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MULTIPLE SITE ESTIMATES OF ERYTHROPOIETIN AND RENIN IN POLYCYTHEMIC KIDNEY TRANSPLANT PATIENTS

JEAN-M. AEBERHARD,[1] PIERRE-A. SCHNEIDER,[2] MICHEL B. VALLOTTON,[1] ARMIN KURTZ,[3] AND MICHEL LESKI[4]

Département de Médecine[1] and Département de Radiologie[2], Hôpital Cantonal Universitaire, Geneva; and Physiologisches Institut[3], Zurich, Switzerland

The development of polycythemia after renal transplantation is a well known phenomenon and its etiology remains controversial. In particular, it is unknown whether inappropriate erythropoietin (EPO) production could be a reason. Utilizing a sensitive radioimmunoassay for EPO we have measured EPO concentrations in venous blood from native and grafted kidneys in three normocythemic and seven polycythemic patients. We found that (1) in our posttransplantation polycythemic patients there are inappropriately high systemic EPO levels, hence that posttransplantation polycythemia is related to EPO overproduction. (2) This high EPO production comes from the native kidneys. (3) There is a correlation between EPO and renin levels in the peripheral as well as in native and transplanted kidneys' venous samples.

End-stage renal disease patients on chronic peritoneal or hemodialysis, if not given erythropoietin (EPO) as a substitute,* almost invariably have a moderate-to-severe degree of anemia. After renal transplantation, when successful, with good kidney function, these patients again raise their hematocrits, which reach normal or near-normal levels. In a number of instances however, polycythemia may develop. This polycythemia is not an infrequent phenomenon and may appear in up to 17.3% of the cases (*1*)-6.8% in our experience. Although spontaneously reversible after a certain time span (3-12 months in our cases), its clinical importance has to be emphasized because it has been clearly demonstrated that thromboembolic accidents are directly correlated with hematocrit value (*2*)—a 52% limit being found by most kidney transplantation teams. If the clinical importance of polycythemia is admitted, there is no consensus about its etiology. A number of hypotheses do exist; some have been proved to be etiological or contributive under certain circumstances: graft artery stenosis (*3*), hydronephrosis (*4*), smoking (*1*), and diuretic treatment (*5*)—however, this last condition provokes a pseudopolycythemia. Others remain speculative: a direct effect of cyclosporine (*6*) and graft rejection (acute or chronic) (*7*). Finally, some of them were formulated on a theoretical basis: androgenic steroids, EPO stimulation threshold resetting after long pretransplant anemia (*8*), resolution of hyperparathyroidism with its associated fibrosis of the bone marrow (*9*), and hepatic EPO production (*10*).

EPO production by the native kidneys was first advocated by Dagher et al. (*11*) in 1979. To our knowledge only one other study was performed with similar conclusions since then, in 1983 (*12*).

A sensitive radioimmunoassay for EPO developed recently (*13*) allowed the present study to be done. Its purposes were (1) to test whether polycythemia in renal transplant recipients is related to an exaggerated EPO production; (2) if so, to locate its origin; (3) to examine whether there is any correlation between erythropoietin and renin production; and (4) to eventually define the role of interventional radiology (nonsurgical nephrectomy).

MATERIALS AND METHODS

Cyclosporine was introduced as an immunosuppressive treatment on a routine basis in September 1983; this time was chosen to start the study so that all patients were comparable for immunosuppressive treatment.

Patients. Group 1 (patients 1-7): Of 148 patients transplanted since September 1983 through August 1989 (72 months), 10 (6.8%) developed polycythemia (Ht >52%, Hb >17.5 g%). Among these 10 polycythemic patients, 7 were included in this study (the 3 patients not included in the study had developed a transitory polycythemia before the above-mentioned sensitive radioimmunoassay became available). These 7 patients were male, aged 42-59 years when transplanted (mean: 48.7), duration of dialysis before transplantation ranged from 2 to 32 months (mean: 37), none showed clinical evidence of dehydration, 5 were hypertensive but none had diuretic treatment, all were nonsmokers. Renal function was good in all cases (creatinine levels below 140 μmol/L). Basic disease was variable in its etiology. None had acquired cystic disease of the native kidneys. Onset of polycythemia after transplantation was between 2 and 42 months (mean: 17). Of these 7 patients, 6 underwent spontaneous remission of their polycythemic state within 3-12 months (mean: 6.2). Phlebotomy was performed iteratively whenever Ht value exceeded 52%.

