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# Effect of erythropoietin on cardiovascular prognosis parameters in hemodialysis patients

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**Effect of erythropoietin on cardiovascular prognosis parameters in hemodialysis patients.**

*Background.* Renal anemia is an important determinant for left ventricular hypertrophy in dialysis patients and an independent prognosis parameter for the cardiovascular survival in dialysis patients. In addition, an autonomic dysfunction is associated with the uremic state and influences the cardiovascular risk in patients with end-stage renal disease (ESRD).

*Methods.* We investigated in this prospective longitudinal study the effect of hemoglobin normalization by a chronic treatment with recombinant human erythropoietin (rhEPO) on cardiovascular prognosis parameters in 23 patients on chronic hemodialysis with renal anemia (hemoglobin concentration ≤10.5 g/dL) and echocardiographically proven left ventricular hypertrophy. We studied muscle sympathetic nerve activity measured by microneurography; cardiopulmonary baroreflex activity by lower-body negative pressure (LBNP−) testing; left ventricular structure and mass index (LVMI) by echocardiography; blood pressure by 24-hour readings; peripheral blood flow and vascular resistance by plethysmography before (U1) and after 7 months of chronic rhEPO treatment (U2).

*Results.* In the anemic state, mean (± SD) muscle sympathetic nerve activity in ESRD was elevated (U1 rest, 34 ± 13 bursts per minute) and cardiopulmonary baroreflex response during LBNP markedly lacking (U1 –15 mm Hg, 34 ± 13 bursts per minute) reflecting a severely impaired autonomic function. Normalization of the hemoglobin concentration by chronic rhEPO treatment (U1, 10.5 ± 0.9 g/dL versus U2, 13.4 ± 3.1 g/dL, $P < 0.001$) did not influence sympathetic nerve activity (U2, 34 ± 15 bursts per minute, NS) and cardiopulmonary baroreflex sensitivity did not change (U2 –15 mm Hg, 37 ± 16 bursts per minute, NS). LVMI decreased significantly after chronic treatment with rhEPO (U1, 134 ± 26 g/m$^2$ versus U2, 97 ± 25 g/m$^2$, $P < 0.001$) and left ventricular geometry developed from an asymmetric to a symmetric configuration (U1, relative wall thickness 0.58 versus U2, 0.43, $P < 0.001$). Under treatment with rhEPO, 24-hour systolic and diastolic blood pressure did not increase (systolic U1, 132 ± 4 mm Hg versus U2, 128 ± 3 mm Hg, NS, and diastolic U1, 76 ± 2 mm Hg versus U2, 73 ± 2 mm Hg, NS). Peripheral blood flow (U1, 6.1 ± 3.3 mL/100 mL/min versus U2, 6.2 ± 0.6 mL/100 mL/min, NS) as well as forearm vascular resistance (U1, 15.7 ± 3.3 mm Hg/mL/100 mL versus U2, 14.9 ± 3.1 mm Hg/mL/100 mL, NS) did not change by chronic rhEPO treatment.

*Conclusion.* Normalization of hemoglobin by chronic rhEPO treatment in dialysis patients has beneficial cardiovascular effects with regression of left ventricular hypertrophy and improvement of left ventricular geometry. However, a reduction of sympathetic overactivity or a resetting of baroreceptor sensitivity by a rhEPO treatment in dialysis patients in the medium-term could not be demonstrated. The reason for this may be the complex and multifactorial pathomechanism of autonomic dysfunction and cardiovascular disease in ESRD.

The enormous cardiovascular morbidity and premature mortality in patients with end-stage renal disease (ESRD) is due to several risk factors which are associated with the uremic state [1–3]. Of those, an increased sympathetic nerve activity has been recognized as an important pathomechanism causing cardiovascular complications in dialysis patients [3]. A sympathetic overactivity is an important pathogenetic factor for blood pressure elevation in renal insufficiency and for left ventricular hypertrophy (LVH) which is itself an independent determinant of survival in dialysis patients [4–6]. There is consistent evidence that a high sympathetic tone predicts mortality in dialysis patients [3]. Physiologically, an important contributor to the control of the sympathetic outflow is the cardiopulmonary baroreflex [7]. Yet, the effects of the autonomic dysfunction in the state of chronic renal failure on the cardiopulmonary baroreflex control are not clear.

