University of Manitoba

IS HYPERTENSION A DETERMINANT OF ERYTHROPOIETIN REQUIREMENTS IN HEMODIALYSIS?

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Department of Community Health Sciences

by

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Is Hypertension a Determinant of Erythropoietin Requirements in Hemodialysis?

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A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University of Manitoba in partial fulfillment of the requirements of the degree

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Abstract:

Statement of Problem

Post-hoc analysis of results from a pilot study of erythropoietin (Epo) use in hemodialysis (HD) suggested that, to achieve a target hemaglobin (Hg) level, higher doses of Epo were required in patients with hypertension (HTN). There was a need to clarify the role of HTN in determining Epo responsiveness, which led to the hypothesis that the presence of HTN predicts the need for higher Epo doses to achieve a target Hg.

Methods

The study retrospectively reviewed HD patients treated with Epo using an observational, descriptive study design. Data for a single therapeutic inception cohort were collected from the first 4 to 6 months (4-6m) of uninterrupted Epo administration. Parametric methods were used to analyze the data to determine whether the postulated relationship between HTN and Epo requirements existed, as well as the impact of other intervening factors.

Results

The final cohort consisted of 111 patients whose data revealed no relationship between Epo dose and any of the many measures of HTN collected in this study. Multiple regression and other parametric methods consistently pointed to the role of angiotensin-converting enzyme inhibitor (ACEI) use as a

predictor of the 4-6m Epo requirement. The data demonstrated that patients receiving ACEI therapy received less Epo and, independently, achieved lower 4-6m Hg levels. Postulated mechanisms for this phenomenon are discussed.

Conclusions

While HTN does not appear to influence Epo dosing requirements, patients receiving ACEIs for the treatment of HTN may require a modified approach to therapy to achieve the full benefits of Epo in a cost-effective manner.

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1.0 Introduction:

Human recombinant erythropoietin (Epo) is a hormone medication which stimulates the production of red blood cells (RBCs) and is used to overcome the severe anemia associated with chronic renal failure (CRF). There is no question that, quantitatively, Epo is capable of increasing hemoglobin (Hg) and hematocrit (Hct) levels to offset the underlying hormone deficiency that exists in these patients (1,2).

The high acquisition cost of the drug, significant increases in its utilization, and questioning of the relative clinical benefits achieved (3) have made Epo the subject of intense examination since its introduction. This has prompted the development of prescribing guidelines for Epo to restrict its use to certain patient populations (see Appendices I and II). These guidelines implicitly define those patients most likely to benefit from Epo.

The results of a pilot study, however, made it clear that there remains a group of patients who do not exhibit the expected hematologic response to Epo. *Post hoc* analysis of data from that study indicated that this may be related to the effects of hypertension (HTN; i.e., underlying sympathetic tone) on Epo receptor function. These results were indicative of a significant **gap** in our knowledge, in that definitive evidence of the reason for this discrepancy in Epo requirements was not available.

There was a need to formally investigate the role of HTN in Epo dosing using a more stringent study design applying greater statistical power. Clarification of the role of blood pressure (BP) in a patient's ability to respond to Epo therapy might allow a ready means of correcting one source of Epo resistance and improve outcomes overall. There was a need to continue to identify factors that function as indicators of expected Epo responsiveness, to ensure that Epo would be prescribed in a cost-effective manner.

2.0 Background:

2.1 Defining the underlying problem

Erythropoietin is a hormone synthesized by the kidney that stimulates the production of red blood cells (RBCs) by bone marrow (4,5). In the patient with chronic renal failure (CRF), damage to the kidneys is the main factor responsible for decreases in the amount of endogenous Epo produced (6,7) leading to chronic anemia and impaired blood oxygen carrying capacity with associated fatigue, impaired quality of life (QOL), and damage to other organ systems (8,9). Human recombinant Epo has proven to be a viable method of treating the anemia, avoiding the hazards associated with transfusion therapy, and improving the patient's QOL and care (10,11,12,13,14).

Cost-effectiveness studies based on efficacy information from randomized controlled trials have reported the economic viability of prescribing Epo; and drug use evaluation studies have shown that the drug is effective when used appropriately (13,15). The literature, however, has been devoid of post-marketing data bringing together clinical and economic outcome issues in patients treated with Epo. Because of this gap in our understanding of Epo's effectiveness, a pilot study was carried out by the investigator in 1994 to determine the relationship of these outcomes (16).

2.2 The Pilot Study

The pilot study involved the review of hematologic and economic outcomes associated with the use of Epo in 68 hemodialysis (HD) patients at the Health Sciences Centre, Winnipeg. While analyzing the data from this review, a phenomenon never before demonstrated in the Epo literature was observed (see Table 1).

Table 1. Effect of HTN on Epo dosing requirements.

Outcomes of Main Groups:

	Mean Current Epo Dose (u/kg 3x/w) (Mean ± SD*)	Mean Current Hg (g/L)@	Mean Current Hct
Underlying HTN (n=39)	45.7 ± 22.2#	95.7 ± 16.3	0.283 ± 0.049
No Underlying HTN (n=31)	33.8 ± 17.9#	97.0 ± 11.0	0.286 ± 0.029

Outcomes of HTN Subgroups:

Worsening of Underlying HTN (n=16)	46.5 ± 22.0&	96.9 ± 16.6	0.287 ± 0.048
No Worsening of Underlying HTN (n=23)	45.2 ± 22.8&	95.0 ± 16.5	0.280 ± 0.051
New Onset HTN (n=14)	40.4 ± 18.5$	94.4 ± 11.8	0.281 ± 0.032
No New Onset HTN (n=17)	28.3 ± 15.8$	99.1 ± 10.2	0.29 ± 0.027

@ patient groups had comparable baseline Hg and Hct levels
* SD = standard deviation
\# $p < 0.05$ (independent t-test, df = 68, power in the range of 80%)
$ $p < 0.1$ (independent t-test, df = 29, power in the range of 60%)
& not significantly different

Thus, the pilot study results indicated that, within the overall population of patients on HD receiving Epo therapy, there were four distinct subgroups of

patients: those with underlying HTN (which worsened with Epo administration or, alternatively, remained stable), initially normotensive patients who developed HTN after initiation of Epo, and consistently normotensive patients. A conceptual model of the four mutually exclusive HTN groupings implied by the pilot study is presented in Figure I.

		HTN at Baseline	
		Yes	No
HTN at Follow-up	Yes	**group 1** - underlying HTN which worsened with Epo	**group 2** - initially normotensive, with Epo-induced HTN
	No	**group 3** - underlying HTN which remained stable	**group 4** - consistently normotensive

Figure I. Conceptual model of HTN groupings.

The data suggested that the presence of underlying HTN in general (n = 39 patients, i.e., 55.7% of all cases) or the development of new onset drug-induced HTN (n = 14, i.e., 46.7% of initially normotensive cases)[a] influenced Epo

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a Pre-existing HTN was defined by a diagnosis of HTN in the patient's chart and the presence of antihypertensive medications in the patient's therapeutic regimen. The development of Epo-induced HTN was defined by the diagnosis of new-onset HTN in the patient's chart and/or the addition of (or the use of higher doses or more aggressive) antihypertensive agents to the patient's current therapeutic regimen. Absolute BP measurements were not recorded during the course of the pilot study, as the HTN-Epo dose phenomenon was not anticipated at the study's outset.

dosing requirements. Patients who had underlying HTN at baseline required 35% more Epo (+ 11.9 units) per kilogram three times per week (3x/w) to maintain their hematologic indices (i.e., Hg, Hct) than those without underlying HTN at baseline ($p < 0.05$). Further subgroup analysis indicated a trend in initially normotensive patients who developed new onset HTN in that they demonstrated a need for almost 43% more Epo (+ 12.1 units/kg 3x/w) versus those who did not develop elevations in BP ($p < 0.1$). Thus, to achieve the same clinical endpoint (i.e., Hg/Hct levels), the pilot study showed that there was a need for significantly more Epo if HTN (pre-existing or new-onset) was involved. As noted, this phenomenon was identified during *post hoc* analysis.

