low-positive (1+) (59%)<br>Negative (0) (33%) |

LV – left ventricle; RV – right ventricle; RC – renal cortex; RM – renal medulla; SHR – spontaneously hypertensive rats; VEGF – vascular endothelial growth factor

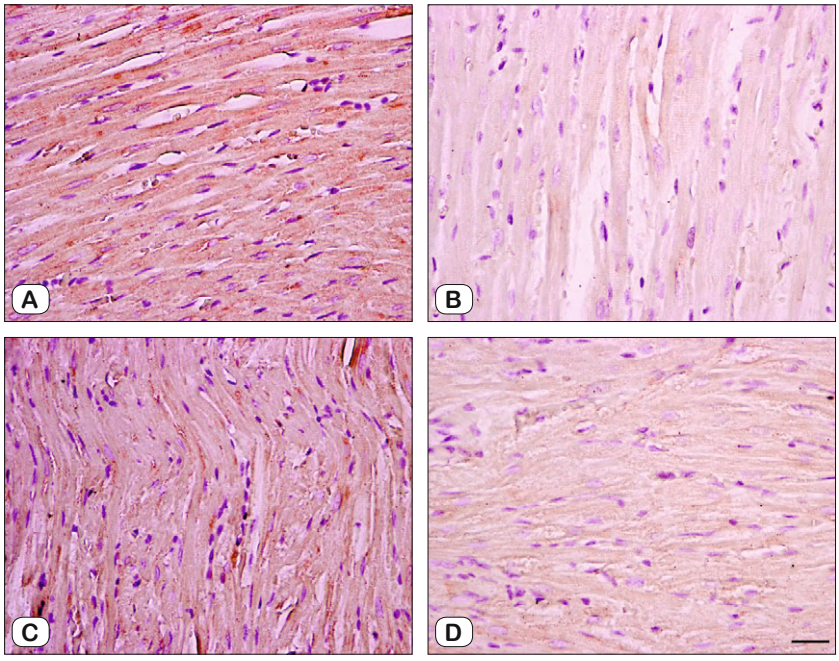


**Fig. 1. Immunohistochemical staining for vascular endothelial growth factor (VEGF) in the myocardium of spontaneously hypertensive rats (SHR). Size bar – 50 µm. A. Left ventricle (LV), 6-month-old SHR; B. Left ventricle (LV), 12-month-old SHR; C. Right ventricle (RV), 6-month-old SHR; D. Right ventricle (RV), 12-month-old SHR.**

*Immunohistochemistry*

The immunohistochemical study was conducted through the heat-induced epitope retrieval (HIER) technique as previously described (24). We used mouse monoclonal anti-VEGF-A IgG antibody (Santa Cruz Biotechnology Catalogue No. sc-7269, Santa Cruz Biotechnology, Inc., Heidelberg, Germany) at concentration 1:250. Further, incubation with mouse IgG kappa binding protein (m-IgGκ BP) conjugated to horseradish peroxidase (HRP) (Santa Cruz Biotechnology Catalogue No. sc-516102) at concentration 1:75 for 2 h was performed. All other procedures followed the standardised protocol described in our previous work (23, 24).

*Semi-quantitative analysis of VEGF expression*

The protocol for the semi-quantitative analysis of the expression of VEGF followed the standardised procedure described in our previous works (23).

*Immunofluorescence*

Paraffin-embedded sections were dewaxed using xylene and rehydrated through usage of ascending series of alcohols, with final rehydration steps using $H_2O$. Slides were put in 10 mM sodium citrate buffer (pH 6.0) for HIER technique to recover antigens. Slides were incubated overnight at 4°C with the monoclonal anti-VEGF-A IgG antibody (Santa Cruz Biotechnology Catalogue No. sc-7269) at concentration 1:250. The slides were rinsed in PBS (Merck Catalogue No. 6505-4 L, Merck KGgA, Darmstadt, Germany) and then treated with fluorescently labelled secondary antibody: a green fluorescent dye for VEGF-A (m-IgGκ BP) conjugated to CruzFluor™ 488 (CFL 488, Santa Cruz Biotechnology Catalogue No. sc-516176) at a dilution of 1:250 in the dark chamber for 1h. After rinsing the slides with PBS (Merck Catalogue No. 6505-4 L), cover slips were mounted with a hard-set mounting medium.

*Morphometric analysis of CD*

The morphometric analysis was performed on H&E-stained slides from each organ of each animal. Quantitative data were obtained with a computerised system for image analysis NIS-Elements Advanced Research (Ver. 2.30). CD was quantified according to well-established protocols (25, 26).

*Statistical analysis*

The statistical analysis was performed with the SPSS software (IBM Corporation, Armonk, New York, United States). Data distribution was not normal, as assessed through the Kolmogorov-Smirnov test. The Mann-Whitney test was then used to test for statistically significant differences between CD at age 6 and 12 months in the LV, RV, RC and RM. Spearman's correlation was used to test whether a correlation exists between CD per slide and VEGF (expressed as the total score of the examined area on the slide). A standard level of significance α (p value) = 0.05 was used in all tests.