It has been suggested that renal anemia stimulates sympathetic nerve activity due to peripheral hypoxia [8, 9]. Correction of renal anemia by the use of recombinant human erythropoetin (rhEPO) has been proven to be an effective treatment with several beneficial effects on exercise capacity, reduction of left ventricular mass and improvement of cardiac function [10–12]. Retrospective studies showed a risk reduction of the cardiovascular mortality by a long-term anemia correction with rhEPO

**Key words:** renal insufficiency, anemia, erythropoietin, sympathetic nerve activity.

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[13, 14]. However, beside these beneficial effects, potential risk factors of a rhEPO treatment have been reported. In the Normal Haematocrit Trial, Besarab et al [15] studied the effect of a normal target hematocrit of 42% in dialysis patients with high cardiac risk (i.e., patients with ischemic heart disease or congestive heart failure) compared to a target hematocrit of 30%. Based on the interim results with even a borderline higher mortality in the normal hematocrit group this study has been aborted prematurely [15]. Therefore, despite of various favourable effects of a treatment with rhEPO, aiming for a normal hematocrit level is still a controversial issue in the management of anemia in renal failure. Beyond this, the development or deterioration of arterial hypertension is a noted complication of the substitution of rhEPO [16]. The mechanisms of rhEPO to increase blood pressure are not completely understood. Hemodynamic effects with an increased blood viscositiy may lead to an augmentation of the total peripheral resistance [17–19]. Whether these hemodynamic changes during rhEPO therapy are substantiated in an increased sympathetic activation, which is an important factor for the cardiovascular prognosis, is still to be determined.

Therefore, the objectives of our study were to prove the effects of a long-term treatment with rhEPO on important cardiovascular prognosis parameters in dialysis patients. We investigated the sympathetic nerve activity (measured directly by microneurography) and the autonomic regulation by experimental unloading of cardiopulmonary baroreceptors before and after chronic rhEPO substitution. Our other objective was to examine left ventricular structure and hemodynamic effects of a chronic rhEPO treatment on blood pressure and peripheral blood flow in dialysis patients.

## METHODS

### Study subjects

Twenty-three patients with ESRD were consecutively enrolled in this prospective longitudinal study. In a screening phase, a total of 252 patients undergoing hemodialysis at 3 centers were screened for inclusion and exclusion criteria. Inclusion criteria were the presence of a renal anemia, defined as a blood hemoglobin concentration before a dialysis session of $\leq$10.5 g/dL and a LVH, assumed by an echocardiographically proved thickening of interventricular septum (IVS) and left ventricular posterior wall (PW) >12 mm, respectively. Iron deficiency and other reasons for anemia had to be excluded. Further exclusion criteria were the presence of a manifest peripheral polyneuropathy, occlusive arterial disease, a history of a malignant tumor, or a thrombosis. Twenty of the 23 patients were already receiving rhEPO before enrollment. All patients gave written informed consent. The study protocol was approved by the Ethical committee of the University of Erlangen-Nuremberg.

### Study protocol

After enrollment, substitution of rhEPO was stopped for at least 14 days in all patients. After this EPO-free interval, baseline examinations of the study parameters were performed (U1). The following parameters were determined at U1: sympathetic nerve activity (SNA) analyzed by microneurography at rest and during cardiopulmonary baroreflex testing, echocardiographic measurements, systolic and diastolic blood pressure by 24-hour ambulatory blood pressure readings, peripheral blood flow by venous occlusion plethysmography, and laboratory tests (EPO and noradrenaline plasma levels). The patients were studied on a day between two dialysis sessions. One day before U1, predialysis concentration of hemoglobin and hematocrit were measured at the patient's dialysis center. Subsequently, all patients received doses of EPO beta (rhEPO) to achieve and maintain hemoglobin concentrations of $\geq$12.5 g/dL over a time period of at least 5 months. Fourteen patients received rhEPO intravenously, in nine patients rhEPO was administered subcutanously. Application frequency was three times a week in all patients. According to the European Best Practice Guidelines for the management of anemia in patients with chronic renal failure [20], the initial dose of rhEPO was determined as 50 to 150 U/kg per week. Doses of rhEPO were augmented by 25% of the baseline dose if the hemoglobin concentration had not increased by at least 0.7 g/dL during a treatment period of 2 weeks. If the hemoglobin concentration increased by more than 2.5 g/dL in a 4-week period under treatment, the dose of rhEPO was reduced by 25%. Iron status (including serum ferritin concentration and serum transferrin saturation) was monitored according to [20] and iron substituted if indicated. After achieving the target hemoglobin of $\leq$12.5g/dL, tests of the hemoglobin concentration and hematocrit were performed every 2 weeks and doses of rhEPO modificated to maintain the target hemoglobin concentration. After a time period of at least 5 months with maintained correction of anemia by rhEPO, measurements of all study parameters were repeated (U2). One day before U2, predialysis concentration of hemoglobin and hematocrit were performed at the patient's dialysis center. During the whole study period, antihypertensive medication was retained and adjusted according to clinical indication. Dialysis dose remained unchanged in all subjects during the study period. Thrombotic complications as arteriovenous shunt thrombosis or embolism did not occur.