2.3 Reports of Epo and HTN

Hypertension is a known adverse effect associated with Epo therapy, occurring in 30 to 40% of CRF patients. The mechanism for this effect is largely unknown, although theories abound and are often contradictory (17,18,19,20,21, 22,23,24), the latter of which has caused some to question the existence of Epo-induced HTN (25). Early Epo studies showed that increases in the diastolic component of BP correlated with changes in Hg levels (26); but that the relationship disappeared when Epo was initiated in lower doses (27,28), suggesting that the rate of rise of Hg was related to the incidence of HTN. The

latter Canadian study also noted that the development of severe HTN in patients receiving Epo was associated with a history of receiving antihypertensive medications or having native kidneys *in situ*. In addition, patients with a positive family history of HTN have been suggested to be more susceptible to Epo-induced HTN (29).

Caravaca *et al.* examined the incidence and risk factors associated with the development or aggravation of HTN due to Epo in the first 6 months of therapy (30). The overall incidence of HTN was 37% (compared to 43% overall in the pilot study). Multiple logistic regression suggested that age, a history of HTN and the use of antiplatelet therapy were the best predictive variables for the development of HTN. When separated into HTN outcome groups (those who developed HTN and those who did not; the authors did not differentiate patients based on baseline HTN status), there was no apparent difference in Hct outcomes at 12 and 24 weeks of therapy; but there was a trend towards a significant difference in the Epo dose required to achieve the outcome ($p<0.09$). The authors also found that concommitant antiplatelet treatment was associated with a significantly diminished the frequency of HTN development (5.8% vs. 56% in those patients not receiving antiplatelet agents; no difference in Hct outcomes between antiplatelet and no antiplatelet subgroups). They suggested that changes in platelet aggregability induced by Epo (as demonstrated in animal

studies [31]) may be involved in the pathogenesis of HTN seen in the early stages of treatment with this hormone.

In a review of Epo utilization, Wolfson *et al.* (32) reported that subcutaneous (SC) Epo dosing requirements to maintain a target Hg level increased over time (higher doses in patients on Epo over 24 months); but did not comment on the Epo dose in relation to changes in BP status. Baskin and Lasker, on the other hand, did note a connection between Epo and the development of HTN (33). They reported a series of patients in whom HTN developed after the initiation of Epo, while Hg and Hct levels remained the same and often decreased from baseline levels. All patients were African American, had a history of HTN and exhibited no increase in body weight due to circulatory overload (i.e., not an issue of inadequate dialysis). These authors argued that their results contradict the Hct-increase theory for Epo-induced HTN.

There are no specific reports of the HTN-Epo dose phenomenon in the literature (33,34,35). Since the pilot study, several reports have surfaced citing the fact that 40 to 60 percent of US dialysis patients have persisting anemia (Hct < 30) and/or continue to require blood transfusions despite therapeutic doses of Epo; and the frequency of functionally debilitated patients remains high (36,37,38,39,40). While none of these reports refer to the possible role of high BP in blunted patient response, it is clear that there is a continuing need to

evaluate the factors associated with Epo responsiveness to overcome the overwhelming persistence of anemia in this patient population.

So, in summary, while the phenomenon of Epo-induced HTN is indirectly reported, few have commented on the blunted hematologic response which appears to accompany this phenomenon.

2.4 Potential explanations for the observed phenomenon

The reasons for the discrepancy in Epo requirements described in the pilot study may lie in some underlying pathophysiologic phenomenon particular to HD patients. Muirhead *et al.* (18) made note of the fact that Epo-induced HTN is a phenomenon specific to the uremic state (i.e., HTN has not been reported as a side effect in patients with normal renal function receiving short or long term Epo therapy [33]), a fact which has been reinforced in animal studies (41,42).

In trying to determine the possible underlying mechanism for the pilot study's observation, the literature was approached from a more theoretical perspective. First, there is evidence of a relationship between the physiologic effects of Epo and a patient's underlying level of sympathetic nervous system (SNS) activity. Biaggioni *et al.* (43) postulated that autonomic nervous system failure is related to decreased Epo production. They proposed that, in normal circumstances, the SNS stimulates red blood cell production through increased

endogenous Epo production via beta-2 adrenergic receptors. Although they did not study CRF patients, they postulated that the development of anemia in CRF may be related to autonomic failure wherein decreased endogenous Epo secretion is a manifestation of renal denervation linked to reduced renin secretion (44); that is, a reduction in sympathetic stimulation of endogenous Epo production at the kidney production site. The authors suggested that elucidation of the mechanisms for anemia in patients with autonomic failure may provide insight into the causes for Epo-induced HTN, despite the fact that earlier work by Roger *et al.* had found contradictory results (45).

In related research, Hoeldtke *et al.* used Epo to treat orthostatic hypotension caused by autonomic neuropathy (46). Short term treatment in a small group of patients proved effective, with some patients developing HTN in the process. The postulated mechanisms for the pressor effects of Epo included: increased production of red cells, expansion of the red cell mass, and direct vascular activity (possibly related to increased Hg or increased blood viscosity).

More recent research has examined the role of endothelin-1 (as well as other vasoactive substances, such as serotonin [47]) in the pathogensis of Epo-induced HTN (48). Endothelin-1 (ET-1) is a local vasoactive mediator involved in the regulation of basal vascular tone at the level of the vascular endothelium

(i.e., a paracrine hormone system). Plasma levels of ET-1 have been reported to be elevated in HD and CAPD patients, with further elevations (with concurrent BP elevations) demonstrated in those receiving Epo therapy (49,50).

Base on this indirect evidence, the following scenario may provide a theoretical explanation for the phenomenon demonstrated in the pilot study. In an environment of increased vasoactive tone and HTN (as seen in CRF), it could be that the increase in vasoactivie activity associated with HTN (either preexisting or developing with the initiation of Epo therapy) causes a resistance to the effects of Epo at the RBC progenitor cell receptor level. This would be similar to the mechanism postulated to explain the association of insulin resistance with HTN (51). Such a situation might arise if the following conditions were to hold true:

1) patients with CRF cannot produce endogenous Epo due to kidney damage;
2) patients with CRF with preexisting HTN or predisposed to Epo-induced HTN have an elevated underlying sympathetic tone relative to patients who do not have (or develop) HTN;
3) constant exposure to an elevated sympathetic tone in the absence of an endogenous source of Epo results in down-regulation of Epo receptor sites;
4) down-regulation of Epo receptor sites results in suboptimal hematologic response when exogenous Epo is administered; and,
5) down-regulation of Epo receptor sites may or may not be a reversible phenomenon.

2.5 Other factors affecting Epo responsiveness

In addition to the possible role of HTN identified in the pilot study, it is necessary to take into consideration other factors which have been demonstrated to impact on the patient's response to Epo therapy. Several determinants of Epo resistance have been described in the literature (52,53,54). (Epo resistance has been loosely defined as the need for larger than normal doses of Epo to maintain a target Hg [35].) In patients with CRF, resistance is well known with iron or folate deficiency, both resulting in Epo-induced production of red blood cells that do not contain an adequate amounts of Hg; with aluminum toxicity or secondary hyperparathyroidism, the latter two causing iron replacement in the bone matrix and bone marrow fibrosis, respectively, and thus impeding the bone marrow response to Epo in terms of RBC production; and L-carnitine deficiency (55,56,57,58,59). Overcoming these factors so as to ensure the most benefit from Epo therapy is a routine consideration in the treatment regimen of patients on HD.

