

*Original Article***Is endogenous erythropoietin a pathogenetic factor in the development of essential hypertension?**

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**Abstract**

**Background.** Recent experimental studies have found that erythropoietin elicits vasoconstriction and proliferation of endothelial cells. We conducted the following study to assess the possible interactions between endogenous erythropoietin, systemic and renal haemodynamics at different stages of essential hypertension.

**Methods.** We examined 47 patients with borderline essential hypertension (age  $26 \pm 3$  years) and 49 patients with established essential hypertension WHO stage I–II (age  $52 \pm 10$  years), and compared them to 42 normotensive individuals (age  $26 \pm 3$  years). The concentration of erythropoietin (radioimmunoassay), 24-h ambulatory blood pressure (Spacelab 90207), systemic haemodynamics (Doppler sonography) and renal haemodynamics (para-aminohippuric acid and inulin clearance) were determined.

**Results.** Erythropoietin was within normal range and similar among the three groups. In patients with established essential hypertension, a close correlation was found between erythropoietin and systolic ( $r=0.45$ ,  $P<0.002$ ) and diastolic ( $r=0.51$ ,  $P<0.001$ ) ambulatory blood pressure. In contrast, ambulatory blood pressure was not correlated with erythropoietin in subjects with borderline hypertension. Total peripheral resistance ( $r=0.41$ ,  $P<0.02$ ) was linked to erythropoietin in established but not in borderline hypertension. However, erythropoietin was inversely correlated with renal plasma flow in both established and borderline hypertension ( $r=-0.33$ ,  $P<0.05$ , and  $r=-0.34$ ,  $P<0.05$  respectively). In normotensive subjects, in contrast, erythropoietin was not correlated with any of the determined variables. In neither group erythropoietin was linked to the haematocrit or hemoglobin concentration.

**Conclusion.** The correlation between erythropoietin and renal vascular changes which is already present in borderline hypertension and is confirmed in established hypertension indicates an involvement of erythropoietin in the development of essential hypertension.

The presence of normal concentrations of endogenous erythropoietin in all groups suggests a dysregulation of erythropoietin in patients with essential hypertension as the pathophysiological link between erythropoietin and vascular changes.

**Key words:** essential hypertension; erythropoietin; renal haemodynamics

**Introduction**

Progressively increased total peripheral resistance is a characteristic haemodynamic feature in the development of essential hypertension even in the early stage of essential hypertension [1]. Human recombinant erythropoietin (Epo), administered for the correction of anaemia in patients with end-stage renal disease, elevates arterial blood pressure in 25–30% of patients on maintenance haemodialysis [2,3] which seems to be due to a parallel increase of total peripheral resistance [4,5]. The elevation of the haematocrit, however, with the subsequent reduction of luxury perfusion in the periphery, does not or, if at all, only in part, account for the increase of total peripheral resistance after treatment with Epo [6,7].

A possible explanation for the blood pressure increase by Epo may be the fact that erythropoietin has been described to have vasoconstricting properties on isolated renal resistance vessels [8]. Moreover, endothelial cells have erythropoietin receptors and respond with proliferation to Epo stimulation [9]. These non-haemodynamic effects may lead to elevated vascular resistance and increased blood pressure.

Of note, patients on maintenance haemodialysis with a positive family history of arterial hypertension are at higher risk to respond with an increase of blood pressure to Epo treatment [10]. Therefore, genetic predisposition to hypertension is an important factor not only for essential hypertension, but also for the development of arterial hypertension secondary to Epo administration.

These studies led us to hypothesize that Epo might play a pathogenetic role early in the development of

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essential hypertension. In a cross-sectional study we investigated the relation between endogenous Epo, blood pressure and systemic haemodynamics as well as renal haemodynamics in normotension, early essential hypertension, and established essential hypertension.

## Subjects and methods

### *Study population*

Three groups were recruited: first, patients with established essential hypertension (EH), second subjects with borderline essential hypertension (BH), and third normotensive subjects as controls (NT).