## Results

*Blood pressure measurement*

Table 1 presents the mean systolic and diastolic blood pressure (as average values) of the two age groups of SHR.

*VEGF expression*

*Immunohistochemical analysis and semi-quantitative analysis of VEGF expression*

VEGF immunoreactivity in the myocardium of both ventricles was predominantly observed in the walls of blood vessels, perivascular areas and ubiquitously across the cytoplasm of cardiomyocytes. Stronger expression was noted in the LV compared to the RV in both age groups and was less pronounced in the group of advanced cardiac hypertrophy (Fig. 1). In the RC, staining was most prominent in the visceral layer of Bowman's capsule and in the epithelial cells of proximal and distal tubular segments and was stronger in younger animals. Immunoreactivity in the glomerular capillary tufts was negative in both age groups (Fig. 2A and 2B). In the RM, VEGF expression was reported predominantly in the collecting ducts and to a lesser extent in the loops of Henle and was again more pronounced in 6-month-old SHR (Fig. 2C and 2D).

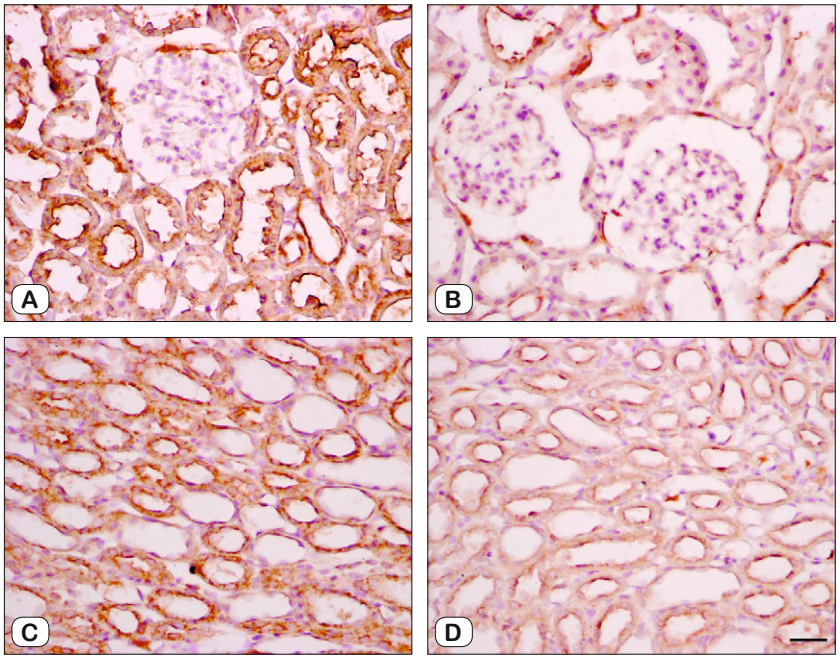


**Fig. 2. Immunohistochemical staining for vascular endothelial growth factor (VEGF) in the kidney of spontaneously hypertensive rats (SHR). Size bar – 50 µm. A. Renal cortex (RC), 6-month-old SHR; B. Renal cortex (RC), 12-month-old SHR; C. Renal medulla (RM), 6-month-old SHR; D. Renal medulla (RM), 12-month-old SHR.**

