

Myocyte/Capillary Mismatch in the Heart of Uremic Patients

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Abstract. Experiments indicate that capillary density is reduced in the hypertrophied left ventricle of rats with subtotal nephrectomy compared to control rats with similar BP and left ventricular hypertrophy, suggesting that in uremia, hypertrophying cardiomyocytes outgrow their capillary supply. No information on myocardial capillary supply in humans is currently available. The hearts of nine dialyzed patients, nine patients with essential hypertension, and 10 normotensive control subjects at postmortem were obtained. Subjects with stenosing coronary lesions and left ventricular pump failure were excluded. Special sampling procedures were used to exclude stereologic artefacts. Capillaries were specifically stained with ulex lectin and analyzed by stereologic techniques. Length density of

myocardial capillaries (L_V ; mm/mm³) was significantly ($P < 0.001$) lower in dialyzed patients (1483 ± 238) than in patients with essential hypertension (1872 ± 243) or in normotensive control patients (2898 ± 456). In parallel, myocyte diameter and volume density of myocardial interstitial tissue were significantly ($P < 0.001$) increased in uremic patients compared to patients with essential hypertension and control patients, respectively. Diminished left ventricular capillary supply in renal failure must increase critical oxygen diffusion distance in the myocardium, thus exposing cardiomyocytes to the risk of hypoxia. It is unknown whether such reduced ischemia tolerance can be reversed by increasing oxygen supply (e.g., by reversing anemia). (J Am Soc Nephrol 9: 1018–1022, 1998)

Ischemic heart disease is frequent in dialyzed patients and is a common cause of death (1,2). There is considerable evidence for a higher prevalence (3,4) and possibly more aggressive evolution (5) of coronary lesions in the uremic patient or signs of cardiac ischemia even in the absence of coronary lesions (6).

In addition, ischemia tolerance of the heart is apparently reduced in renal failure (5,7). This abnormality may have both functional and structural causes, as suggested by nuclear magnetic resonance spectroscopy in the Langendorf heart preparation (7) and by the demonstration of microvascular abnormalities in the heart of subtotally nephrectomized rats, *i.e.*, arteriolar thickening (8) and reduced capillary supply (9).

To date, these arguments are based on animal experiments and no evidence has been provided in humans. Studies in humans must consider a number of potential confounders, including a history of hypertension, the extent of left ventricular hypertrophy, the presence of coronary lesions or of pumping failure, anemia, and other factors.

To obtain quantitative information on the capillary supply in the left ventricle of dialyzed patients, we used random sampling and stereologic techniques (the orientator method) to quantify capillary density in the left ventricular wall. Capillaries were specifically decorated by the endothelial, cell-specific ulex europaeus lectin. Because cardiomyocyte fiber diameter

affects the ratio of oxygen demand and supply, we also measured this parameter.

Materials and Methods

At postmortem, all consecutive cases meeting the entry criteria that came to autopsy in the Department of Pathology Darmstadt were examined. These comprised dialysis patients ($n = 9$), hypertensive patients ($n = 9$), and nonhypertensive control patients ($n = 10$). All had died from noncardiac causes. Exclusion criteria were insulin-dependent diabetes mellitus, coronary stenoses, or left ventricular pumping failure. Among hypertensive patients, all cases with severe nephrosclerosis were excluded. Medical history was examined with respect to antihypertensive therapy, *i.e.*, the use of angiotensin-converting enzyme (ACE) inhibitors. Dialyzed patients had to be on dialysis for more than 1 yr; the duration of dialysis ranged from 1 to 18 yr. Underlying diseases were glomerulosclerosis (three patients), non-insulin-dependent diabetes mellitus (three cases), polycystic kidney disease (two patients), and renal failure of unknown etiology (one patient). In only one patient with renal failure due to chronic glomerulonephritis, hypertension was reported to be a clinical problem. Control patients were mainly patients with malignancy and liver cirrhosis.

The left ventricle (including the septum) was sectioned from the apex to the basis into 1-cm-thick transversal slices. For the first section, the distance from the apex between 0 cm and 1 cm was randomly selected to ascertain randomness of sampling. The transversal slices were positioned on a plastic sheet, on which a grid of squares (1 × 1 cm) was imprinted. The points of intersection were numbered. Using random numbers, a total of four sites was selected from the transversal slices.