Patients with established essential hypertension (EH,  $n = 49$ ) had been referred to our University Hypertension Clinic for evaluation and treatment of elevated blood pressure. All of the participating patients (aged  $52 \pm 10$  years) were diagnosed to have arterial hypertension according to WHO criteria, i.e. the average of four casual blood pressure (BP) readings on two different occasions (at least 2 weeks apart) was  $\geq 160/95$  mmHg. Casual BP was taken from the sitting patient after 5 min of rest with a standard sphygmomanometer. Cuff size was adjusted according to the patient's arm circumference. All patients either had never received any cardiovascular medication or the treatment had been discontinued at least 4 weeks before the study began and casual BP measurements were taken. None of the patients had previously received angiotensin II antagonists or ACE inhibitors known to interact with erythropoietin [11].

Each participating patient underwent a complete routine clinical workup including a complete medical history, physical examination, urine analysis (dipstick and microscopic evaluation), and blood tests (serum concentration of urea, creatinine, sodium, and potassium) to exclude WHO stage III of hypertensive disease and secondary hypertension. In particular, 12-lead electrocardiogram at rest and with exercise, fundoscopy, and sonography of the kidneys were performed. If indicated, intra-arterial digital subtraction arteriography and endocrine investigations were carried out to exclude secondary causes of hypertension. Further exclusion criteria were advanced hypertensive fundoscopic changes, myocardial infarction or other evidence of coronary artery disease, congestive heart failure (New York Heart Association classes II to IV), and previous cerebrovascular event. All patients had a normal renal function (by inulin clearance) and were free of any renal disease according to clinical investigation. Forty-two of 49 patients were life-time non-smokers, 7 had a history of smoking (1 active smoker and 6 ex-smokers). All patients had no history or clinical signs of pulmonary disease, in particular no patient had a history of chronic obstructive airway disease.

Subjects with borderline essential hypertension (BH,  $n = 47$ , age  $26 \pm 3$  years) were recruited from the Campus of the University Erlangen-Nuremberg at the occasion of screening for high blood pressure. Borderline hypertension was defined as casual BP  $\geq 140$  mmHg systolic or  $\geq 90$  mmHg diastolic. Blood pressure measurements were taken according to the protocol described above. Secondary hypertension, WHO stage III of hypertensive disease and other cardiovascular or pulmonary disorders were excluded according to the protocol for established essential hypertension, as outlined above. No subject had received medical treatment for high blood pres-

sure in the past. Fifteen of 47 patients had a smoking history with nine ex-smokers and six active smokers.

Normotensive controls (NT,  $n = 42$ , age  $26 \pm 3$  years) had all casual blood pressure readings of  $\leq 140/90$  mmHg according to the protocol described above. All normotensive individuals were healthy with no abnormal clinical or chemistry findings following the same protocol as hypertensive patients. No subject of this group had a family history of arterial hypertension. Seven of 42 persons were ex-smokers, and six were active smokers.

### *Measurements*

The following investigations were carried out in all participants. Non-invasive ambulatory BP monitoring was conducted with an automatic portable device (Spacelab 90207, Redmond, USA). Measurement intervals were every 15 min during daytime (defined from 06:00 to 22:00) and every 30 min during night-time. The 24-h BP profiles were analysed according to an individualized approach [12].

Measurements of renal haemodynamics were carried out in a semirecumbent position in a quiet room under resting conditions at the same time of the day. We applied the constant infusion input technique (Perfusor secura, Braun Melsungen AG, Germany) to determine renal perfusion (para-aminohippuric acid- [PAH] clearance) and glomerular filtration rate (inulin-clearance) as suggested by Cole and coworkers [13], and previously described and revalidated in detail [14]. Blood samples for the determination of inulin and PAH were collected twice at minutes 105 and 120 and were measured in duplicate for each sample. Coefficient of variation was less than 5% [14]. Filtration fraction was calculated by dividing glomerular filtration rate by renal plasma flow. Renal blood flow was estimated by dividing renal plasma flow by 1 minus the haematocrit. Renal vascular resistance was calculated as mean arterial BP divided by renal blood flow. Renal haemodynamic data were indexed to body surface area to allow comparison between groups with various body constitution.