Group 2 (patients I-III): 3 nonpolycythemic patients who had severe hypertension despite treatment (three antihypertensive drugs, including minoxidil in all cases) were used as the control group (the indication for catheterization being hypertension).

Methods. Sampling: Selective renal venous catheterization was performed in all 10 patients. Samples for EPO and renin plasma activity were obtained upstream from the graft in the femoral vein (considered systemic samples [SS]), in the vein of the transplanted kidney (TK), and in the veins of both native kidneys (NK). As far as possible all different tributaries of the renal veins were catheterized (permitting more sample data points). Renal arteriography by the Seldinger method was performed in all cases.

Erythropoietin estimation: Plasma EPO titers were estimated by a sensitive radioimmunoassay (*13*). The assays were calibrated against an EPO standard (2nd International Reference Preparation of human

[1] Département de Médicine., Hôpital Cantonal Universitaire.

[2] Département de Radiologie., Hôpital Cantonal Universitaire.

[3] Physiologisches Institut.

[4] Address correspondence and reprint requests to Prof. Michel Leski, Département de Médecine, Division de Néphrologie, Hôpital Cantonal Universitaire, 1211 Geneva 4, Switzerland.

* Abbreviations: EPO, erythropoietin; NK, native kidney; SS, systemic sample; TK, transplanted kidney.

urinary EPO) provided by the National Institute for Biological Standards and Control, London. The results are expressed in milliunits per milliliter and matched with Ht values measured the day of sampling, which permits determination of whether the latter are appropriate. By analogy with what is done with renin activity, the ratios were calculated between EPO titers measured at different sites in the native and transplanted kidneys and those measured systemically.

Renin activity estimation: A radioimmunological estimation of angiotensin I generated during 1 hr of incubation was performed (*14*)—the normal range supine with normal salt intake being 0.58±0.4 ng/ml/hr.

Statistics: Log EPO values were plotted against log renin activity values, and statistical analysis was performed by means of a linear regression.

RESULTS

Figure 1 shows sytemic EPO levels matched to Ht measured the day of catheterization; it clearly demonstrates that all polycythemic patients are above the upper normal range while the 3 nonpolycythemic patients have EPO titers within the normal range. This range has recently been shown to be the same in normal subjects and in renal transplant recipients with good graft function (*15, 16*).

Table 1 shows that the ratio of EPO titers measured in the native kidneys to those measured systemically (NK/SS) is higher than 1.5 in 6 of the 7 polycythemic patients. This NK/SS ratio is less than 1.5 in the nonpolycythemic patients. The ratio of EPO titers measured in the transplanted kidneys to those obtained systemically (TK/SS) was always less than 1.5 in both groups.

In the polycythemic group a linear regression analysis of log EPO values on log renin activity values showed a very strong correlation for the values measured systemically—a correlation that is again found in the native kidneys as well as in the transplanted kidneys (Figs. 2–4). This analysis could not be performed in the nonpolycythemic group, the number of patients being insufficient.