Angiotensin-converting enzyme inhibitors (ACEIs) are a common modality used in the treatment of HTN in both CRF and non-CRF patients. They lower BP by inhibiting the production of angiotensin II (a potent direct and indirect vasopressor). In addition, BP reduction is also thought to be achieved through increased production of vasodilatory prostaglandins (60). Given that HTN is a

common cause of CRF, and that other diseases known to lead to CRF are often treated with ACEIs (e.g., to delay diabetic nephropathy, to treat congestive heart failure), the likelihood that a HD patient will be on an ACEI is high. ACEIs have been studied to determine their effect on endogenous Epo production in both healthy volunteers and CRF patients (61,62,63,64). These studies have consistently demonstrated that an activated renin-angiotensin system stimulates endogenous Epo secretion, while production is blunted with the introduction of ACEIs.

Inadequate dialysis may affect both BP control and the anemic state in patients on HD. It has been demonstrated that insufficient removal of fluids through suboptimal dialysis is a factor contributing to HTN in these patients, although Ifudu *et al.* (65) did not specifically examine the interaction of this relationship with Epo dosing requirements. In addition, undertreated uremia through inadequate HD has a direct impact on the bone marrow's ability to produce adequate numbers of RBCs.

In both CRF (66,67) and non-CRF patients (the latter with anemia of chronic disease [68]), the presence of inflammatory cytokines has been demonstrated to suppress endogenous Epo production as well as inhibiting the action of Epo at the receptor site. And finally, antiEpo antibodies have been cited as being responsible for a blunted response to endogenous Epo in patients

with pure red-cell aplasia (69). (There has been no evidence of such antibodies as a cause for resistance in CRF patients treated with exogenous Epo.)

Thus, the literature has not identified a role for BP, or any other related phenomenon, in determining the Epo dose required to achieve a given hematologic response. It is important, however, that these other factors related to Epo resistance be incoporated into any evaluation of the HTN-Epo relationship.

3.0 Description of the Research:

3.1 Statement of purpose

The purpose of this project was to determine if there were additional factors (particularly, HTN) which could serve as indicators of the need for Epo dose modification to achieve target Hg levels in HD patients. If HTN were one such indicator, caregivers would be able to determine *a priori* those patients likely to respond inadequately to Epo. The next logical step would then be to investigate the possiblity of correcting this Epo resistance by treating the HTN so as to achieve improved outcomes and to ensure the efficient use of Epo overall.

3.2 Objectives

The objectives of the study were to:

a) assess the dose of Epo therapy required to achieve target Hg levels in HD patients;

b) collect data regarding physiologic and other parameters related to BP control; and,

c) assess the strength of the association between Epo dose and BP levels.

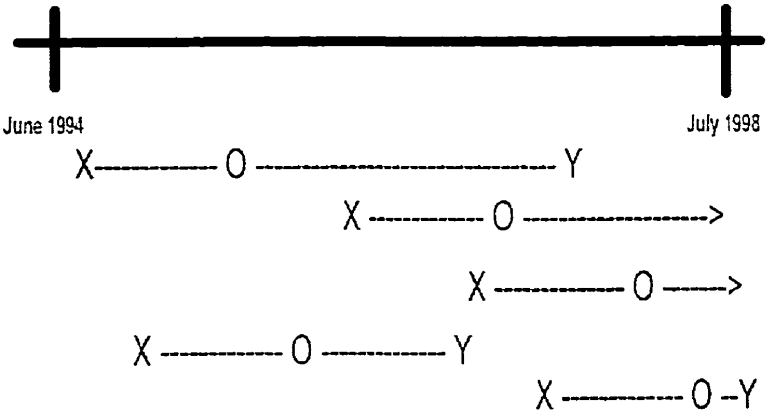
3.3 Hypothesis

The hypothesis for this project is that, after controlling for the initial level of anemia, the presence of HTN predicts the need for higher Epo doses in order to achieve the target Hg level.

The theoretical model and conceptual framework for this project are outlined in Appendix III. The underlying rationale supporting the plausibility of causation in the HTN-Epo relationship is summarized using Bradford-Hill's criteria in Appendix IV.

3.4 Research design

A graphical representation of the overall study design is outlined in Figure II. The study involved a retrospective review (in an historically prospective manner) of all HD patients commencing treatment (X) with Epo on or before July 31, 1998, going back in time as far as June 1,1994. Data for a single therapeutic inception cohort (i.e., all patients entered the study from the date of onset of Epo therapy) were collected over the first 4 to 6 months (4-6m) of uninterrupted Epo administration, until the target Hg level (approximately 115 g/L) was achieved (O). (If the defined target Hg level was not reached, the highest Hg level during the 4-6m window was used as the endpoint for the study.)



Legend: X = patient starts Epo therapy; O = patient's Hg level at 6 months; Y = Epo discontinued (ie. transplant, death, etc.)

Figure II. Research design.

3.5 Target population

The research was designed to evaluate the impact of a hemodynamic variable on Epo therapy requirements and hematologic outcomes in HD patients. The prescribing of Epo was centralized within two campuses of Ottawa's largest hospital (i.e., The Ottawa Hospital - General Campus and Civic Campus, serving a catchment area of 1 million people in the National Capital Region), with the former serving as the primary data collection site. Such centralization allowed for the generation of complete lists of HD patients and patients receiving Epo

(i.e., a clearly defined target population).

3.6 Inclusion criteria

The records of the following patients were reviewed: those on HD and receiving their initial 4-6m of Epo therapy between June 1, 1994 and July 31, 1998; and whose care was directed by physicians from The Ottawa Hospital - General Campus Nephrology group. All HD patients were assessed based on the intent to treat them with Epo, with prescribing being tightly controlled on a prospective basis and most patients meeting the criteria for Epo use outlined in Appendix II. Patient accrual started from July 31, 1998 and worked backwards in time in an effort to collect data for the required sample size (see Appendix V regarding sample size calculation).

3.7 Outcome measurements

Appendix VI outlines the outcome measures[b] that were established *a priori* for data collection, based on previous experience and potential Epo response modifiers as identified in the literature. All information was collected using standardized data collection formats within two databases (Appendix VII).

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[b] As appropriate, outcome measures (i.e., Hg, BP, albumin, etc.) reflect pre-dialysis measurements so as to control for the potential effects of changing extracellular volume as a result of the dialysis process.

For the purposes of this study, the main independent variable was BP (i.e., level after 4-6 months of Epo therapy) while the dependent variable was the Epo dose (in units/kg per week), both measured at the time that the target Hg level was achieved.[c] Both were measured as continuous variables, while BP was also evaluated based on categorical variables (see Appendix VIII for a complete description of methods related to the BP variable). In order to rule out disease progression as the major cause for new onset or worsened HTN, only BP elevations which occurred within the initial 4-6m of Epo treatment time frame were considered.

3.8 Analytic approach

Collation and analysis of the data were facilitated using a relational database (Access®) with its partner spreadsheet program (Excel®), and SPSS for Windows® for statistical analysis. Frequency distributions and descriptive statistics were used to summarize the data, and help determine the best approach to further analysis.

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[c] An adequate response to Epo was defined as achieving a target Hg of 115 g/L within four to six months of initiation of therapy on the usual therapeutic doses of 50-100u/kg SC 3 times per week.

The following summarizes the major analyses carried out:[d,e]

- a paired t-test was used to determine whether or not the use of Epo resulted in statistically significant increases in Hg levels (i.e., assessing the therapeutic effectiveness of the product);
- univariate analysis (one-way analysis of variance) was used to determine the impact of various categorical variables (e.g., baseline and 4-6m BP status and other BP categorizations, gender, smoking status, adequacy of HD, use of iron therapy, ACEI use, whether or not target Hg was achieved) on the dependent variable, as well as other related outcome variables (e.g., 4-6m Hg level, change in Hg, days to target Hg, BP increment);
- analysis of covariance was used to compare the 4 HTN groups in the conceptual model and their Epo dosing requirements, after adjusting for baseline Hg levels;
- correlation analysis was used to describe the relationship between the two continuous variables (i.e., BP increment and 4-6m Epo dose); and to determine the degree to which the various parameters used to measure

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[d] As noted in Appendix V, the samples size was calculated to provide sufficient power for these analyses to detect differences where they exist.