Systemic haemodynamics were assessed non-invasively by continuous wave Doppler sonography (Picker-Hitachi CS 192, Tokyo, Japan, 2.5 MHz probe). The echocardiographic recordings were obtained at rest with the probe in the third or fourth intercostal space lateral to the left sternal border and the patient recumbent in the half left-sided position [15]. All echocardiographic tracings were read independently by two physicians and in accordance with the American Society of Echocardiography (ASE) convention [16]. To assess stroke volume, continuous pulsed-wave doppler sonography was subsequently used. Stroke volume was calculated as the product of cross-sectional area of the aortic valve (long axis parasternal M-mode view) and the velocity time integral of left ventricular outflow obtained from the five-chamber view [17]. Cardiac output was calculated as stroke volume multiplied by heart rate, and total peripheral resistance was calculated as mean blood pressure divided by cardiac output.

Blood samples were always collected at the same time in the morning in each patient for the determination of erythrocyte count, haemoglobin, haematocrit, and endogenous Epo after the patients had rested for 1 h in the supine position. The blood samples for the determination of endogenous Epo were centrifuged at room temperature ( $18^\circ\text{C}$ ) immediately after being taken from the patients, and plasma was rapidly frozen and stored at  $-20^\circ\text{C}$ . Endogenous Epo was measured within 3 months with a commercially available radioimmunoassay kit (EPO-Trac RIA Kit (23200), Sorin Bio Medica

AG, Germany). Coefficient of variation was less than 6%. The mean endogenous Epo concentration was  $15.0 \pm 3.6$  mU/ml in the group with established hypertension,  $16.0 \pm 7.6$  mU/ml in the group with borderline hypertension, and  $13.5 \pm 5.3$  mU/ml in normotensive controls. The concentrations of endogenous Epo were within normal limits in all examined subjects [18].

All statistical analysis was carried out using the SSPS-PC program [19]. ANOVA and ANCOVA testing was applied to compare the groups followed by the Scheffe test for comparison between groups. Pearson correlation coefficients and multiple regression analysis was done when indicated. A value of  $P < 0.05$  (two-sided significance) was considered to be statistically significant. All values are given as mean  $\pm$  standard deviation.

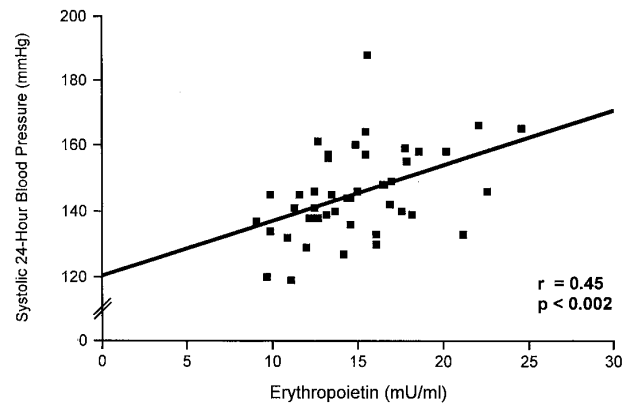
## Results

### Clinical data

Twenty-four-hour ambulatory blood pressure differed between groups ( $P < 0.001$ , see Table 1). Casual BP in EH was  $165 \pm 21/106 \pm 12$  mmHg, in BH  $145 \pm 11/90 \pm 11$  mmHg, and in NT  $121 \pm 11/75 \pm 9$  mmHg, which was different by study design ( $P < 0.001$ ). Body mass index was  $27.5 \pm 4.1$  kg/m<sup>2</sup> in EH,  $24.6 \pm 2.8$  kg/m<sup>2</sup> in BH, and  $23.1 \pm 1.8$  kg/m<sup>2</sup> in NT ( $P < 0.001$ ). No correlation was found between Epo and body mass index or age in the entire population. The mean Epo concentration in EH ( $15.0 \pm 3.6$  mU/ml) was equal to the one in BH ( $16.0 \pm 7.6$  mU/ml) and NT ( $13.5 \pm 5.3$  mU/ml). In neither of the groups nor in the total study group Epo concentration correlated with hematocrit, haemoglobin concentration, or erythrocyte count.