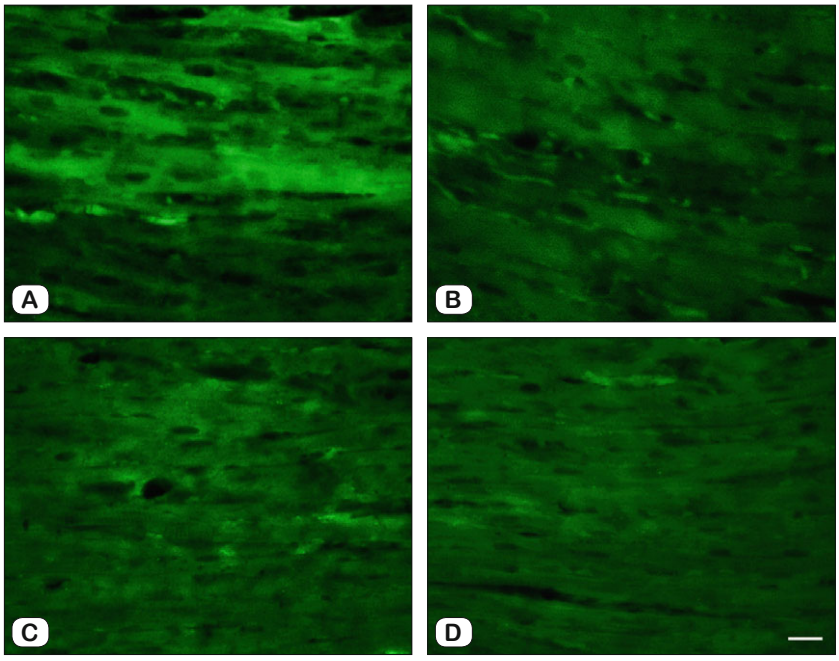


**Fig. 3. Immunofluorescent labelling of vascular endothelial growth factor (VEGF) in the myocardium of spontaneously hypertensive rats (SHR). Size bar – 30 µm. A. Left ventricle (LV), 6-month-old SHR; B. Left ventricle (LV), 12-month-old SHR; C. Right ventricle (RV), 6-month-old SHR; D. Right ventricle (RV), 12-month-old SHR.**

The results of the semi-quantitative analysis of the intensity of immunoreactivity of VEGF are shown in Table 2. VEGF expression in the LV of 6-month-old SHR was positive (2+) in almost 50 % and high positive (3+) in nearly one third of the examined fields, whereas in the 12-month-old animals the predominant expression was low-positive (1+), while more than a third of the fields stained negatively (0). A similar tendency was noted in the RV, where in 6-month-old animals a similar percentage of fields were either high-positive (3+), positive (2+) or low-positive (1+), while in 12-month-old SHR, nearly half the fields were low-positive (1+) and another 46 % – negative (0). The RC of younger animals was characterised by mostly positive (2+) to low-positive (1+) expression, while in the older group it was predominantly low-positive (1+) to negative (0). Interestingly, in the RM of 6-month-old animals, expression was reported to be strongest, with more than a third of the fields read as high-positive (3+) and another 38 % as positive (2+). Conversely, in older animals, it was low-positive (1+) in nearly 60 % of fields and negative (0) in a third of them.

*Immunofluorescence*

Localisation of VEGF expression in the heart and kidney was further confirmed with an immunofluorescence study. The immunofluorescence reaction in the myocardium of 6-month-old animals in both ventricles showed a clear staining pattern, with predisposition for the perinuclear area in cardiomyocytes and the perivascular zone, and was stronger in the LV. This reactivity was reduced in the group of 12-month-old SHR (Fig. 3).

In the RC, VEGF staining in a granular pattern was noted in the visceral layer of Bowman's capsule and in tubular epithelial cells, where the reaction was observed in the perinuclear area. Again, this expression was reduced in 12-month-old animals (Fig. 4A and 4B). The immunofluorescence reaction in the RM was similar, more prominent in the perinuclear area of epithelial cells in the walls of collecting ducts and less strong in the older age group (Fig. 4C and 4D).

*Comparative analysis of CD in 6- and 12-month-old SHR*

CD in the myocardium, RC and RM was evaluated on sections stained with H&E (Fig. 5). Capillaries were observed as oval or circular structures with a lumen, sometimes filled with red blood cells and an endothelial cell lining the lumen.

Highest CD was reported for the LV, followed by the RV and an approximately identical value in the RC and RM in both age groups. A marked decrease in CD in 12-month-old SHR compared to younger animals was noted in all examined structures. The conducted statistical analysis confirmed that decrease to be statistically significant (Tab. 3 and Fig. 6).

*Correlations between CD and VEGF expression*

In all examined structures, a positive correlation was established between the CD per section and the expression of VEGF, assessed semi-quantitatively as score per section. In the heart, this correlation was statistically significant only in the LV, both in the group of established hypertension ($r_s$ = 0.80338, p = 0.00031) and advanced hypertension ($r_s$ = 0.71264, p = 0.00287) (Fig. 7A and 7B). In the RV, the correlation was found not to be statistically significant ($r_s$ = 0.46565, p = 0.08024 in 6-month-old SHR and $r_s$ = 0.44666, p = 0.0951 in 12-month-old SHR, respectively) (Fig. 7C and 7D). In the RC, the positive correlation was statistically significant in both age groups ($r_s$ = 0.59556, p = 0.01915 in 6-month-old SHR and $r_s$ = 0.65794, p = 0.00767 in 12-month-old SHR, respectively) (Fig. 8A and 8B). Finally, in the RM, a statistically significant positive correlation was established in younger animals ($r_s$ = 0.64001, p = 0.01018) but not in the group of 12-month-old SHR ($r_s$ = 0.4579, p = 0.08609) (Fig. 8C and 8D).