### Recordings of SNA

Multiunit recordings of postganglionic SNA were obtained by unipolar tungsten microelectrodes inserted selectively into muscle-nerve fascicles of the peroneal nerve posterior to the fibular head by the microneurographic

technique of Vallbo et al [21]. The electrodes were connected to a preamplifier, and the nerve signal was fed through a bandpass filter and routed through an amplitude discriminator to a storage oscilloscope and loudspeaker and recorded by a polygraph. The recording of sympathetic activity was considered acceptable when the neurogram revealed spontaneous, pulse-synchronous bursts of neural activity, with the largest bursts showing a minimal signal-to-noise ratio of 3:1. In each study we confirmed that sympathetic outflow to skeletal muscle rather than sympathetic discharge to skin is recorded by demonstrating that the neural activity did not change in response to arousal stimuli or a pinch of the skin but showed a characteristically biphasic response to the Valsalva maneuver [22]. For analysis, sympathetic bursts were identified by inspection of the filtered and mean-voltage neurograms. The rate of sympathetic nerve discharge was expressed as the number of bursts per minute (burst frequency). SNA was also corrected for the heart rate and expressed as bursts per 100 heartbeats (burst incidence) [23]. All nerve recordings were analyzed by two independent investigators who were unaware of the study protocol. The data on the SNA given in this study represent the mean for the two observers. All patients were studied between 1:00 p.m. and 5:00 p.m. without sedation on a day between two dialysis sessions. The studies were performed with the patient supine in a calm room with a temperature of 23 to 25°C.

### Testing of cardiopulmonary baroreflex function by the lower body negative pressure test

Unloading of cardiopulmonary baroreceptors by reduction of left ventricular preload leads to an increased sympathetic efferent outflow to the effector organs heart, kidneys, and resistance vessels [24]. To assess the autonomic neural response, a cardiopulmonary baroreflex test by using the lower body negative pressure (LBNP−) test was performed [25]. At this, each subject was submitted on a negative pressure device consisting of an airtight box in which the lower half of the body, down from the iliac crests, was enclosed [24]. The subjects remained in a supine position during the whole study period. The vacuum in the box was achieved by the use of a standard vacuum cleaner motor. The level of the vacuum was continuously measured by a standard mercury manometer connected to the inside of the box. Each subject was submitted to a level of –5 mm Hg for 2 minutes with a stepwise enhancement of the lower body negative pressure by further –5 mm Hg subsequently every 2 minutes. The target level of LBNP amounted to –15 mm Hg. Each LBNP− level was achieved within 2 seconds. SNA was measured by microneurography under steady-state conditions. Symptoms as syncope or vertigo did not occur.

### Echocardiographic studies

Two-dimensional echocardiography was performed with an ultrasonoscope (Picker-Hitachi CS 192) with a 3.5 MHz probe. Echocardiograms were recorded at rest in the third or fourth intercostal space lateral to the left sternal border with the patient recumbent in the supine or half-sided position. Left ventricular chamber recording was obtained at the tip of the mitral valve leaflet. To characterize left ventricular structure, measurements of IVS, posterior wall thickness (PWT), and left ventricular internal end systolic diameter (ESD) as well as end diastolic (EDD) diameter were performed according to the recommendations of the American Society of Echocardiography (ASE) using the leading edge to leading edge convention [26]. All parameters were registered over three cardiac cycles by two independent investigators and subsequently mean values were calculated. Left ventricular mass index (LVMI) was calculated conformable to the standard formula of Troy, Pombo, and Rackley [27], which takes both septal and PWT into account. To avoid overestimation of the "true" left ventricular mass, the values based on the ASE convention were corrected by the anatomically applying regression equations of Devereux and Reichek [28]: Left ventricular mass = 0.8 (ASE cube left ventricular mass) + 0.6 g (PENN convention). Relative wall thickness (RWT), a measure of left ventricular geometry, was calculated as: RWT = (2 PWT/EDD). Concentric hypertrophy was assumed when RWT >0.45 in the presence of LVH and eccentric hypertrophy as RWT <0.45 in the presence of LVH, respectively [29].

### Laboratory analysis

Blood samples for EPO and noradrenalin plasma levels were withdrawn into cooled tubes [containing ethylenediaminetetraacetic acid (EDTA) for noradrenaline], immediately centrifuged at 0°C and stored at –21°C (EPO) or –70°C (noradrenaline), respectively, and analyzed within 3 months of storage. Measurements of EPO plasma levels were performed at the laboratory of the University of Erlangen-Nuremberg using an enzyme-linked immunoassay (EPO-Immulite) (Diagnostic Products Corporation, Biermann GmbH, Germany). Plasma noradrenaline levels were measured by high-performance liquid chromatography (HPLC) with electrochemical detection (ClinRep) (Recipe Chemicals and Instruments GmbH, München, Germany) [30].