Stereologic Techniques

Each sample was processed according to the orientator method (10). The orientator method generates planes of section without spa-

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Table 1. Patient data

| Characteristic | Controls (n = 10) | Essential Hypertension (n = 9) | Uremia (n = 9) | ANOVA |
|---|----------------------|-----------------------------------|--------------------------|------------------|
| Age (years) | 59.7 ± 15.8 | 75.4 ± 9.6 | 69.1 ± 5.5 | NS |
| Gender (M/F) | 5:5 | 4:5 | 3:6 | |
| Relative heart weight (g/kg) | 4.3 ± 1.1 | 5.8 ± 1.5 ^a | 8.1 ± 1.2 ^{b,c} | <i>P</i> < 0.001 |
| Relative left ventricular weight (g/kg) | 2.2 ± 0.5 | 3.0 ± 0.6 | 4.3 ± 0.9 ^{b,c} | <i>P</i> < 0.001 |
| Left ventricular wall thickness (mm) | 13.8 ± 3.5 | 18.0 ± 2.8 ^a | 16.5 ± 2.7 | <i>P</i> < 0.05 |
| Right ventricular wall thickness (mm) | 3.1 ± 1.0 | 4.6 ± 1.5 | 5.4 ± 0.5 ^a | <i>P</i> < 0.05 |

^a *P* < 0.05 versus controls.

^b *P* < 0.001 versus controls.

^c *P* < 0.01 versus essential hypertension.

Table 2. Capillarization, myocyte diameter, and cardiac interstitium in essential hypertension and uremia

| Parameter | Controls (n = 10) | Essential Hypertension (n = 9) | Uremia (n = 9) | ANOVA |
|--|----------------------|-----------------------------------|---------------------------|------------------|
| Length density of myocardial capillaries (mm/mm ³) | 2898 ± 456 | 1872 ± 243 ^a | 1483 ± 283 ^{a,b} | <i>P</i> < 0.001 |
| Myocyte diameter (μm) | 13.8 ± 1.0 | 23.7 ± 2.2 ^a | 26.1 ± 1.3 ^{a,c} | <i>P</i> < 0.001 |
| Volume density of nonvascular interstitium (%) | 14.5 ± 2.2 | 16.7 ± 2.3 | 24.0 ± 5.6 ^{a,d} | <i>P</i> < 0.001 |

^a *P* < 0.001 versus controls.

^b *P* < 0.05 versus essential hypertension.

^c *P* < 0.001 versus essential hypertension.

^d *P* < 0.01 versus essential hypertension.

cial predilection. This permits one to estimate the length of capillaries from two-dimensional histologic sections without any distortion. The specific aim of the method is to reduce variance by systematic generation of planes of section without violating the principle of randomness (10). Using the above method, four sections were taken from each of the four sampling sites.

The myocardial capillaries were visualized using ulex europaeus lectin, as described by Möller and Lennert (11). It has been validated in hearts taken at autopsy and cross-validated with factor VIII antibody (data not given). Even 20 h postmortem, the capillaries of the heart are mostly well delineated. This stain is superior to the CD-31 and factor VIII stains, because the immunohistochemical signal is stronger and more intense. Quantitative comparisons in pilot experiments showed similar capillary counts, using immunostain of factor VIII and ulex europaeus lectin stain in all groups including uremic patients (data not given), thus excluding staining artefacts secondary to an effect of uremia.

Morphometry

All morphometric measurements were performed in a blinded manner, *i.e.*, without knowledge of which group the patient was from. Capillaries were assessed using a magnification of ×400. From each section, two fields were selected using the systematic random sampling procedure (*i.e.*, 32 fields per heart).

The length of capillaries per unit volume of myocardial tissue (L_V)

was calculated according to the formula $L_V = 2 Q_A$, where Q_A is the number of capillaries per area (for details, see reference 10).

The volume fraction of myocardial interstitium (V_V) was assessed using a 100-point grid and the point-counting method (12). From each section, two fields were randomly selected at ×400 magnification.

In transverse sections of the heart muscle, the diameters of the cardiomyocytes were measured at the level of the nucleus, using a semiautomatic image analyzing system (IBAS II, Kontron, Eching, Germany) at a magnification of ×1000. For each patient, 100 cardiomyocytes were randomly selected and measured. It is noteworthy that measurements of capillary density and myocyte diameter (at the level of the nucleus) are very reproducible from one observer to another (interobserver error: 1%).