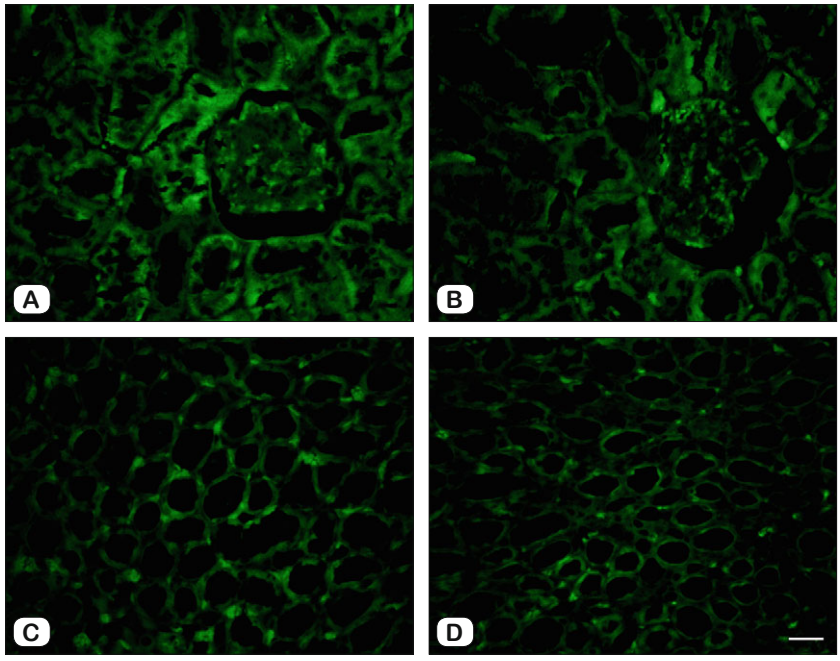


**Fig. 4. Immunofluorescent labelling of vascular endothelial growth factor (VEGF) in the kidney of spontaneously hypertensive rats (SHR). Size bar – 50 µm. A. Renal cortex (RC), 6-month-old SHR; B. Renal cortex (RC), 12-month-old SHR; C. Renal medulla (RM), 6-month-old SHR; D. Renal medulla (RM), 12-month-old SHR.**

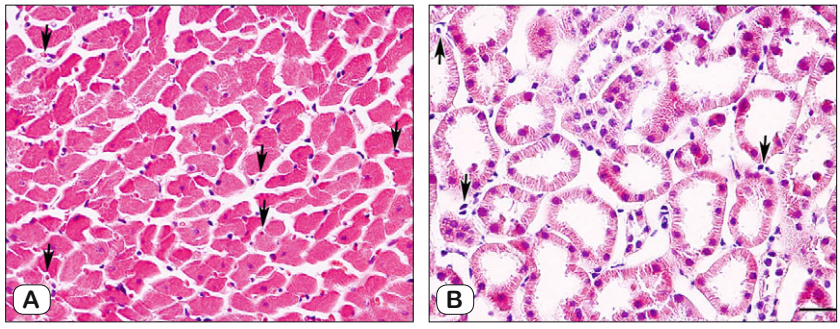


**Fig. 5. Routine haematoxylin and eosin (H&E) staining used to identify capillaries and calculate capillary density (CD). Arrows – capillaries. Size bar – 50 µm. A. Myocardium; B. Kidney.**

## Discussion

The present study addresses a key adaptive mechanism in the heart and kidney under hypertensive conditions, namely the formation of new blood vessels and its depletion with the progression of hypertension-induced injury. The expression of VEGF-A, a key protein in angiogenesis, was shown to decrease in the LV, RV, RC and RM as hypertension-induced injury progressed. We have also demonstrated a statistically significant decrease in CD in all examined structures in SHR with advanced hypertension compared to SHR with early (established) hypertension. In addition, positive correlations between CD and VEGF expression were reported in all examined structures during both established and advanced hypertension.

Reduced cardiac angiogenesis is a major factor in the transition from adaptive cardiac hypertrophy to heart failure (27, 28). The significance of VEGF in maintaining cardiac function in pressure overload has been highlighted by the fact that its inhibition promotes the transition to heart failure in the hypertrophied myocardium (29). Duan et al (4) suggested that the upregulation of VEGF-A leads to an increase in the capillary/cardiomyocyte ratio but noted an overall net reduction in CD since the increased angiogenesis could not match the simultaneous myocyte growth (described as

**Tab. 3. Descriptive statistics for the parameter capillary density per high-power field on hematoxylin and eosin-stained slides in the heart and kidney of 6- and 12-month-old SHR.**

| Capillary density | N | 6-month-old SHR | | | | | 12-month-old SHR | | | | | p-value |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Mean | SD | Median | Min | Max | Mean | SD | Median | Min | Max | |
| LV | 150 | 79.1 | 8.4 | 79 | 55 | 98 | 63.3 | 11.2 | 65.5 | 43 | 81 | p<0.05 |
| RV | 150 | 39.7 | 7.9 | 39 | 24 | 60 | 28.6 | 5.4 | 28 | 18 | 42 | p<0.05 |
| RC | 150 | 17.8 | 3.3 | 17 | 11 | 26 | 9.1 | 2.9 | 9 | 5 | 16 | p<0.05 |
| RM | 150 | 22.6 | 3.7 | 23 | 14 | 30 | 14.9 | 3.8 | 14 | 8 | 26 | p<0.05 |