[e] It should be noted that no adjustment was made to the alpha levels of univariate tests reported by this project in consideration of the fact that they were used repeatedly.

BP were redundant, so as to determine which BP measures would be most useful in the multiple regression analysis.

On the basis of the results of these analyses, those variables with significant effects on the 4-6m Epo dose were selected for inclusion in the multiple regression analysis. This approach to analysis was chosen as it would allow evaluation of the degree and importance of the influence of the various factors affecting the HTN-Epo dose relationship, as well as the interactions amongst these factors themselves. A stepwise approach was used to identify which independent variables would be useful predictors in the multiple regression model (70,71,72).

4.0 Results:

4.1 Sampling frame

The population of patients considered for inclusion in the study consisted of those beginning Epo therapy between June 1, 1994 and July 31, 1998. A total of 253 patient charts were reviewed to determine eligibility for inclusion, with a total of 111 (44%) meeting the criteria for the study. An initial investigation of treatment patterns in the Ottawa region had suggested that patient accrual would take place over a much shorter time frame. The reasons for the low accrual/high exclusion rates from the identified sampling frame are outlined in Figure III.

Figure III. Reasons for exclusion from sampling frame.

a)	the patient had not received Epo for a sufficient period of time (i.e., 4-6m of continuous therapy);
b)	there were insufficient data in the patient chart to allow evaluation of the Epo dose - BP relationship;
c)	the patient had not been treated with HD during the first 4 to 6 months of Epo therapy (e.g., epo used pre-dialysis or transplantation);*
d)	the patient was treated via continuous ambulatory peritoneal dialysis (CAPD; the drug database used was unable to differentiate patients based on the type of dialysis treatment); and,
e)	the patient received Epo for a non-renal indication (e.g. cancer; the drug database used to identify the population for the study was unable to exclude all non-renal patients).

* while the use of Epo in pre-dialysis patients has been reported, it was not a common method of treatment in this patient population (73)

4.2 Patient sample

Table 2 summarizes the general demographic data for those included in

the study. General summary information regarding dialysis and iron therapy for this sample of 111 patients is provided in Appendices IX and X.

Table 2. General demographic data.

Parameter	Description
gender	54% male
race	5% afro-Canadian 4% arabic 9% asian 82% caucasian (55% male)
age at time of dialysis (mean)	57 years (range: 18-88)
age at time of Epo initiation (mean)	57.5 years (range: 18-88)
mean baseline weight; BMI*	71.2 kg (range: 43.5-122); 26.6 kg/m^2 (range: 18.2-49.0)
mean 4-6m weight; BMI	70.2 kg (range: 41-116); 26.2 kg/m^2 (range: 16.3-43.6)
disease causing CRF	26% diabetes 13% HTN 7% IgA nephropathy 5% polycystic kidney disease 3% Wegner's granulomatosis 3% cancer-related 43% "other" (e.g., post-operative renal failure, chronic pyelonephritis, hydronephrosis, lupus, scleroderma, membranous glomerulopathy, post-stroke or myocardial infarction, failed transplant, Alport's syndrome, unknown)
smoking status	16% yes (mean 32-pack-year history of smoking) 15% former smoker (duration not well documented) 32% no 36% unknown

* body mass index

4.3 Descriptive Epo treatment data

Table 3 summarizes the data regarding Epo therapy for the study sample

overall. All patients received Epo via the SC route, reflecting the current thinking on the most cost-effective method of administration (74,75). Analysis demonstrated that the absolute values for the dependent variable (i.e., the 4-6m Epo dose u/kg per week [/w]) were not normally distributed (see Appendix XI). Logarithmic (base 10) transformation of these data created a normal distribution and decreased the number of extreme values that could influence analytic outcomes (see Appendix XI). Analyses were carried out using both the absolute and log transformed values of the dependent variable. As the findings from these analyses were quite similar in magnitude, presentation of results has been limited to those based on the absolute values as the latter are more transparent and intuitive from a clinical perspective.

Table 3. Epo therapy information.

Parameter	Description
mean time between initiation of dialysis and initiation of Epo therapy	103.4 days (range: -239 to 3787)
mean Epo dose (per week)	initial: 8,946 units (range: 2,000-30,000) 4-6m: 10,149 units (range: 2,500-30,000)
mean weight-adjusted Epo dose (per week)	initial: 130.3 units/kg (range: 40-446.8)* 4-6m: 150.7 units/kg (range: 30.7-501.0)* ** p<0 .008, 95% CI of difference 5.3 to 35.3*
adverse events while on Epo	16% vascular access blockage 1% deep vein thrombosis and AV fistula occlusion

4.4 Descriptive outcomes data

As noted in the Background material, measures of hematologic and hemodynamic outcomes were key variables that could play a role in the HTN-Epo dose relationship. Table 4 summarizes the baseline and 4-6m Hg outcomes, while Figure IV depicts the baseline and 4-6m BP outcomes in terms of whether or not Epo-induced HTN developed during the treatment period.[f] Frequency distributions for baseline and 4-6m Hg levels and the absolute change in Hg are provided in Appendix XI. Data summarizing baseline and 4-6m BP control (Yes/No), categories (based on JNC-VI), the severity of Epo-induced HTN by category, and absolute BP increments are depicted in Appendices XII and XIII.

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[f] No patients had Epo therapy permanently withdrawn during the study period due to severe and uncontrollable HTN.

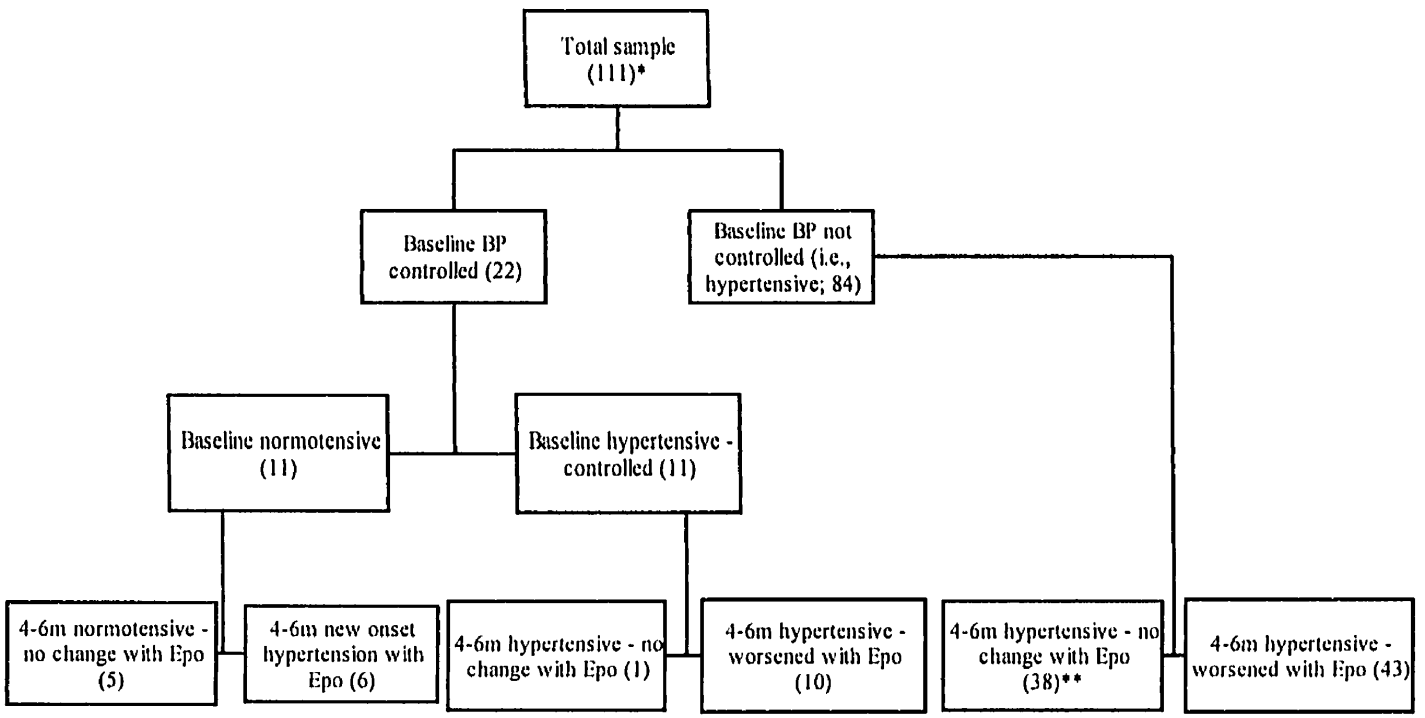
Table 4. Overall hemoglobin data (g/L).