### Blood pressure

In EH, a strong correlation was found between Epo concentration and 24-h blood pressure. The higher the concentration of Epo, the higher was 24-h systolic ( $r = 0.45$ ,  $P < 0.002$ , see Figure 1) and diastolic ( $r = 0.51$ ,  $P < 0.001$ ) blood pressure in this group. When analys-



**Fig. 1.** Relation of endogenous erythropoietin concentration and 24-h systolic blood pressure in patients with established essential hypertension (EH).

ing average daytime and night-time blood pressure separately, Epo still correlated with the respective values (data not given). In a stepwise multiple regression analysis, Epo was the strongest determinant of diastolic 24-h blood pressure ( $R^2 = 0.30$ ,  $\beta = 0.49$ ,  $P = 0.0009$ ) compared to age ( $\beta = 0.19$ , n.s.) and body mass index ( $\beta = -0.06$ , n.s.). In the same model, Epo proved to be a potent determinant of systolic 24-h blood pressure ( $R^2 = 0.35$ ,  $\beta = 0.39$ ,  $P = 0.0053$ ) compared to age ( $\beta = 0.39$ ,  $P = 0.0042$ ) and body mass index ( $\beta = -0.22$ , n.s.). Casual blood pressure did not show a correlation with Epo in this group.

In BH, as opposed to EH, neither 24-h systolic nor diastolic blood pressure correlated with Epo concentration. Similarly, no correlation was found between Epo and 24-h systolic or diastolic blood pressure in NT.

Hence, a strong correlation between 24-h blood pressure and Epo was found in established essential hypertension, but not at the early stage of hypertension nor in the normotensive healthy state.

### Systemic haemodynamics

The concentration of Epo was correlated with total peripheral resistance in EH. The higher Epo concentra-

**Table 1.** Characteristics of patients with established essential hypertension (EH), borderline essential hypertension (BH), and normotensive controls (NT) (mean  $\pm$  standard deviation)

Characteristics	NT	BH	EH
Age (years)	26 $\pm$ 3	26 $\pm$ 3	52 $\pm$ 10**§§
Weight (kg)	76.3 $\pm$ 9.0	81.9 $\pm$ 8.6###	83.6 $\pm$ 15.6*
Body mass index (kg/m <sup>2</sup> )	22.9 $\pm$ 1.7	24.6 $\pm$ 2.8###	27.7 $\pm$ 4.2**§§
Ambulatory SBP (mmHg)	118 $\pm$ 6	132 $\pm$ 7###	145 $\pm$ 13**§§
Ambulatory DBP (mmHg)	69 $\pm$ 5	78 $\pm$ 7###	92 $\pm$ 8**§§
Cardiac output (ml/min)	6669 $\pm$ 2334	7844 $\pm$ 2216#	7478 $\pm$ 2104
Total peripheral resistance (U)	15.2 $\pm$ 5.5	14.9 $\pm$ 4.6	17.5 $\pm$ 5.8**§§
Glomerular filtration rate (ml/min/1.73 m <sup>2</sup> )	106 $\pm$ 17	105 $\pm$ 15	97 $\pm$ 14*§
Renal plasma flow (ml/min/1.73 m <sup>2</sup> )	577 $\pm$ 107	572 $\pm$ 92	457 $\pm$ 93**§§
Renal blood flow (ml/min/1.73 m <sup>2</sup> )	1128 $\pm$ 196	1039 $\pm$ 178	790 $\pm$ 157**§§
Endogenous erythropoietin (mU/ml)	13.5 $\pm$ 5.3	16.0 $\pm$ 7.6	15.0 $\pm$ 3.6
Haematocrit (%)	43.7 $\pm$ 2.1	44.7 $\pm$ 2.7#	42.7 $\pm$ 3.1§§

\* $P < 0.05$  NT vs. EH; \*\* $P < 0.01$  NT vs. EH; § $P < 0.05$  BH vs. EH; §§ $P < 0.01$  BH vs. EH; # $P < 0.05$  NT vs. BH; ### $P < 0.01$  NT vs. BH. SBP, systolic blood pressure; DBP, diastolic blood pressure.