### Hemodynamic parameters

Twenty-four–hour blood pressure measurements were achieved by using an automated portable device (Spacelabs Medical, Redmond, WA, USA). The measurements were carried out every 20 minutes during day time and

every 30 minutes during night time. Beat-to-beat arterial pressure was taken noninvasively by the validated method of a photoplethysmographic finger device (Finapres) (Ohmeda, Englewood, CO, USA) [31]. Heart rate was counted from the electrocardiogram. Forearm blood flow was measured by the technique of venous occlusion plethysmography with a calibratable mercury-in-Slastic strain gauge [32]. Measurements were made in supine position at the forearm contralateral to the arteriovenous fistula of the dialysis patient. The forearm was positioned at the heart level in 60° adduction. A pneumatic cuff was placed around the upper arm for venous occlusion. During the phases of venous occlusion the hand was excluded from circulation by inflating a wrist cuff to suprasystolic pressure. Forearm vascular resistance was calculated from arterial blood pressure (measured by Finapres) and forearm blood flow [33].

### Statistical analysis

All results are presented as means ± SD. Paired *t* tests (two-tailed) were used for the comparisons of study parameters before and after correction of renal anemia in dialysis patients. All data were analyzed by using the SPSS/PC version of the statistics package for social sciences [34]. Statistical significance was indicated by a *P* value of less than 0.05.

## RESULTS

Patients characteristics are presented in Table 1. The duration of the study period was 7.9 ± 2.6 months. Causes for the ESRD were as follows: clinical suspected or biopsy proven chronic glomerulonephritis (ten patients), hypertensive nephrosclerosis (five patients), diabetic glomerulopathy (four patients), interstitial nephritis (two patients), polycystic kidney disease (one patient), and Alport syndrome (one patient). Patients' concomitant antihypertensive treatment at the beginning of the study without rhEPO (U1) and after correction of anemia under chronic substitution of rhEPO (U2) is shown in Table 2, separately for different antihypertensive drug classes. Serum creatinine concentrations did not change significantly (U1 versus U2) (Table 3) and extracellular fluid volume remained normal in all 23 hemodialysis patients.

During therapy with rhEPO, the predialytic hemoglobin concentration increased significantly from 10.5 ± 0.9 g/dL (U1) to 13.4 ± 3.1 g/dL (U2) ($P < 0.001$) (Table 3). As well, the hematocrit values increased from 31.4 ± 3.1% (U1) to 40.0 ± 2.6% (U2) ($P < 0.001$) (Table 3). Serum levels of EPO increased under substitution of rhEPO significantly from 9.9 ± 6.3 U/L (U1) to 29.9 ± 23.0 U/L (U2) ($P < 0.05$).

**Table 1.** Patient characteristics[a]

| | |
|---|---|
| Age *years* | 52.3 ± 14.7 |
| Gender *male/female* | 17/6 |
| Body mass index $kg/m^2$ | 24.6 ± 3.8 |
| Smoker/non-smoker | 8/15 |
| Duration of end-stage renal disease *months* | 42.6 ± 19.8 |
| Dialysis dose *hours/week* | 12.9 ± 0.4 |
| Dose of erythropoietin before the study (20 patients) *U/week* | 3600 ± 650 |
| Dose of erythropoietin during the study *U/week* | 6474 ± 2294 |
| Duration of recombinant human erythropoietin treatment *months* | 7.9 ± 2.6 |

[a]Plus-minus values are mean ± SD.

**Table 2.** Concomitant antihypertensive medication before (U1) and after (U2) correction of renal anemia

| | U1 *number* | *U2 number* |
|---|---|---|
| β blocker | 14 | 13 |
| Angiotensin-converting enzyme inhibitor | 14 | 14 |
| Angiotensin II type 1 receptor antagonist | 5 | 4 |
| Central sympatholytic agent | 6 | 6 |
| Calcium channel blocker | 11 | 12 |
| Diuretics | 10 | 9 |

Abbreviations are: U1, renal anemia, no therapy of recombinant human erythropoietin (rhEPO); U2, renal anemia corrected under chronic substitution of rhEPO.