Statistical Analyses

Data are given as mean ± SD. After testing for normality, ANOVA or Kruskal-Wallis test was chosen for analysis of variance, followed by Scheffé test to determine whether differences between the groups were significant. The results were considered significant when *P* was <0.05.

Results

Heart weight and relative left ventricular weight, *i.e.*, left ventricular weight/body weight ratio, were higher in patients with renal failure or essential hypertension compared with

control subjects. Heart weight and relative left ventricular weight were slightly but significantly higher in uremic patients compared to patients with essential hypertension (Table 1). Left ventricular wall thickness was significantly higher in patients with essential hypertension than in uremic patients or control subjects, respectively (Table 1). In contrast, right ventricular wall thickness was significantly higher in uremic patients than in the other two groups (Table 1).

Myocardial capillary length density (L_v) was significantly lower in patients with essential hypertension compared to control subjects and was also significantly lower in dialyzed patients compared to patients with essential hypertension (Table 2). Representative examples of myocardial capillary density are given in Figures 1 through 3.

The diameter of cardiomyocytes was significantly higher in hypertensive patients compared to control subjects and was again significantly higher in uremic patients. By linear regression analysis, no significant correlation was found between length density of myocardial capillaries and cardiomyocyte diameter ($r = 0.02$) or left ventricular wall thickness ($r = 0.18$), respectively. In contrast, a significant correlation was found between myocyte diameter and volume density of the cardiac interstitium ($r = 0.55$, $P < 0.05$).

In dialyzed patients, the volume fraction of interstitial tissue was significantly increased compared with control subjects, but this was not the case in patients with essential hypertension (Table 2). The finding in patients with renal failure may be explained partly by edema and partly by interstitial fibrosis. These two factors were not differentiated in the present analysis. By linear regression analysis, no significant correlation was found between volume density of the cardiac interstitium and length density of myocardial capillaries ($r = -0.19$). Also, no significant correlation was found between relative left ventricular weight and myocardial capillary length density either in hypertensive ($r = 0.25$) or uremic ($r = 0.39$) patients. If all data are combined, however, a significant nonlinear relationship is noted between left ventricular weight/body weight ratio and capillary length density ($r = -0.80$). Figure 4 demonstrates the relationship between relative left ventricular weight and capillary length density in the three groups. In contrast, no significant correlation was found between age of the patients and capillary length density.

Discussion

The major finding of this study is the observation that length density of capillaries (L_v), *i.e.*, conceptually the total length of all capillaries added one to the other that are contained in one unit volume of left ventricular tissue, is significantly reduced in dialyzed patients. In such patients, capillary supply was markedly less than in the left ventricle of patients with essential hypertension, although pronounced left ventricular hypertrophy had also been present in the latter group. This finding is in agreement with the results of previous experimental studies (9) and indicates that in uremia, capillary growth does not keep pace with cardiomyocyte hypertrophy. This defect in uremia seems to be restricted to the heart, because the capillary supply