Parameter	Mean	Median	Standard Deviation	95% CI	Range
initial Hg	88.05*	87	13.29	2.47	52-136
4-6m Hg	122.11*	123	14.6	2.71	92-157
absolute change in Hg	34.05*	34	18.27	3.4	-9 to 78
relative change in Hg (%)	41.5	37.84	26.17	4.87	-6.62 to 128.07
days to target Hg	121.15	119	26.55	4.94	84-188
rate of rise of Hg/month	8.93**	8.93	5.35	0.995	-2.27 to 24.64
relative rate of rise of Hg/month (%)	10.85	10.97	10.97	1.36	-1.67 to 36.59

* *p<0.0001, 95% CI of difference 30.62 to 37.49*

** *dose of Epo to increase Hg by 10 g/L calculated to be 44u/kg/w for the time frame of the study*

Figure IV. Overview of Epo-induced hemodynamic outcomes.



* unable to assess initial BP status of 5 patients in sample
** unable to assess 4-6m BP status of 8 patients in sample

4.5 Data excluded from further analysis[g]

A number of the data parameters outlined in Appendix VI were either not readily and/or consistently accessible during the study period; or were common to almost all patients and, thus, were not useful as discriminating factors regarding the dependent variable of concern (i.e., 4-6m Epo dose u/kg/w). In addition, parametric analysis of the relationship between the major outcomes of interest and some of the other parameters listed in Appendix VI demonstrated no statistically significant relationship. Figure V summarizes the parameters excluded from further analysis (particularly the multiple regression analysis), and reasons for exclusion.

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[g] There are a number of additional analyses that could be carried out using the data available through this study (i.e., iron status and/or administration, nutritional status/serum albumin, mean arterial pressure, etc.). It was beyond the scope of the study to evaluate all aspects of care in these patients at this time.

Figure V. Data excluded from further analysis.

Parameter	Reason for exclusion
adverse events	low frequency
aluminum therapy	only prescribed in two patients in sample
anabolic steroids	not prescribed to any patients in sample
antihypertensive medications	ACEIs were evaluated (see analysis below); too wide a variety of medications and combinations involved to go beyond descriptive reporting
antiplatelet therapy	data lost
BMI	no significant change in this parameter over study period
dialysis frequency per week	vast majority of patients on HD 3x/week
dialyzer	vast majority of patients used a single model of dialyzer
gender	no significant impact of gender on 4-6m Epo dose, or other related parameters (Hg, change in Hg, days to target Hg, BP increment)
hematocrit	not measured by hospital laboratory
hyperparathyroidism	not routinely assessed and/or documented
other underlying disease states	wide-ranging and multiple concomitant diseases documented for most patients, with too many different scenarios to allow evaluation of influence of other disease states on outcome (only 10% of patients had no concomitant disease documented along with CRF)

Parameter	Reason for exclusion
presence of residual renal function	vast majority of patients fully HD-dependent
smoking status	no significant impact of smoking status on 4-6m Epo dose, or other related parameters (Hg, change in Hg, days to target Hg, BP increment)
time on dialysis (per session)	more important indicator is dialysis efficiency, Kt/V (see analysis and discussion re: adequacy of HD)
use of iron therapy (and, by implication, the dose of iron therapy and total IV iron)	no significant impact of use of iron therapy on 4-6m Epo dose, or other related parameters (Hg, change in Hg, days to target Hg, BP increment)

4.6 Outcomes based on Hg target

As previously noted, the target Hg level typically used as the treatment goal for patients receiving Epo therapy is 115 g/L. Data were analyzed to determine if there were any systematic differences between patients who were able to achieve this target within the 4-6m time frame and those who did not. The analysis (see Table 5) did not reveal any statistically significant differences in 4-6m Epo requirements, days to target Hg (or highest Hg achieved within 4-6m time frame for those with Hg <115), total IV iron dose administered or BP increment. There was a statistically significant difference in the absolute Hg difference between baseline and the 4-6m endpoint ($p<0.0001$, 95% CI 13.2 to 25.1), consistent with the case definition of reaching the therapeutic target.

Table 5. Outcomes based on Hg target.

Parameter (mean)	Hg <115 n=31	Hg≥115 n=80	Significance
4-6m Epo dose u/kg/w	145	152.9	NS (p=0.664)
change in Hg	20.3	39.4	p<0.0001
days to target Hg	122.8	120.5	NS
total IV iron dose (mg)	1075	993.9	NS*
BP increment (mm Hg)	15.3	15.5	NS

* There has not been a complete analysis of all of the iron related data collected as a part of this study (i.e., iron stores, transfusions, etc.). Thus, the "not significant" status of this variable must be interpreted with caution.

4.7 Outcomes based on conceptual model of HTN

The conceptual model of HTN groupings derived from the pilot study (outlined in Figure I above) formed the basis of the initial analysis to examine the relationship between HTN and the Epo dosing data (see Table 6). Analysis of variance demonstrated that there was a statistically significant difference in the 4-6m Epo dose required (p=0.042) between groups 2 and 3 (i.e., between initially normotensive patients who developed Epo-induced HTN versus those with underlying HTN whose BP remained stable).

Table 6. 4-6m Epo dose based on conceptual model of HTN.

Parameter	Number of patients	4-6m Epo dose u/kg/w (mean)
group 1 - underlying HTN which worsened with Epo	53	149.8
group 2 - initially normotensive, with Epo-induced HTN	6	242.6*
group 3 - underlying HTN which remained stable	38	138.7*
group 4 - consistently normotensive	5	176.4

* p=0.042, based on 102 evaluable cases

4.8 Outcomes based on development of Epo-induced HTN

In addition to using the conceptual model as a means of evaluating the data, one can also approach the data from the perspective of the 6 subgroups

outlined in the 4-6m time frame of Figure IV to give a more refined assessment of the HTN-Epo dose relationship. As noted in Appendix XIV, there was a trend in the relationship between the Epo-induced HTN outcome category and the dependent variable, but it was not statistically significant (p=0.097). Further analysis based on a simple dichotomous variable (Epo-induced HTN - Yes/No) revealed no significant relationship with the 4-6m Epo dose required (p=0.349).

4.9 Outcomes based on other measures of BP status

There were several other BP-related variables for which data were collected to allow for an evaluation of the relationship between BP status and Epo dosing requirements from a number of angles (see Appendices XII to XV for descriptive data summaries of these variables). Initial BP status was evaluated based on a dichotomous variable (BP controlled - Yes/No), as well as being categorized on the basis of the JNC-VI classification system (see Appendix VIII). Analysis of the impact of baseline BP on the dependent variable indicated no difference in mean 4-6m Epo dose based on initial BP control status or JNC-VI category. A similar analysis using 4-6m BP control and categories also demonstrated no impact on the 4-6m Epo dose (see Appendix XV). Finally, the BP increment and the dependent variable were found to be poorly correlated (Pearsons correlation, r= -0.023).