**Table 3.** Mean systolic and diastolic blood pressure (determined by 24-hour ambulatory readings), heart rate, peripheral blood flow, serum creatinine, and noradrenaline plasma levels before (U1) and after (U2) correction of renal anemia

| | U1 | U2 | *P* value |
|---|---|---|---|
| Mean systolic blood pressure *mm Hg* | 132 ± 4 | 128 ± 3 | NS |
| Mean diastolic blood pressure *mm Hg* | 76 ± 2 | 73 ± 2 | NS |
| Heart rate *beats/min* | 69 ± 11 | 66 ± 7 | NS |
| Peripheral blood flow *mL/100 mL/min* | 6.1 ± 0.7 | 6.2 ± 0.6 | NS |
| Forearm vascular resistance *mm Hg/mL/100 mL* | 15.7 ± 3.3 | 14.9 ± 3.1 | NS |
| Hemoglobin concentration *g/dL* | 10.5 ± 0.9 | 13.4 ± 3.1 | $P < 0.001$ |
| Hematocrit% | 31.4 ± 3.1 | 40.0 ± 2.6 | $P < 0.001$ |
| Serum erythropoietin *U/L* | 9.9 ± 6.3 | 29.9 ± 23 | $P < 0.5$ |
| Serum creatinine *mg/dL* | 9.2 ± 3.4 | 10.5 ± 2.8 | NS |
| Serum noradrenaline (70 to 750) *pg/mL* | 724.6 ± 140.2 | 699.3 ± 170.1 | NS |

Abbreviations are: U1, renal anemia, no therapy of recombinant human erythropoietin (rhEPO); U2, renal anemia corrected under chronic substitution of rhEPO.

### Effect of anemia correction with rhEPO on SNA

At baseline (U1), muscle sympathetic nerve activity (MSNA) frequency was 34 ± 4 bursts per minute and MSNA incidence 48 ± 10 bursts per 100 heartbeats. After correction of renal anemia by chronic substitution of rhEPO, the rate of sympathetic discharge did not change significantly (U2, MSNA frequency 34 ± 4 bursts per

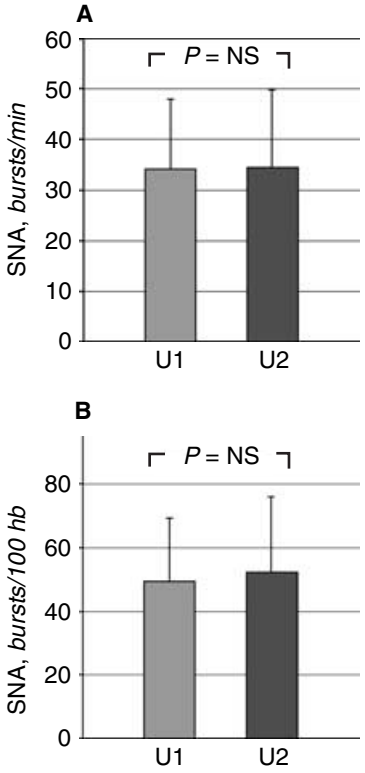


**Fig. 1. Effect of correction of anemia with chronic recombinant human erythropoietin (rhEPO) substitution on sympathetic nerve activity (SNA).** (*A*) Burst frequency. (*B*) Burst incidence. Abbreviations are: U1, renal anemia, no therapy of rhEPO; U2, renal anemia corrected under chronic substitution of rhEPO.

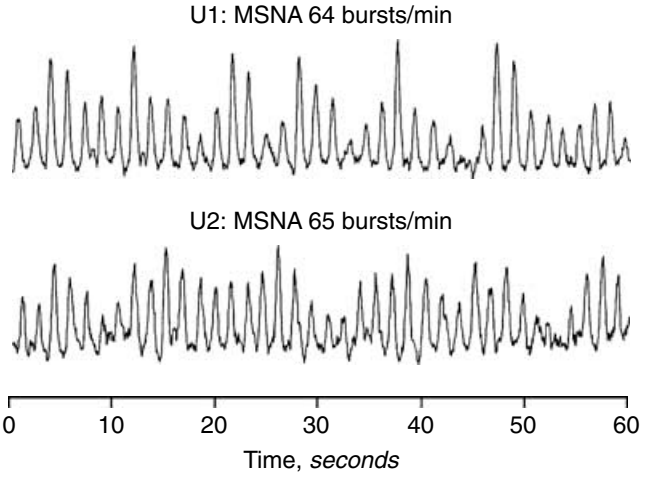


**Fig. 2. Representative single microneurographic recording of the muscle sympathetic nerve activity (MSNA) of a dialysis patient before and after correction of renal anemia by recombinant human erythropoietin (rhEPO).** Abbreviations are: U1, renal anemia, no therapy of rhEPO; U2, renal anemia corrected under chronic substitution of rhEPO.

minute; MSNA incidence 51 ± 11, NS) (Fig. 1). A representative single microneurographic recording of the efferent SNA of a hemodialysis patient before and after correction of renal anemia is shown in Figure 2. As well, plasma noradrenaline levels did not change before and after treatment with rhEPO (724.6 ± 140.2 pg/mL (U1) versus 699.3 ± 170.1 pg/mL (U2), NS) (Table 3).