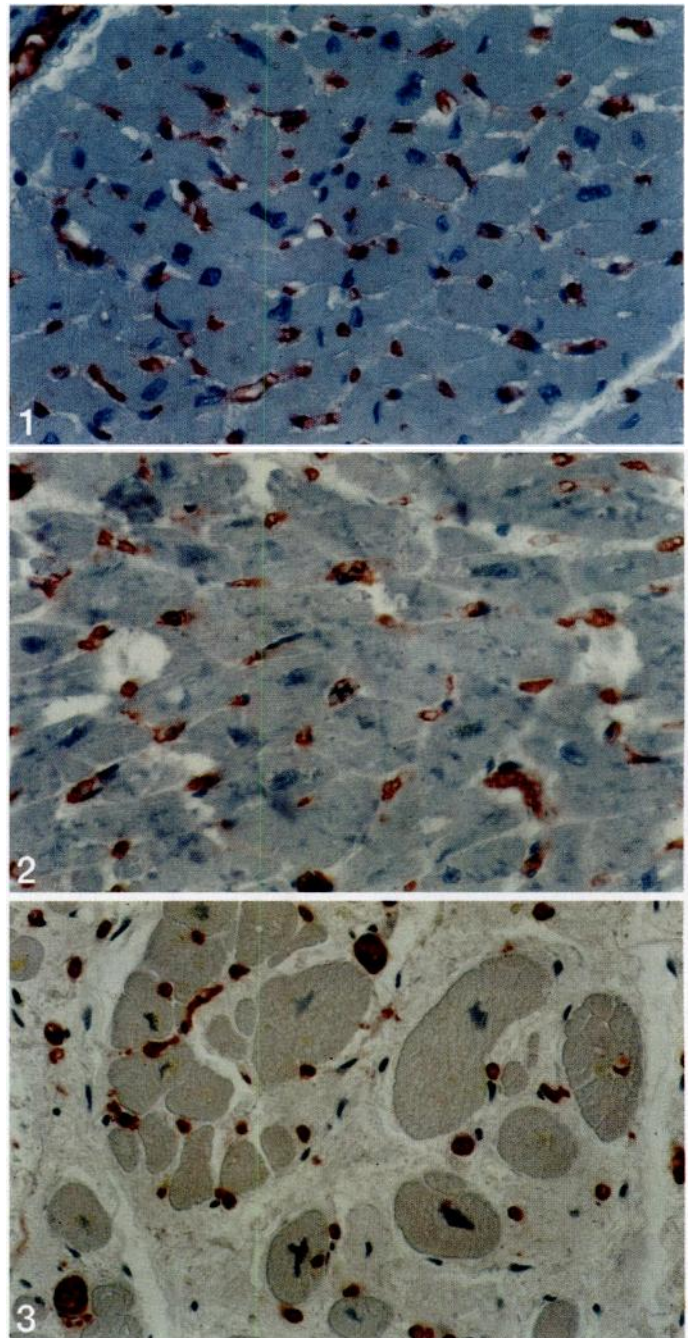


Figure 1. Myocardium of a control patient. Compare size of cardiomyocytes and number of vessels per area myocardium (red) with Figures 2 and 3. Paraffin section, immunohistochemistry using ulex europaeus. Magnification, $\times 200$.

Figure 2. Myocardium of a patient with essential hypertension. Myocyte cross-sectional area is increased, and the number of capillaries per area myocardium is decreased. Interstitial tissue is only slightly increased. Paraffin section, immunohistochemistry using ulex europaeus. Magnification, $\times 200$.

Figure 3. Myocardium of a patient with chronic renal failure. Myocyte cross-sectional area is more markedly increased than in essential hypertension, whereas the number of capillaries is lower. Note the bizarre shape of several myocyte nuclei and the pronounced expansion of interstitial space. Paraffin section, immunohistochemistry using ulex europaeus. Magnification, $\times 200$.

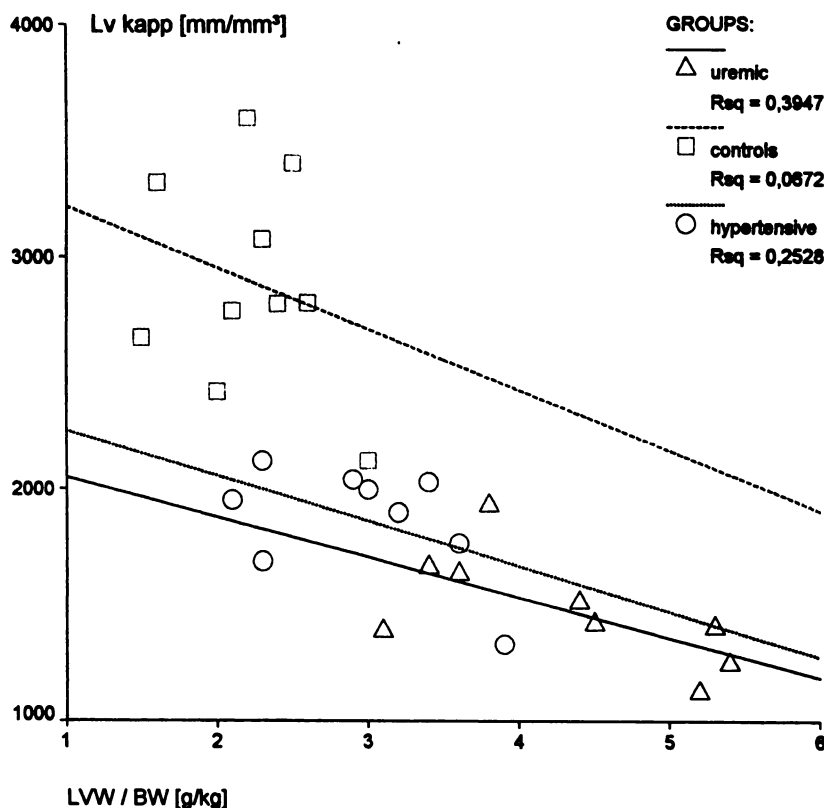


Figure 4. Relationship between relative left ventricular weight/body weight (LVW/BW) and capillary length density (L_v) in all three groups by linear regression.

of other organs was not altered when examined using similar stereologic techniques (13).