Baseline and 4-6m BP control status (dichotomous variable) were also evaluated to determine if BP status had an impact on other parameters related to the dependent variable. Both baseline and 4-6m BP control had a statistically significant impact on the BP increment (see Appendix XIII), but not on the baseline or 4-6m Hg, change in Hg or days to target Hg.

4.10 Outcomes based on use of ACEI therapy

Twenty percent of patients receiving Epo were also on ACEI therapy at some point during the 4-6m evaluation period. An assessment was made of the impact of ACEI therapy on the dependent variable, as well as on other related parameters, with statistically significant results shown in Table 7. There were no significant differences in baseline Hg levels, days to target Hg and total IV iron therapy based on the presence or absence of ACEI therapy.

Table 7. Impact of ACEI use on outcomes.

Parameter (mean)	ACEI - yes (n=22)	ACEI - no (n=88)	Significance
4-6m Epo u/kg/w*	112.7	160.6	p=0.018, 95% CI 8.3 to 87.3
4-6m Hg level*	115.7	123.8	p=0.018, 95% CI 1.41 to 14.9
change in Hg (g/L)	21.9	37.1	p=0.001, 95% CI 7.1 to 23.5
BP increment**	24	13.6	p=0.025, 95% CI 1.3 to 19.4

* ACEI therapy impacts on the 4-6m Epo dose independently of whether or not the target Hg (115) is achieved.

** 59% of those on ACEIs had worsening of their HTN upon introduction of Epo therapy.

Based on the impact of the presence of ACEI therapy on the dependent variable, analysis of variance was carried out to determine whether ACEI use interacted with any of the other parameters shown to affect the 4-6m Epo dose requirement. There was particular focus on the measures of BP, given the common links between ACEIs, BP/HTN, anemia and Epo in the treatment of CRF patients.

There was no difference in baseline BP control or BP category; BP outcomes (based on the conceptual model for HTN); the development of Epo-induced HTN; 4-6m BP control, JNC-VI BP category or degree of change in BP from baseline, based on the use of ACEI therapy. There was a trend (p=0.055) towards a relationship between ACEI use and overall BP outcomes, and ACEI

use did appear to be significantly related to the severity of Epo-induced HTN (p=0.018).

4.11 Other outcomes

There was a trend towards a relationship between the adequacy of HD and the 4-6m Epo dose, but it was not statistically significant (p=0.159). The adequacy of HD was not shown to have a significant impact on any other related parameter (i.e., 4-6m Hg, change in Hg, days to target Hg, or BP increment).

4.12 Results of regression analysis

As a result of the analyses above, the variables outlined in Figure VI were selected for evaluation by stepwise multiple regression analysis. These variables were found to have a statistically significant effect (or a strong trend towards an effect) on the dependent variable in the aforementioned analyses; or were impacted significantly by ACEI use, the main independent variable found to have an effect on the 4-6m Epo dose in the analyses above.

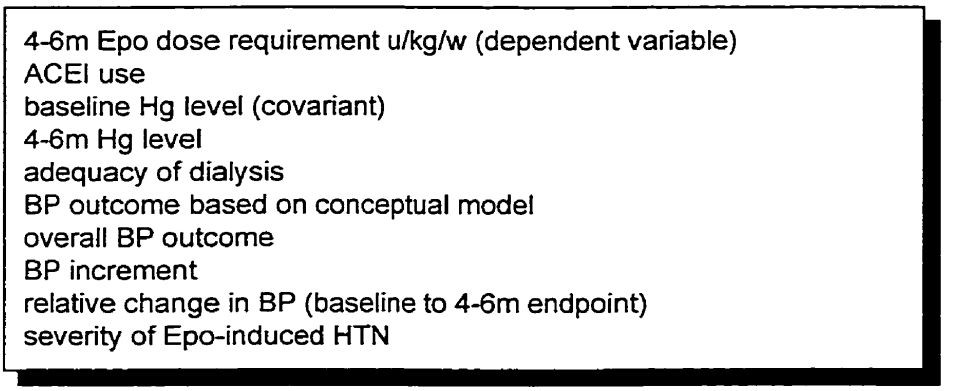

Figure VI. Variables for inclusion in stepwise multiple regression.

Several of the HTN-related variables in Figure VI reflect different measures of BP outcome subsequent to Epo therapy. The degree of correlation and covariance amongst these variables was assessed using both Pearson and Spearman methods (as both continuous and categorical variables were involved). The results of this assessment are outlined in Table 8. Given the high degree of correlation amongst these variables, it was necessary to chose one variable for use in the regression model. As the BP conceptual model variable[h] was strongly correlated with all of the other BP variables, it was chosen to represent BP within the regression model.[i] Finally, as a means of determining

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[h] This variable represents the 4 main HTN groups described in the conceptual model underpinning this project.

[i] The impact of changing the BP variable was assessed *post hoc* by running the multiple regression using all variables concurrently, as well as only one BP outcome variable at a time. These additional analyses did not change the results.

the impact of extreme values of the dependent variable (i.e., 4-6m Epo dose > 300u/kg/w), such cases were excluded from the regression analysis in additional *post hoc* tests.

Table 8. Correlations between BP outcomes (Pearson/Spearman's).

	overall BP outcome	BP conceptual model	severity of Epo-induced HTN	relative change in BP	absolute BP increment
overall BP outcome					
BP conceptual model	0.416**/ -0.637**				
severity of Epo-induced HTN	0.264**/ 0.522**	-0.732**/ -0.873**			
relative change in BP	-0.312**/ -0.201	-0.440**/ -0.395**	0.243*/ 0.362**		
absolute BP increment	0.055/ 0.277**	-0.715**/ -0.815**	0.560**/ 0.795**	0.631**/ 0.649**	

** correlation significant at 0.01 (2-tailed)
* correlation significant at 0.05 (2-tailed)

The primary regression analysis indicated that ACEI use (Yes/No) and the adequacy of HD (Yes/No) were the two main predictors of the 4-6m Epo dose based on the data available from 97 cases in the study. The following is the regression equation describing the relationship amongst these variables:

4-6m Epo dose = 149.7 - 57.4 (ACEI use: Y=1, N=0) + 40.6 (HD adequacy: Y=1, N=0) **requirement**

Table 9 provides a summary of the model components (#1) and the statistical significance. Overall, the model described 10.7% of the variability in the dependent variable, with 5.7% being attributable to the ACEI use variable and 5% to the HD adequacy variable.

Table 9. Multiple regression models and significance.

Model description (number of cases)	R/R^2	Significance	Constant	Coefficient(s)
#1) predictors = ACEI use and HD adequacy (n=95)	0.326/0.107	p=0.006	149.7	ACEI = -57.4 HD = 40.6
#2) predictors = ACEI use (n=91)	0.246/0.061	p=0.019	144.6	ACEI = -34.2

When the extreme 4-6m Epo dose values (4 cases) were removed from the analysis, the HD adequacy variable disappeared from the model (#2) leaving ACEI use as the main predictor of the 4-6m Epo dose (accounting for 6.1% of the variability in the dependent variable).

5.0 Discussion:

5.1 Overall results

Patients evaluated as a part of this study were in many ways fairly typical of HD populations seen in the literature regarding the use of Epo to achieve the target Hg levels described, in terms of gender distribution, age range, reported underlying disease states, and dialysis prescription. (Studies evaluating the use of Epo to "normalize" hematologic indices in CRF are beyond the scope of this project; but it is of note that early results point to problems with this approach in patients with concurrent cardiac disease [76,77].)