## Effect of anemia correction with rhEPO on cardiopulmonary baroreflex control

We compared efferent sympathetic nerve activity during the unloading of cardiopulmonary baroreceptors using the LBNP– test at the phase of renal anemia (U1) and after chronic rhEPO treatment (U2). In comparison with normal subjects [26], the response of the SNA discharge during the stepwise increased levels of lower body negative pressure was found to be reduced in dialysis patients in the stage of renal anemia (U1 rest, 34 ± 13, NS; U1 LBNP– level, –5 mm Hg, 34 ± 15, NS; U1 LBNP– level, –10 mm Hg, 36 ± 17, NS; and U1 LBNP– level, –15 mm Hg, 34 ± 13, NS, bursts per minute, respectively) (Table 4). Normalization of the hemoglobin concentration by chronic rhEPO treatment did not influence the cardiopulmonary baroreflex response during the LBNP– test (U2 rest, 34 ± 15; U2 LBNP– level, –5 mm Hg, 35 ± 15; U2 LBNP– level, –10 mm Hg, 36 ± 17; and U1 LBNP– level, –15 mm Hg, 37 ± 16, bursts per minute, respectively, *P* not significant for all comparisons at different levels) (Table 4).

**Table 4.** Effect of anemia correction by recombinant human erythropoietin (rhEPO) treatment on the response of the sympathetic nerve activity (SNA) during cardiopulmonary baroreflex testing in dialysis patients

| | Rest | –5 mm Hg | LBNP– level –10 mm Hg | –15 mm Hg | *P* value |
|---|---|---|---|---|---|
| SNA U1 bursts/min | 34 ± 13 | 34 ± 15 | 36 ± 17 | 34 ± 13 | NS |
| SNA U2 bursts/min | 34 ± 15 | 35 ± 15 | 36 ± 17 | 37 ± 16 | NS |
| Hear rate U1 beats/min | 69 ± 11 | 70 ± 8 | 70 ± 9 | 69 ± 10 | NS |
| Heart rate U2 beats/min | 67 ± 7 | 68 ± 8 | 68 ± 7 | 66 ± 8 | NS |

Abbreviations are: LBNP–, lower-body negative pressure; U1, renal anemia, no therapy of rhEPO; U2, renal anemia corrected under chronic substitution of rhEPO.

## Effect of anemia correction with rhEPO on cardiac structure

At baseline, a LVH (U1 IVS 15.4 ± 2.8 mm; PW 14.1 ± 2.5 mm) as well as an enhanced LVMI (U1 LVMI 134 ± 26 g/m$^2$) was present in all dialysis patients. During treatment with rhEPO, left ventricular septal and PW thickness decreased significantly (U2 IVS 11.9 ± 2.8 mm, $P < 0.001$; PW 11.1 ± 1.6 mm, $P < 0.001$) (Fig. 3). As well, LVMI was reduced significantly after correction of anemia (U2 97 ± 25 g/m$^2$, $P < 0.001$) (Fig. 4). Left ventricular ESD and EDD were not enlarged at the beginning of the

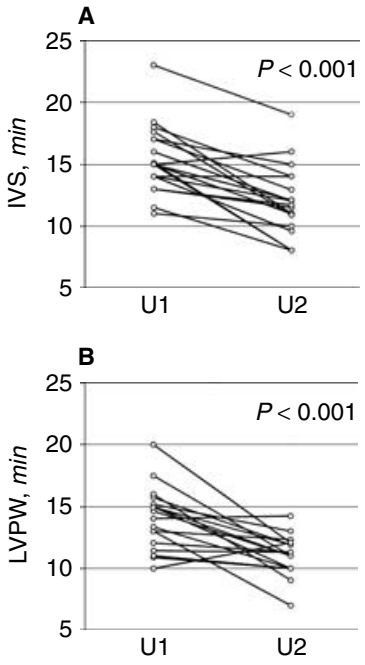


**Fig. 3. Effect of anemia correction by chronic recombinant human erythropoietin (rhEPO) treatment on left ventricular septal (IVS) and posterior wall thickness (LVPW).** Abbreviations are: U1, renal anemia, no therapy of rh-Epo; U2, renal anemia corrected under chronic substitution of rh-Epo.