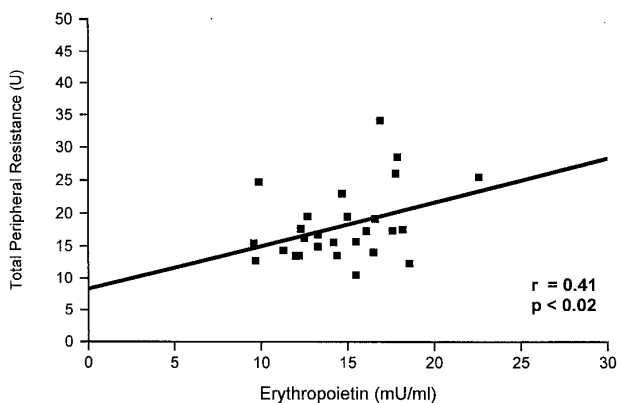
tions, the higher was total peripheral resistance determined by dopplersonography ( $r=0.41$ ,  $P<0.02$ , see Figure 2). Epo concentration did not show a correlation with cardiac output ( $r=-0.10$ , n.s.). In BH, no correlation was found between Epo and total peripheral resistance ( $r=0.08$ , n.s.) or cardiac output ( $r=-0.01$ , n.s.). Similarly, Epo was neither correlated with total peripheral resistance ( $r=-0.16$ , n.s.) nor cardiac output ( $r=0.26$ , n.s.) in NT. Thus, Epo correlated with total peripheral resistance in EH only.

### Renal haemodynamics

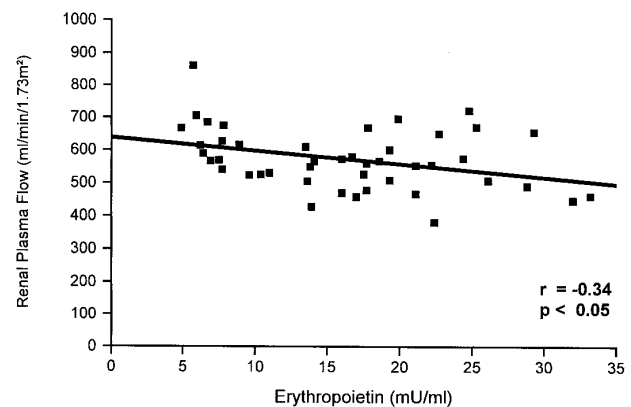
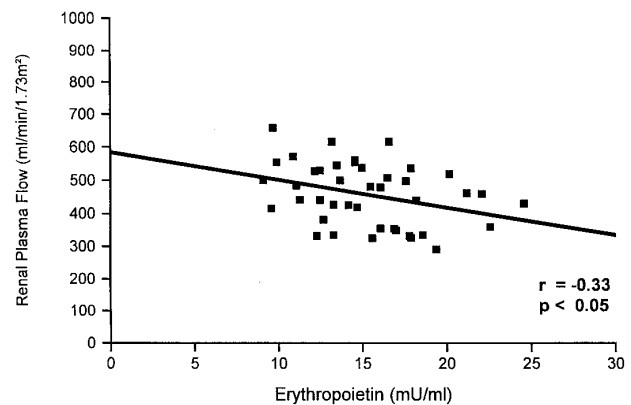
Renal plasma flow was inversely correlated with Epo concentration in EH. The higher Epo, the lower was renal plasma flow in patients with essential hypertension (EH;  $r=-0.33$ ,  $P<0.05$ , see Figure 3a). Renal blood flow was linked to high Epo concentration ( $r=-0.35$ ,  $P<0.05$ ) as was renal vascular resistance ( $r=0.38$ ,  $P<0.02$ ). Glomerular filtration rate only tended to be correlated with Epo ( $r=-0.30$ ,  $P=0.052$ ), and filtration fraction was not correlated with Epo ( $r=0.12$ , n.s.).

In patients with borderline hypertension (BH), high Epo concentrations were inversely related to renal plasma flow ( $r=-0.34$ ,  $P<0.05$ , see Figure 3b), renal blood flow ( $r=-0.34$ ,  $P<0.05$ ), and glomerular filtration rate ( $r=-0.58$ ,  $P<0.001$ ) but in a direct manner to renal vascular resistance ( $r=0.50$ ,  $P<0.001$ ). This contrasts the findings in the systemic circulation found for this group (lack of correlations of Epo with 24-h blood pressure and total peripheral resistance). In contrast to both hypertensive groups, in normotensive controls Epo did not correlate with renal vascular resistance ( $r=0.10$ , n.s.), renal plasma flow ( $r=-0.12$ , n.s.), nor renal blood flow ( $r=-0.09$ , n.s.). However, glomerular filtration rate correlated inversely with Epo ( $r=-0.54$ ,  $P<0.01$ ).