The acuity of this sample of patients was not measured (e.g., APACHE scores, Karnofsky scale, etc.) as part of the study; but anecdotal reports, the low accrual rate and the frequency of CAPD initiation and/or switches during the same time frame (due to HD bed shortages) suggest that this group may have contained relatively more unstable patients than would otherwise have been the case. This factor must be kept in mind while interpreting the results of this study.

Another indicator that this is likely a high acuity patient population is the low rate of adequate dialysis[j] reported in the sample (see Appendix IX).[k] It may

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[j] Inadequate HD is defined as a <65% reduction in the blood urea nitrogen [BUN] concentration subsequent to a dialysis session, or a Kt/V ≥1.3 for ≥3.5 hours of HD.

[k] Note: the adequacy of HD (i.e., Yes/No) was determined on the basis of the Kt/V calculated for HD sessions where a pre and post BUN level was assessed. If the majority of Kt/V values during the period of Epo therapy were ≥1.3, then the HD was

be more difficult to adequately dialyze unstable patients, as they may experience more frequent episodes of adverse events related to HD. Given that more intense dialysis (i.e., "adequate" dialysis) has been associated with some degree of correction of anemia, the need for lower doses of and/or elimination of the need for Epo (78,79,80) and has been used as a mechanism for BP control (81), this aspect of care (as well as better ways of measuring dialysis adequacy [82]) may need more attention as we endeavour to overcome factors contributing to Epo resistance (65). This has been reinforced by a recent study by Lebel *et al.* In studying the mechanism of Epo-induced HTN within the first three to four months of Epo therapy, they concluded that "optimally reducing extracellular fluid volume to prevent, at least in part, the development of hypertension" was an important factor in the improved uremia anemia in HD patients (83). Volume-independent meachnisms for the Epo-induced BP increase were not excluded by the authors.

5.2 The HTN-Epo relationship

As demonstrated in Table 6 above, there was a significant difference in the 4-6m Epo dose requirements of initially normotensive patients who developed Epo-induced HTN vs. patients with underlying HTN whose BP

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considered to be adequate overall.

remained stable throughout the treatment period. It is of note that the group that remained normotensive throughout also had higher (but not statistically significant) 4-6m Epo requirements relative to the two baseline HTN groups.

It is difficult to compare these results to those from the pilot study, as there is much greater skewing of the patient population in this project (i.e., many more patients were baseline hypertensive [n=91] vs. baseline normotensive [n=11], compared to a more even split in the pilot study). However, these results are similar to the pilot study (see Table 1 above) to the extent that Epo requirements did not appear to differ in those with pre-existing HTN regardless of the development of Epo-induced HTN in either case. However, unlike the data in the pilot study, normotensive patients appeared to require relatively more Epo to reach target Hg levels than baseline hypertensive patients regardless of whether or not they developed Epo-induced HTN.

As noted above, there were a small number of patients in the baseline normotensive group and the presence of extreme values for the dependent variable could not be excluded as a factor in the results obtained. This, and the fact that this phenomenon is counter-intuitive to the original theory, prompted a reassessment of the impact of HTN on Epo dose using an analysis of variance which excluded 4-6m Epo dose requirements greater than 300u/kg/w (i.e., an arbitrary cut off to capture the majority of the extreme values shown in the

frequency plot in Appendix XI). By excluding these data, 2 of the 6 patients were lost from group 2 and the analysis no longer demonstrated a significant difference in the dependent variable amongst the groups in the original model (data not shown).

Thus, based on all of the measures of HTN used in this study (i.e., the 4 groupings in the conceptual model for HTN, outcomes based on the development of Epo-induced HTN [refined categories and dichotomous variable], outcomes base on initial and/or 4-6m BP status [categories and dichotomous variable], or absolute BP increment), there was no demonstrable evidence of the impact of HTN status on Epo dosing requirements as had been implied in the pilot study.

5.3 Impact of angiotensin-converting enzyme inhibitors

Analysis of the impact of ACEIs on some of the parameters of interest in this study (see Table 7 above) demonstrated some expected as well as some unexpected results. The impact of ACEI on Hg levels was, in isolation, consistent with documented cases of ACEI-induced anemia as previous discussed; that is, the use of ACEI therapy resulted in lower 4-6m Hg levels and a smaller relative change in Hg over the treatment period. The other factor to consider was the concommitantly and significantly lower dose of Epo seen in

patients on ACEI therapy. In this situation, analysis revealed that the lower Hg levels attributable to ACEI therapy were independent of the effect of ACEI therapy on the dependent variable.

As noted in the Background material, the blunting effect of ACEIs on endogenous Epo production has been consistently demonstrated in both healthy volunteers and CRF patients (see Figures VII and VIII), often with anemia developing over time no matter the patient population. Pratt *et al.* postulated that ACEI-induced decreases in endogenous Epo levels in health volunteers was caused by inhibition of angiotensin II production, thus inhibiting the enzyme's direct effect on Epo secretion (61). Two points must be noted, however. First, this and the previously cited studies only describe the Epo-ACEI relationship in those patients capable of producing sufficient amounts of their own Epo to maintain Hg levels. In addition, the effect appears to take place at the point of Epo secretion or production (84), rather than at the effector level. This may be an important distinction for HD patients on Epo therapy, as their need for Epo may be as much related to decreased endogenous Epo levels (due to renal disease and, thus, interference in the renin-angiotensin-aldosterone axis) as it is to red blood cell destruction by dialyzers (85).

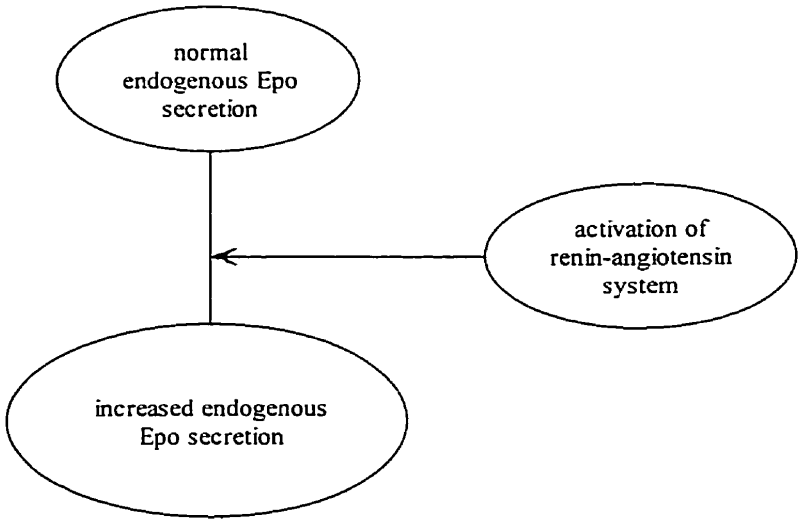

Figure VII. Normal endogenous Epo stimulation.

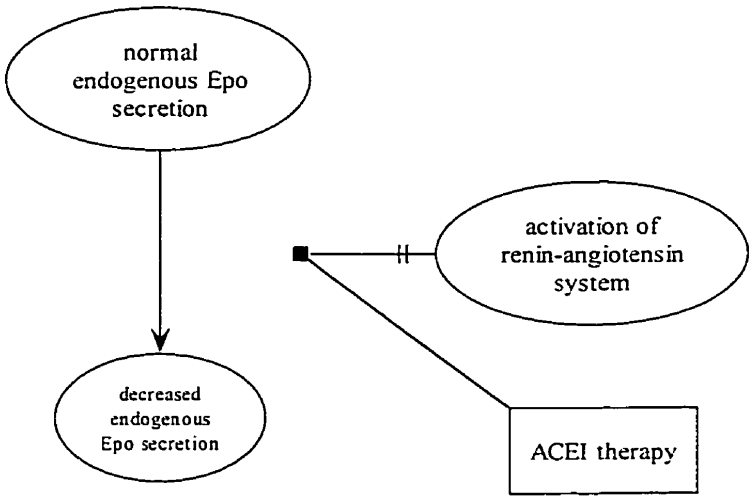

Figure VIII. ACEI-blunted endogenous Epo stimulation.