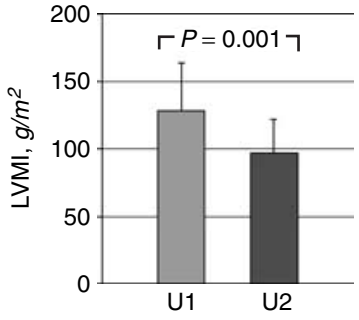


**Fig. 4. Effect of anemia correction by chronic recombinant human erythropoietin (rhEPO) treatment on left ventricular mass index (LVMI).** Abbreviations are: U1, renal anemia, no therapy of rhEPO; U2, renal anemia corrected under chronic substitution of rhEPO.

study and remained unchanged during the study course (U1 ESD 35.2 ± 11 mm versus U2 ESD 33.8 ± 5 mm, NS; U1 EDD 50.1 ± 5.6 mm versus U2 EDD 51.2 ± 4.9 mm, NS). The RWT, a parameter for the left ventricular geometry, decreased under chronic treatment with rhEPO significantly (U1 0.58 versus U2 0.43, $P < 0.001$) indicating a remodelling of the eccentric LVH into a symmetric configuration.

### Effect of anemia correction with rhEPO on hemodynamic parameters

Twenty-four–hour systolic and diastolic blood pressure were normotensive at U1 and did not change after correction of renal anemia by rhEPO treatment (U2) (Table 3). At this, the number and dose of the antihypertensive drugs did not differ significantly during the study course (Table 2). Heart rate was normofrequent at the phase of renal anemia and did not change before and after the treatment with rhEPO (U1 69 ± 11 beats/min versus U2 66 ± 7 beats/min, NS). Correction of anemia did not change forearm blood flow (U1 6.1 ± 0.7 mL/100 mL/min versus U2 6.2 ± 0.6 mL/100 mL/min, *P* not significant) (Table 3). As well, forearm vascular resistance remained unchanged during rhEPO treatment (Table 3).

## DISCUSSION

The main effects of a chronic therapy with rhEPO in dialysis patients are as follows. Normalization of the hematocrit level in dialysis patients by a chronic therapy with rhEPO leads to a significant regression of LVH and also to an improvement of left ventricular geometry. SNA which is elevated in ESRD did not decrease after correction of renal anemia. The impaired cardiopulmonary baroreflex as a part of the autonomic dysfunction in dialysis patients did not change after normalization of the hematocrit level. Under chronic rhEPO treatment, blood pressure did change significantly and antihypertensive medication was continued. Under these conditions, changes of the peripheral blood flow during rhEPO treatment in dialysis patients did not occur. These findings suggest that correction of renal anemia by rhEPO has beneficial effects on cardiovascular prognosis parameter such as LVH and geometry. However, the autonomic dysfunction is not reversible by an effective rhEPO therapy for the medium-term in dialysis patients.

### Effect of correction of anemia with rhEPO on cardiac structure and hemodynamic parameters

LVH, a major cardiovascular risk factor, is present in 74% in patients starting renal replacement therapy [35]. Renal anemia is one important determinant for the development of LVH [3]. It has been demonstrated that effective correction of anemia with rhEPO treatment for 8 months reduces left ventricular mass significantly [3]. In correspondence to these results, we found that normalization of hemoglobin over a time period of 7 months, LVMI decreased significantly in all patients. Over that, we found that the configuration of the left ventricle developed from an asymmetric into a symmetric geometry after correction of renal anemia. Left ventricular geometry in uremic patients has been shown to be a strong predictor of cardiovascular mortality [36]. Thus, these findings may indicate that an effective correction of anemia in dialysis patients has favorable effects beyond the reduction of left ventricular mass with a remodeling of left ventricular geometry in ESRD.

Development or deterioration of a preexisting elevation in diastolic blood pressure is known as a possible side effect of rhEPO therapy [16]. In our study, 24-hour blood pressure readings revealed no change of systolic or diastolic blood pressure during rhEPO treatment. At this, dose and number of antihypertensive drugs did not differ significantly with and without rhEPO therapy. From a hemodynamic viewpoint, a rapid reversal of anemia-induced peripheral vasodilatation by a fast increase in hematocrit with high doses of rhEPO has been presumed as a factor which may contribute to the hypertensive effect of rhEPO [37]. In our study, increases of rhEPO doses were performed carefully striving for a tempered rise of hemoglobin with a close clinical monitoring of the patients at their dialysis centers. Thus, in accordance with previous reports [38], we point out that with a close attention to patient surveillance, hypertensive problems during rhEPO therapy should be treatable.