LV – left ventricle; RV – right ventricle; RC – renal cortex; RM – renal medulla; SHR – spontaneously hypertensive rats; N – number of high-power fields; SD – standard deviation

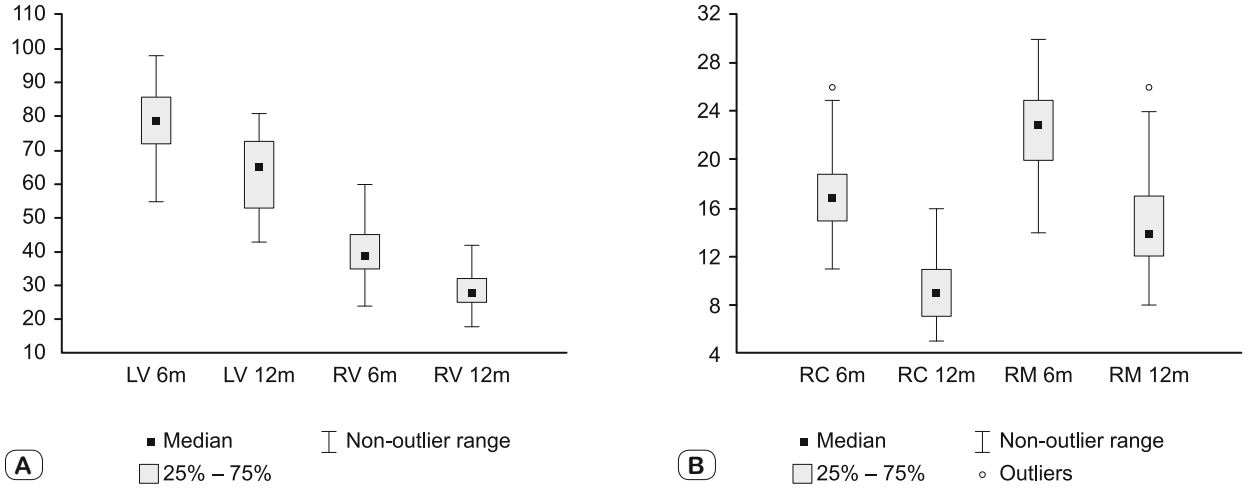


**Fig. 6. Box and whisker plots showing the median (square), surrounded by a 'box', the vertical edge of which is the interval between the lower and upper quartile [25–75 %]. 'Whiskers' originating from this 'box' represent the non-outlier range. Circles – outliers. 6m – 6-month-old spontaneously hypertensive rats (SHR); 12m – 12-month-old SHR; LV – left ventricle; RV – right ventricle; RC – renal cortex; RM – renal medulla. A. Myocardium; B. Kidney.**

increased cross-sectional area of the cardiac muscle cells). The subsequent depletion of VEGF leading to a decreased CD, together with a reduction in myocardial contractility and the progressive interstitial fibrosis would then contribute to the rapid transition to heart failure (4, 29). An earlier work by Gu et al (30) found that VEGF expression in the LV of 18-week-old (4.5-month-old) SHR was 5.05-fold higher compared to normotensive Wistar-Kyoto rats (WKY). These findings were later supported by Jesmin et al (31) in their study of WKY, SHR and stoke-prone SHR (SHRSP), who found that VEGF expression in the LV of SHR was significantly higher compared to WKY at age 6 weeks (1.5 months) before decreasing significantly up until age 40 weeks (10 months). Both studies, however, compared SHR to WKY and did not assess the changes in VEGF expression in specific periods of hypertension-induced myocardial remodelling. In the present study, we noted a decrease in VEGF expression both in the LV and RV of SHR with advanced cardiac hypertrophy compared to younger animals with early hypertension-induced changes. In addition, a positive correlation was established between VEGF expression and CD, which was significant in the LV both at 6 and 12 months of age and not significant in the RV. These findings likely suggest that a depletion of vascular adaptive mechanisms in the myocardium of both ventricles subjected to pressure overload takes place within that time frame and could be one of the key elements in the onset of subsequent heart failure.

A study by Caudron et al (32) found that CD in the hearts of SHR decreased significantly between the age of 3 and 6 months. However, the reduced CD did not correlate with a decrease in cardiac perfusion assessed on magnetic resonance, which prompted the authors to discuss that capillary rarefaction in 6-month-old SHR was of minor extent and was compensated by the effect of vasoreactivity. Furthermore, a recent study by Olianti et al (33) found no decrease in CD up until the age of 6 months in SHR. These findings contradict the earlier results of Engelmann et al (34) who reported a statistically significant decrease in CD in the LV between 1 and 6 months of age in SHR. Moreover, the same paper noted that capillary rarefaction also took place between 6 and 12 months in SHR, again being statistically significant. This trend continued from 12 until 24 months of age, however the decline was no longer statistically significant. Our results focused on the period between 6 months of age (equivalent to early established hypertension and cardiac hypertrophy) and 12 months of age (equivalent to advanced hypertension and cardiac hypertrophy). Supporting the findings of Engelmann et al (34), we reported a statistically