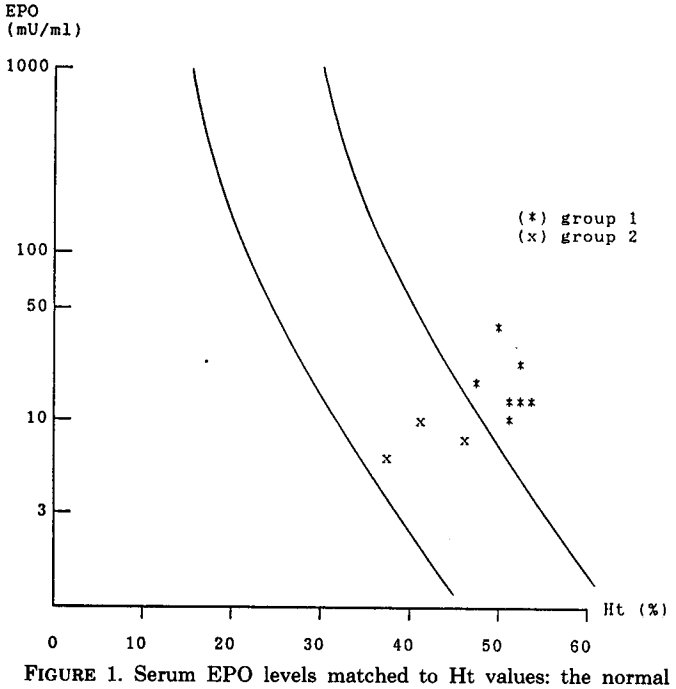

FIGURE 1. Serum EPO levels matched to Ht values: the normal range as defined by Erslev (*20, 23*); the same range after renal transplantation when good graft function is present (*15, 16*). Systemic EPO levels are above the normal range in group 1 and within the normal range in group 2.

DISCUSSION

Impaired EPO production is the primary cause of anemia that nearly always accompanies chronic renal failure. Soon after transplantation—if the result is a rejection-free course and good renal function—these patients build up again their hematocrits due to the presence of appropriate levels of plasma EPO originating in the renal graft. Under normal conditions, after an initial high EPO output, a negative feedback mechanism slows down EPO production to normal values.

All polycythemic patients presented here showed evidence of inappropriately high EPO production; in none was there evidence of an underlying disease or circumstances that have previously been reported to contribute to or cause posttransplantation polycythemia. Renal artery stenosis and hydronephrosis were ruled out by arteriography. Immunosuppressive treatment consisted of prednisone, azathioprine and cyclosporine. Although corticosteroids have some stimulatory effect on bone marrow, an induction of EPO production has not been reported. We did not perform globular mass determination in the absence of clinical signs of dehydration and diuretic treatment in all our studied polycythemic patients.

In 6 of these 7 patients the native kidneys could be proved to be the source of this EPO overproduction (patient 4 had a horseshoe kidney, and not all accessory veins could be catheterized; we probably missed the hypersecretive site of this native kidney, the origin of the inappropriately high EPO level measured systemically not being the transplanted kidney).

One could object that flow has to be measured to assess the production rate of EPO at a given site and then determine whether the increment of EPO produced in the native kidneys is sufficient to account for high titers in the systemic samples. Since these systemic EPO titers are high, the 2 possible origins are the native kidneys or the transplanted kidney. The NK/SS ratio for EPO being high only in the polycythemic group, while the TK/SS ratio was low in the polycythemic as well as in the nonpolycythemic group, we came to the conclusion that the NK are the source of EPO overproduction without flow determination (by analogy with what is currently done in the assessment of renin-dependent hypertension).

Uremic nephric patients have some EPO production, sometimes even with high titers compared with those present in normal subjects, but they are always lower than the titers expected for the hematocrit values. Once the hematocrit reaches normal levels after transplantation, EPO production drops to a steady state. The diseased native kidneys, however, undergo an accelerated ischemia, and the remaining functional nephrons are probably responsible for the high EPO output. This hypothesis is supported by the fact that the state of polycythemia is frequently transitory, occurring during ischemic period until complete atrophy of the remaining functional parenchyma of the native kidneys. Furthermore, we noticed that only certain sites of the native kidneys showed high levels of EPO.