Thus, endogenous Epo concentration was linked to high renal vascular resistance and low renal plasma flow in EH in a similar way as in BH as opposed to normotensive controls where no such correlations were found.



**Fig. 2.** Relation of endogenous erythropoietin concentration and total peripheral resistance in patients with established essential hypertension (EH).



**Fig. 3a.** Relation of endogenous erythropoietin concentration and renal plasma flow in patients with established essential hypertension (EH).

**Fig. 3b.** Relation of endogenous erythropoietin concentration and renal plasma flow in patients with borderline hypertension (BH).

### Discussion

As the most interesting result, the concentration of endogenous Epo was strongly correlated with elevated 24-h blood pressure in patients with established essential hypertension. Total peripheral resistance was identified as the underlying mechanism to which Epo was correlated in patients with established essential hypertension. Consistently, in patients on maintenance haemodialysis there is clear evidence that Epo administration increases total peripheral resistance independently from the rise in haematocrit, thereby causing an increase of arterial blood pressure [4,5]. Also, intravenous Epo infusion lead to reduced muscle blood flow and increased vascular resistance in healthy normotensive individuals [20].

The concentration of endogenous Epo was within the limits of normal in all groups in the present study [18] and is in accordance with values found in essential hypertension in prior studies [21–23]. The correlation between vascular changes and endogenous Epo therefore cannot be explained by elevated levels of Epo.

Furthermore, we found a strong correlation between high endogenous Epo concentration and low renal plasma flow as well as low glomerular filtration rate

and consistently, increased renal vascular resistance in established essential hypertension. Surprisingly, our patients with borderline essential hypertension showed a similar correlation between endogenous Epo and renal plasma flow as did patients with established essential hypertension despite the lack of an association between Epo and total peripheral resistance or ambulatory BP respectively. Therefore an interaction between Epo and renal vascular resistance is present already in borderline hypertension. It is well known that renal vascular changes might be earlier and more pronounced (and thereby better correlated to Epo) at the early stage of essential hypertension than changes of systemic haemodynamics [24,25]. Our data therefore, indicates that the concentration of Epo and renal vascular changes are already related to each other in the early stage of essential hypertension. However, clearly, from this cross-sectional study it cannot be established whether Epo is linked to renal vascular changes in a cause-effect manner.

Several lines of experimental and clinical evidence suggest, however, that Epo might be more than an innocent bystander in the pathogenesis of essential hypertension. One possible explanation is the interaction of Epo with vascular responsiveness. It has been shown that patients on maintenance haemodialysis show an increased vascular responsiveness to intra-arterial infusion of noradrenaline after Epo treatment [26]. In particular, Epo has been shown to have a vasopressor effect on renal resistance vessels in a normotensive animal model [8]. An exaggerated response of the renal vascular bed to Epo might explain the correlation between Epo and renal plasma flow in early essential hypertension. Furthermore, an impaired downregulation of endogenous Epo to the stimulus of hyperbaric oxygenation is found in essential hypertension in contrast to secondary hypertension and normotension [27]. The latter indicates a disarranged regulation of Epo secretion in essential hypertension. Finally, it is interesting to note that endothelial cells have Epo receptors and respond to activation by Epo with proliferation [9]. This trophic effect of Epo might alternatively explain the interaction between Epo and elevated total peripheral resistance.

In conclusion, an interaction between endogenous Epo and vascular changes is found in humans already at an early stage of essential hypertension and is confirmed in established essential hypertension. In particular, the correlation between high endogenous Epo and low renal plasma flow in borderline hypertension indicates an early involvement of Epo in the development of essential hypertension prior to systemic vascular changes. Prospective studies are required to prove the hypothesis that Epo plays a causal role in the pathogenesis of essential hypertension.

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