Before discussing the potential functional implication of this finding, we took care to exclude a number of potential artefacts. Patients with severely stenosing coronary lesions or left ventricular pumping failure were excluded, and qualitative microscopy failed to reveal capillary closure or other pathologies, *e.g.*, gross calcium deposits, β_2 -microglobulin amyloid deposits, etc. Artefacts from anisotropy were avoided by the sampling procedure, *i.e.*, the orientator method (10), and failure of endothelial cells to stain with *ulex europaeus* was excluded by appropriate pilot experiments.

Selection of the control group is crucial for the interpretation of data in uremic patients. We selected individuals with a long-standing history of essential hypertension without evidence of coronary or renal disease. They were largely comparable to the uremic patients with respect to age and gender, but we acknowledge that left ventricular weight was somewhat higher in uremic patients and that we did not have complete data on BP or antihypertensive medication, respectively. We verified, however, that none of the individuals had received ACE inhibitor treatment. Because of the minor age differences between the groups, we emphasize that no significant relation of capillary length density to age was noted.

We acknowledge that cardiac weight might be misleading in the uremic patient with potential interstitial edema. We found no significant correlation between left ventricular weight/body weight ratio and capillary length density in uremic patients.

The failure to confirm a relation to the degree of hypertrophy must be interpreted with due consideration of the β error. We emphasize, however, that no significant correlation was found between cardiomyocyte diameter or left ventricular wall thickness, respectively, and capillary length density within and between the groups. We stress that the cardiomyocyte/capillary interface is the biologically relevant parameter for oxygen exchange.

We confirmed our previous observation (14,15) that uremia is associated with more severe expansion of the interstitial space. We cannot definitely exclude that increased interstitial matrix deposition and diminished capillary supply are interdependent, but no statistically significant correlation was noted between the two parameters. The latter finding provides some evidence that lower capillary density in patients on dialysis is not explained simply by more marked expansion of the nonvascular interstitium. In our study, the volumetric density of the nonvascular interstitial tissue in uremia (25%) is comparable with what has been reported in patients with severe aortic valve disease (16). The observation of a specific reduction of capillary supply, at least in part independent of cardiomyocyte volume and interstitial fibrosis, is in line with animal studies (9,13) that document rarefaction of capillaries despite only minor cardiomyocyte hypertrophy and interstitial volume expansion.

As for the mechanisms that led to reduced capillary supply, no definite conclusions can be drawn from a postmortem study. On the basis of animal experiments, we postulate a role for: (1)

angiotensin II (AngII) and/or bradykinin, because in uremic animals near-normal capillary density is seen after ACE inhibitor treatment (17); (2) sympathetic overactivity, because normal capillary supply was seen after treatment with a central sympatholytic agent (17); and (3) endothelin, because capillary density was also normal after administration of endothelin receptor blockers (18). Because erythropoietin stimulates endothelial cells and is thus a potentially angiogenic factor (19), one must also consider the possibility that lack of erythropoietin contributes to inadequate capillary growth in chronic renal failure. In view of our previous work (20,21), a possible role for parathyroid hormone (PTH) must also be considered, because secondary hyperparathyroidism is nearly universally present in patients with established uremia. One might consider an interaction between PTH and AngII, *e.g.*, downregulation of PTH receptors in the heart allowing unopposed trophic effects of AngII, but this is purely speculative.

Reduction in capillary supply must have functional consequences. If the distance between the center of the cardiomyocyte and the adjacent capillary is increased, the critical oxygen diffusion distance increases in parallel, exposing the cardiomyocyte to the risk of hypoxia under conditions of anemia and low blood flow. The consequences of reduced capillary supply for myocardial perfusion may be aggravated by upstream arteriolar lesions, as seen in this study (data not quantified) and well documented in experimental studies (5,8). Future research is warranted to determine whether the propensity of the myocardium to hypoxia secondary to diminished capillary density can be reversed by increasing oxygen supply, *e.g.*, by recombinant human erythropoietin treatment.

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