The renin-EPO correlation found in our polycythemic patients is a stimulating finding from the physiopathological point of view. Every intrarenal condition of mismatch between oxygen consumption (VO_2) and oxygen delivery (DO_2) provokes an increased EPO release whenever the VO_2/DO_2 ratio increases and the opposite when it falls (*17*). Increased VO_2 with second-

TABLE 1. EPO and renin activity values at different sites[a]

P[b]	A	OD	D	PC	HT	Ht	EPO					Renin		
							SS	NK	TK	N/S	T/S	SS	NK	TK
1	49	CGN	6	+	−	46	21	53	26	2.5	1.2	1.1	4.4	1.3
2	49	SHS	72	+	+	52	27	59	29	2.2	1.1	1.9	2.2	2.3
3	44	BD	2	+	+	51	15	41	19	2.7	1.3	1.2	2.5	1.2
4	42	PGN	36	+	−	52	11	14	11	1.3	1.0	0.3	1.4	0.3
5	59	SGN	5	+	+	54	13	29	14	2.2	1.1	0.7	3.9	0.9
6	49	HTD	42	+	+	52	13	30	14	2.3	1.1	0.6	1.0	0.8
7	49	CGN	38	+	+	50	35	102	37	2.9	1.1	1.2	3.3	1.0
I	43	PHA	3	−	+	41	11	15	—	1.4	—	1.9	1.2	—
II	36	HTD	36	−	+	36	6	7	8	1.2	1.3	3.5	7.3	3.6
III	51	CGN	1	−	+	46	10	10	10	1.0	1.0	2.2	4.0	2.8

[a] Only one sample of EPO originating in one of the NK is presented here, it is the highest EPO level measured in the NK, renin value being the corresponding one at the same site. The NK/SS ratio for EPO was higher than 1.5 in 6 of the 7 polycythemic patients and below 1.5 in the nonpolycythemics; the TK/SS ratio for EPO was always less than 1.5 in both groups.

[b] P: patient, A: age, OD: original disease, D: duration of dialysis before transplantation (months), PC: polycythemia, HT: hypertension, Ht: hematocrit on the day of catheterization (most values below the 52% limit because of phlebotomy), SS: systemic level, NK: native kidney, TK: transplanted kidney, CGN: chronic glomerulonephritis, SHS: Schonlein-Henoch syndrome, BD: Berger's disease, PGN: proliferative glomerulonephritis, SGN: segmental glomerulonephritis, HTD: HT + diabetes, PHA: phenacetin intoxication.

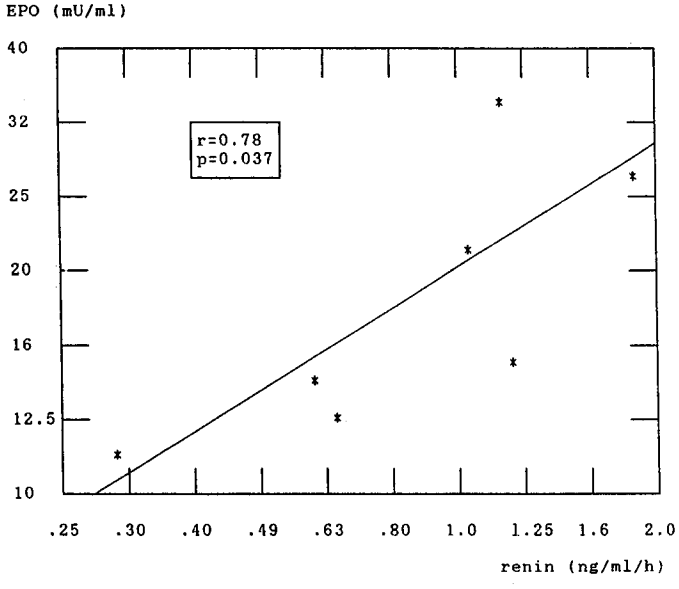

FIGURE 2. Linear regression analysis of log EPO on log renin activity values measured systemically.