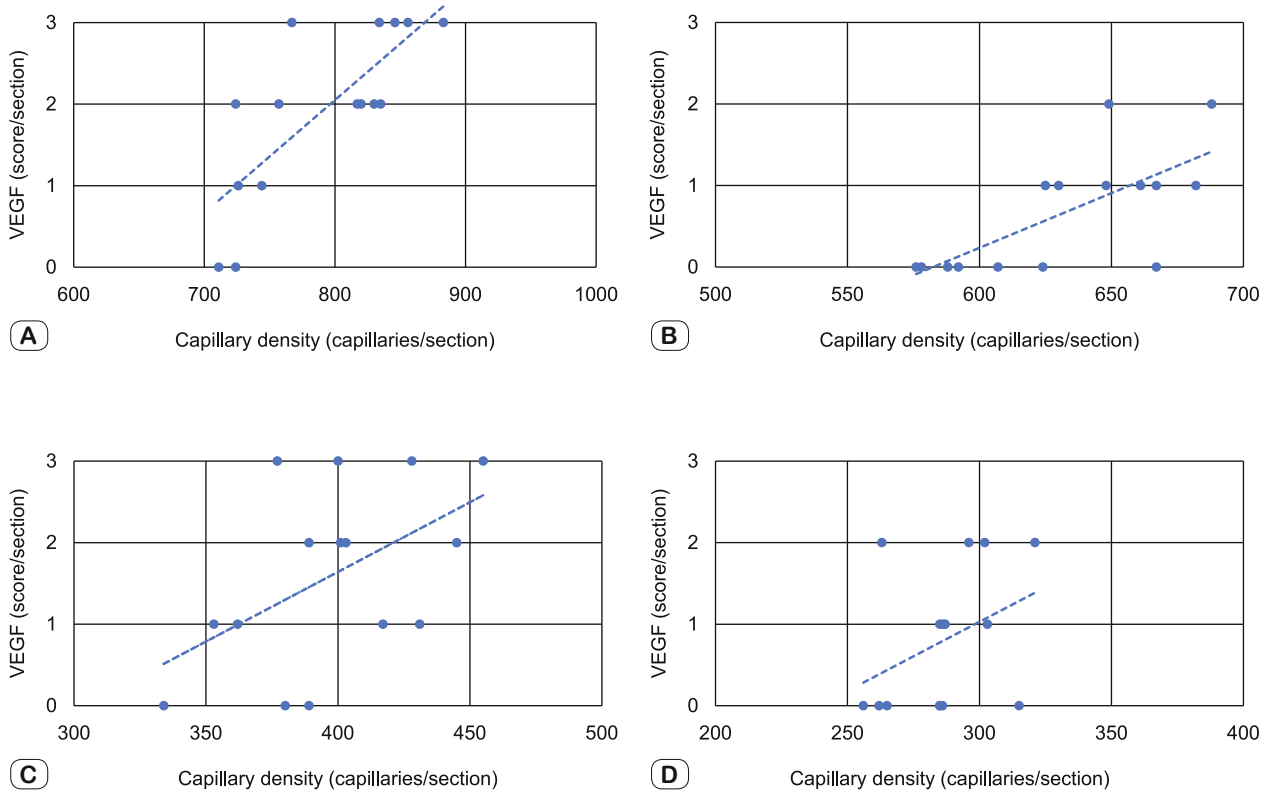


**Fig. 7. Correlation between the immunohistochemical expression of vascular endothelial growth factor (VEGF) and capillary density (CD) in the myocardium of 6- and 12-month-old spontaneously hypertensive rats (SHR). x-axis – CD expressed as total number of capillaries relative to the whole examined area on the slide; y-axis – VEGF expressed as total semi-quantitative score of the whole examined area on the slide. A. Left ventricle (LV), 6-month-old SHR ($r_s$ = 0.80338, p = 0.00031); B. Left ventricle (LV), 12-month-old SHR ($r_s$ = 0.71264, p = 0.00287); C. Right ventricle (RV), 6-month-old SHR ($r_s$ = 0.46565, p = 0.08024); D. Right ventricle (RV), 12-month-old SHR ($r_s$ = 0.44666, p = 0.0951).**

significant decrease in CD in the LV. In addition, we demonstrated a similar tendency in the RV, which represents an original finding and is a continuation of our earlier studies on changes in the RV in the context of systemic hypertension (23, 35, 36).