London et al [39] demonstrated a significant reduction of forearm blood flow and a significant increase in limb vascular resistance after correction of renal anemia in 11 hemodialysis patients. However, in parallel with our microneurographic findings, the peripheral arterial blood flow as well as vascular resistance did not change after correction of anemia in our study. Yet, these results have to be analyzed carefully, since in ESRD the fluid balance fluctuates depending on the duration of the dialysis-free interval. These for the ESRD-specific factors interact with hemodynamics and blood viscosity rendering an interpretation more difficult. Although the "dry" body weight had formally not changed significantly, a transient change of hemodynamics early on induced by the treatment of rhEPO can not be excluded.

### Effect of anemia correction with rhEPO on SNA at rest and during cardiopulmonary baroreflex testing

Converse et al [4] demonstrated a state of sympathetic overactivity in chronically dialyzed patients with a 2.5 times higher rate of sympathetic nerve discharge compared to normal subjects. The mean rate of sympathetic nerve discharge was 2.5 times higher in patients receiving hemodialysis compared to normal subjects [4].

Evaluation of the SNA by the measurement of plasma catecholamines is insensitive and may be influenced by many factors such as synaptic transmitter release, reuptake mechanisms, and regional blood flow [40]. Besides, ESRD may lead to changes in catecholamine metabolism and clearance. In contrast, microneurography allows a precise, directly intraneural, quantitative, and reproducible measurement of postganglionic SNA.

In dialysis patients without rhEPO treatment we found an elevated sympathetic neural vasoconstrictor activity at rest. Renal anemia may be accounted as one potential mechanism for the increased SNA in chronic renal insufficiency. Hypoxia, induced by a significant reduction of the hematocrit concentration due to the lack of erythropoietin, may lead to a stimulation of the sympathetic nervous system [9]. An important aim of our study was to determine the effect of anemia correction by rhEPO on the autonomic function in dialysis patients. Normalization of the hemoglobin concentration did not alter the SNA at rest in dialysis patients. The reason for the lack of decrease of the sympathetic activity despite the improved oxygen supply after anemia correction may be due to a stimulation of the afferent nerves of the failing kidneys which could induce a reflex activation of the efferent sympathetic discharge. A hint for this hypothesis is the observation that in patients who had undergone bilateral nephrectomy no excess sympathetic activity could be documented [7]. As well, experimental denervation of the renal afferent nerves has been shown to reduce sympathetic discharge and blood pressure in animals [41].

In normal subjects, the SNA increases with age and is higher in men than in women [42]. With regard to these results, we found that sympathetic vasoconstrictor discharge in dialysis patients is higher than in normal subjects in consideration of age and gender. However, the extent of sympathetic overactivity in our study population was less than the highly sympathetic activation in dialysis patients described by Converse et al [7]. The reason for this may be the fact that in our study 22 of the 23 study participants received antihypertensive drugs including ACE inhibitors, angtiotensin II type 1 (AT1) receptor blockers, β blockers, and sympatholytic agents, which all may modulate the central sympathetic outflow [43–46]. In comparison with this, in the study of Converse et al [7] only 50% of the hemodialyzed patients were treated with antihypertensive medication. Thus, the more extensive pharmacologic influence in our study may be the cause for the relatively less sympathetic activation compared to earlier results.

To assess the baroreflex-mediated control of SNA we used a challenge maneuver causing a nonhypotensive orthostatic stress. In normal subjects, unloading of cardiac baroreceptors during lower body negative pressure increases sympathetic discharge to skeletal muscle in a potent way [25, 47]. A decreased baroreflex sensitivity has been proven to be associated with a high cardiovascular risk [48]. We found a strongly diminished sympathetic response in the cardiopulmonary baroreflex testing with a lack of increase in sympathetic efferent discharge in anemic dialysis patients. These results indicate a severe autonomic disorder with a markedly reduced baroreflex sensitivity. Of note, after correction of the renal anemia by rhEPO treatment for months, the sympathetic response during the cardiovascular stress was not adequate as well.

Our study is limited by the lack of a crossover design. Nevertheless, these findings may confirm the suggestion that autonomic dysfunction in ESRD is a multifactorial

process at which a number of pathogenetic factors associated with renal failure are involved. Beside secondary organ damage of vessels and heart as LVH and hematologic factors as anemia, uremic toxins may play a role damaging autonomic nerve fibres causing an impairment of baroreflex-mediated sympathetic nerve control [49]. Whether correction of renal anemia yet in an earlier stage of chronic renal failure can prevent or mitigate autonomic disorders remains to be determined.

## CONCLUSION

Normalization of hemoglobin in dialysis patients has beneficial cardiovascular effects with regression of LVH and improvement of left ventricular geometry. However, a reduction of sympathetic overactivity or a resetting of baroreceptor sensitivity by rhEPO treatment in the medium-term could not be achieved. The reason for this may be the complex and multifactorial pathomechanism of cardiovascular disease and autonomic dysfunction in ESRD.

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