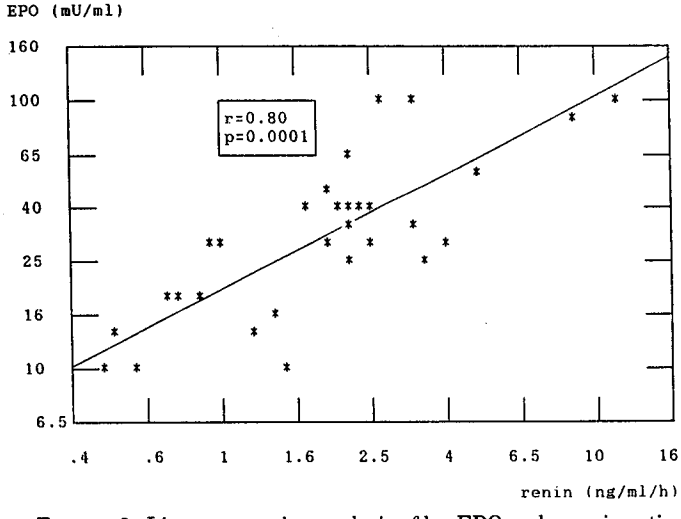

FIGURE 3. Linear regression analysis of log EPO on log renin activity values measured in the native kidneys.

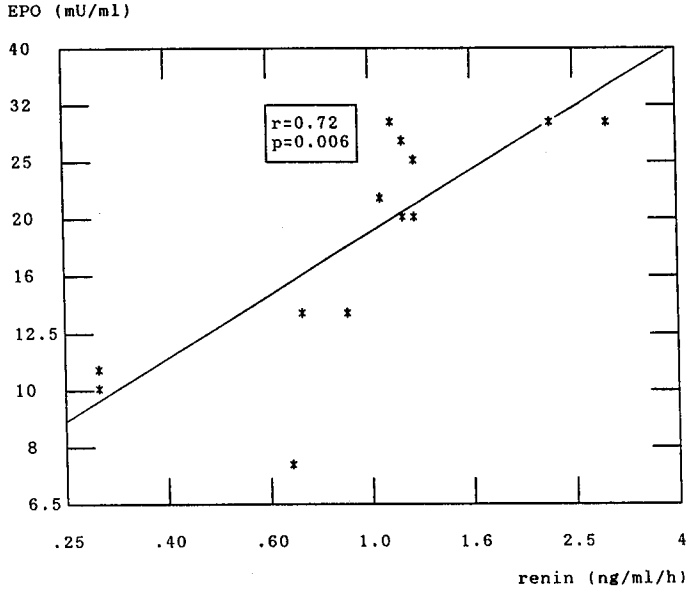

FIGURE 4. Linear regression analysis of log EPO on log renin activity values measured in the transplanted kidneys.

arily increased EPO release can be obtained with stimulation of metabolism by thyroid hormone administration (*18*). Decreased DO_2 with secondary increased EPO release can be obtained either by selective renal flow reduction (*19*) or by a decreased arterial oxygen content (CaO_2), anemia (*20*), diminished arterial partial pressure (PaO_2) of any origin (*21*), functional anemia with CO_2 intoxication (*17*), or increased O_2 affinity of hemoglobin (*22*).

Intrarenal DO_2 is the product of renal flow and the arterial oxygen content (DO_2 = RF × CaO_2); as shown above the stimulus for EPO release is either an enhanced VO_2 or a decreased DO_2. The stimuli for renin release are hyponatremia, hypovolemia, and decreased afferent arteriolar pressure, which partially depends on RF—hence one can stipulate that the correlation found between EPO and renin release has the RF as a common denominator. This finding deserves further investigation especially in conditions where CaO_2 is impaired, with concomitant absence of renin stimulation. Theoretically

the EPO-renin correlation should be lost in this condition; if not, one could suggest that the common pathway for EPO and renin regulation is DO_2.

Because polycythemia mostly resolves by itself, we suggest an observation period of one year with iterative phlebotomies if hematocrit values require it. If polycythemia should continue, phlebotomy being itself a contributive factor for EPO stimulation, we advocate native nephrectomy by alcohol treatment of renal arteries by means of an occlusive catheter. This procedure is safe if performed correctly (only a small amount of ethanol is required), and successful complete infarction is always achieved, unlike the result with particles. Morbidity is much lower than with a surgical procedure. We did not have to use this method for this indication until now, but we have performed it successfully in renal transplant patients with severe renin-dependent hypertension originating in the native kidney(s) (*24*).

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