In addition to the above observations, Olianti et al (33) compared CD in the LV and RV of SHR whose age ranged from 1 to 6 months to age-matched normotensive animals and found it to be significantly increased (at least two-fold). The authors of the study concluded that an increase in CD was already present before the onset of hypertension, i.e. at age 1 month, suggesting an early microcirculation remodelling in SHR as a likely manifestation of a key compensatory mechanism. Their results are in conjunction with earlier reports showing a significantly increased CD in SHR as opposed to normotensive animals (30). On the other hand, these data seem to contradict the earlier report of Pu et al (37), who found a statistically significant lower CD in SHR versus age-matched normotensive WKY at age 5 months (38). These discrepancies merely underline the fact that more studies are needed, despite the support in the pertinent literature of the key role of angiogenesis and increased capillary density as an adaptive mechanism in the myocardium under hypertensive conditions. As shown by our results, however, this mechanism is depleted in advanced cardiac hypertrophy which may be one element in the pathophysiological cascade of subsequent heart failure.

The present study is one of the few reports in the literature on VEGF expression in the renal parenchyma under hypertensive conditions. Our results revealed that a decrease in VEGF immunoreactivity takes place both in the RC and RM with progression of hypertension-induced kidney injury. This process was characterised by a statistically significant reduction in CD in both structures. In addition, we have previously shown a significant increase in the glomerular sclerosis index and tubulointerstitial damage index in 12-month-old SHR compared to 6-month-old animals (24). It has been suggested that the altered expression of VEGF may serve as a trigger mechanism for the development of hypertension-induced renal damage. Advani et al (21) demonstrated an increased VEGF expression in SHR compared to normotensive controls and suggested that an upregulation of VEGF may have a potential renoprotective role under hypertensive conditions. Moreover, VEGF inhibition in SHR led to mild glomerulosclerosis and morphological changes in the podocytes characterised by the presence of protein absorption droplets. Given this protective effect of VEGF on the glomeruli, the fact that they remained negative for VEGF-A immunoreactivity in the present study was

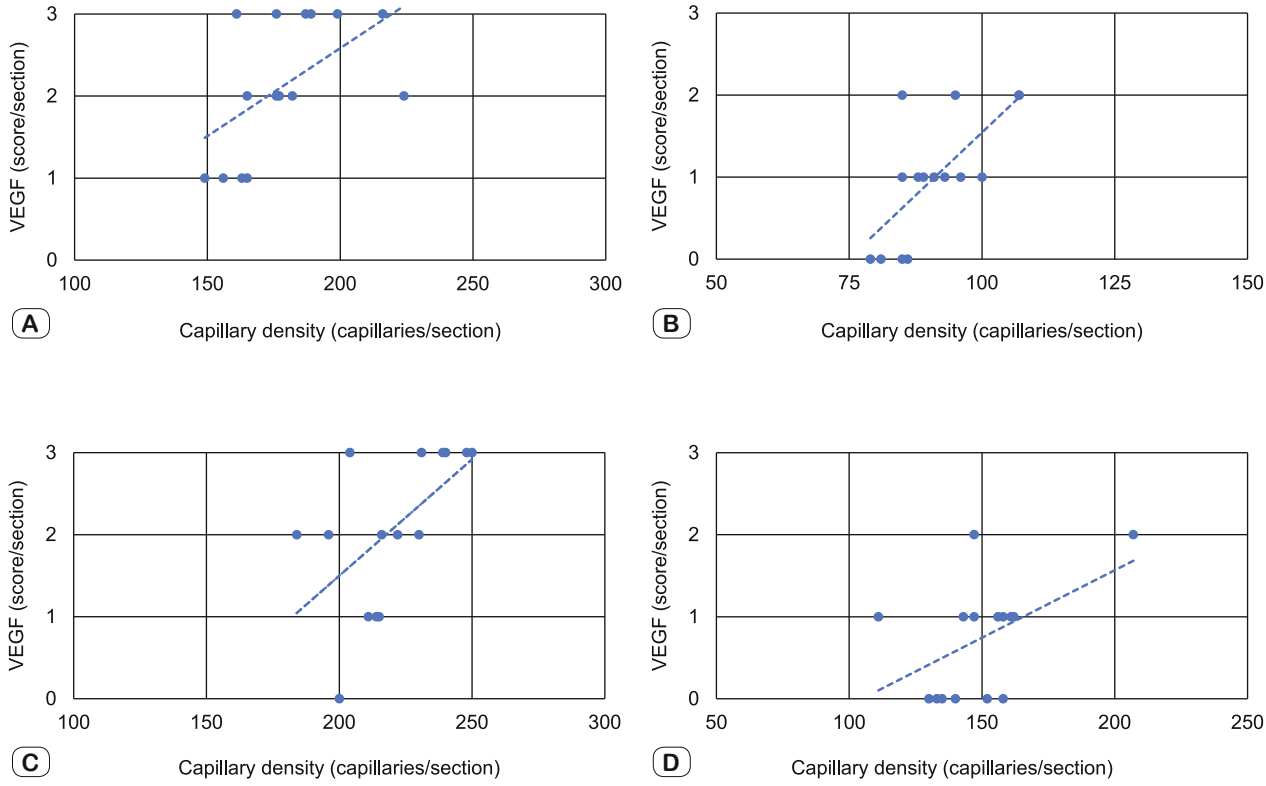


**Fig. 8. Correlation between the immunohistochemical expression of vascular endothelial growth factor (VEGF) and capillary density (CD) in the kidney of 6- and 12-month-old spontaneously hypertensive rats (SHR). x-axis – CD expressed as total number of capillaries relative to the whole examined area on the slide; y-axis – VEGF expressed as total semi-quantitative score of the whole examined area on the slide. A. Renal cortex (RC), 6-month-old SHR ($r_s$ = 0.59556, p = 0.01915); B. Renal cortex (RC), 12-month-old SHR ($r_s$ = 0.65794, p = 0.00767); C. Renal medulla (RM), 6-month-old SHR ($r_s$ = 0.64001, p = 0.01018); D. Renal medulla (RM), 12-month-old SHR ($r_s$ = 0.4579, p = 0.08609).**

somewhat perplexing. Previously, Baderca et al (39) demonstrated a negative immunohistochemical reaction in the region of the renal corpuscles in normal renal parenchyma. More recently, a complex paracrine mechanism providing the passage of VEGF produced by podocytes in opposite direction through the glomerular filtration barrier in order to bind to VEGFR-2 has been described, thus providing a plausible explanation for this phenomenon (40). Under hypoxic conditions, renal VEGF is redistributed in the inner medulla and its expression in the glomeruli diminishes (41) – results that bear certain resemblance to the findings of the present study and likely point to a common effector mechanism to different pathogenic factors.

In a recent paper, Liu et al (42) described the possible renoprotective effect of induced VEGF-A expression in SHR, namely reduced infiltration of inflammatory cells in the tubulointerstitium, as well as better preserved morphology of the glomerular filtration barrier, in particular the endothelial fenestrations and podocyte foot processes. This key role of VEGF in maintaining the renal microvasculature has been highlighted by the study of Dimke et al (43), who found that the selective deletion of VEGF in renal tubular segments was associated with decreased peritubular CD. A reduction in renal VEGF has been associated with a loss of glomerular capillaries and has been implicated in the development of glomerulosclerosis (16). As a result of nephron loss, VEGF mediates the hypertrophy of the remaining functional glomeruli – a compensatory mechanism corresponding to the initial stage of glomerulosclerosis. In time, the enlarged glomerular capillary tuft reaches its initial size due to glomerular shrinkage, before a progressive decrease in glomerular size takes place in the late stage of glomerulosclerosis. Nephron injury is closely associated with renal microvasculature changes and VEGF expression – nephron reduction was accompanied by an initial proliferation of peritubular and glomerular endothelial cells followed by a loss of peritubular and glomerular capillaries together with a reduction in VEGF expression (44). Moreover, peritubular capillary rarefaction has been implicated in the development of hypertensive nephrosclerosis and shows a positive correlation with the severity of tubulointerstitial injury (45). Additional mechanisms affecting peritubular capillary density include endothelial-tubular epithelial cell dysfunction, inflammatory cell response, as well as lack of other angiogenic growth factors (46). These results, along with the findings in the present study, underscore that VEGF and the microvasculature are among the primary targets of various aetiological factors of kidney damage.

There were several limitations to the present study which should be noted. Firstly, the expression of VEGF in the heart and kidney was assessed semi-quantitatively and a separate statistical analysis of quantified values of the strength of expression was thus not possible. Secondly, significant inter-observer variation has been noted in the visual quantification of slides processed for immunohistochemistry. In order to resolve this issue, we used an automated software, which eliminates inter-observer discrepancies in the visual assessment as previously reported (47). Thirdly, the study only included male SHR in order to avoid a possible impact of females sex hormones during the cyclical changes observed in female SHR. Fourthly, the unipapillar structure of the rat kidney, which is a distinct species-related feature, did not allow for a clear extrapolation of our findings to the human kidney, which is multipapillar. Finally, representative fields selected for analysis depended on the quality of the obtained specimens and were limited to a certain extent in the hearts and kidneys of younger animals, where parts of the histological material were damaged as part of the routine tissue processing.

## Conclusion

As hypertension-induced injury of the heart and kidney in the studied experimental model progressed, VEGF-A expression was reduced, along with a statistically significant capillary rarefaction. These changes were observed both in the LV and RV, as well as in the RC and RM. VEGF immunoreactivity was positively correlated with CD in all examined structures and was statistically significant in all but the RV at age 6 and 12 months and the RM at age 12 months. The depletion of these key vascular adaptive mechanisms observed in advanced hypertension in SHR is likely one of the major determinants of subsequent heart failure and nephrosclerosis and provides a potential target for future therapeutic interventions.

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Received September 13, 2022.
Accepted September 21, 2022.