The presence of significantly lower Epo dosing requirements in patients receiving ACEI therapy was not an expected outcome of the study. Based on both theoretical and empirical evidence, the expectation had been that these patients would require higher doses of Epo therapy to achieve similar (or even lower) Hg levels as those not on ACEIs. In addition to the primary analysis, two additional analyses reinforced the relationship observed. *Post-hoc* analysis of the subgroup of patients reaching the target Hg (≥115) revealed a slightly wider difference in mean Epo dose requirements based on ACEI use than that described in Table 7 (ACEI/no = 162.5 vs. ACEI/yes = 107.4 u/kg/w [p=0.047, 95% CI 0.6 to 109.4]). The stepwise multiple regression analysis also reinforced the importance of the concomitant use of ACEIs in HD patients receiving Epo therapy as a determinant of 4-6m Epo dosing requirements (based on the data collected).

Another unexpected outcome seen in this study was the fact that ACEI patients had greater BP increments than their counterparts not on ACEI therapy. The significance of this result is unknown, as data were not systematically gathered to address this phenomenon.

5.3.1 Evidence from the literature

Examination of the literature revealed few investigations examining the

impact of ACEIs on the clinical efficacy of exogenous Epo therapy. Heb *et al.* (86) evaluated the correlation between doses and duration of ACEI therapy and Epo dosage requirements (to a target hematocrit level) in the first 18 months of therapy in HD. All patients were on antihypertensive therapy, with half receiving ACEIs as part of their regimen. The cumulative dose of Epo (in u/kg) was consistently greater in those patients on ACEI therapy, reaching statistical significance at 15 and 18 months of therapy. The authors were able to exclude other potential causes of Epo resistance, and concluded that an interaction between ACEIs and Epo may be responsible for the discrepancy. Similar results have been reported by Albitar *et al.* (87), specifically with enalapril.

A cause for the apparent interaction identified by Heb and Albitar has been postulated by Yaqoob *et al.* (88). In their search to understand the pathophysiology of HTN in patients receiving Epo therapy, the authors initiated Epo in severely anemic (Hg < 70 g/L), normotensive HD patients. They demonstrated decreased plasma renin and aldosterone levels, suggesting that exogenous Epo inhibited the renin-angiotensin system through a negative feedback loop between circulating Epo concentrations and renin secretion (see Figure IX). (Recall from above that, in normal circumstances, an activated renin-angiotensin system would cause angiotensin II secretion which would, in turn, activate secretion of endogenous Epo.)

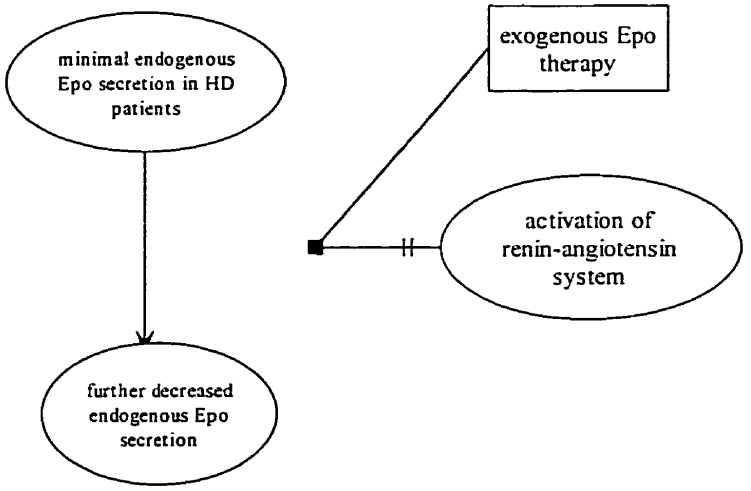

Figure IX. Epo-blunted endogenous Epo stimulation.

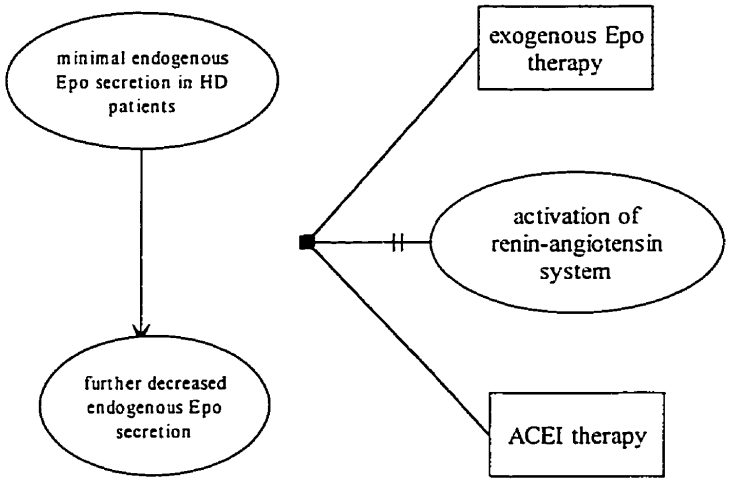

Figure X. Epo + ACEI-blunted endogenous Epo stimulation.

Thus, the suggestion is that ACEIs further suppress the already reduced endogenous Epo production (cause by exogenous Epo administration), leading to increased Epo dosing requirements to achieve the same hematologic outcome (see Figure X). Heb *et al.* suggest that the use of ACEIs to treat Epo-induced HTN in HD patients may, in fact, result in a vicious circle of blunted Epo response, increased Epo dose, increased HTN, increased ACEI dose, blunted Epo response, etc.

It should be noted that the literature is by no means homogeneous in terms of opinions as to the existence of an ACEI-Epo interaction in HD patients. For instance, Schwenk *et al.* (89) examined a cohort of HD patients on Epo therapy for at least one year, and evaluated the impact of the addition of an ACEI over a 3-12 month time frame, controlling for other factors that affect Epo responsiveness. From baseline to the 12-month endpoint, there was no significant change in Epo dose and no decrease in hematocrit levels that could be attributed to the introduction of ACEI therapy.

The most notable problem with all studies (i.e., those supporting and those refuting the ACEI-Epo interaction) is the small number of patients involved in each citation. There is great variability in study design, making comparisons amongst these investigations difficult. The other factor not taken into consideration in this project or in studies from the literature is that of endogenous

Epo levels. Did the patients who did not receive ACEI therapy have systematically lower endogenous Epo levels which resulted in a need for higher exogenous Epo doses? The question then becomes, "should we start measuring baseline endogenous Epo levels in patients to whom we plan to give ACEI therapy?" The absence of a reliable dose-response relationship between plasma Epo levels and RBC production makes that question somewhat of a moot point (90). There is a need, however, for a comprehensive evaluation of this phenomenon using a large group of patients over a clinically significant time frame (e.g., one year) to derive a true characterization of any interaction which might exist between these commonly co-administered drug products.

5.3.2 What does all of this mean?

The underlying significance of this finding is unclear. If the physiologic argument (see above) were to hold true, then the patients in this study who were on ACEIs should have required more Epo to overcome direct ACEI-induced anemia and/or impeded renin-angiotensin system activation of endogenous Epo. The expected physiologic response did not seem to have occurred in this cohort of patients, as demonstrated by the lower Epo dose in ACEI/yes patients. The Epo dose increased (rather than decreased, in relative terms) in those who were not on ACEIs and, at the same time, achieved higher Hg levels. On one hand, a

potential means of determining the "real" outcome would be to normalize all patients to a Hg of 115 and then assess 4-6m Epo dose differences based on ACEI use (this analysis has not be carried out to date). On the other hand, it is entirely possible that, as in the pilot study, this result is purely a phenomenon of the perils of subgroup analysis.

Whether it is the relationship described in the literature or that described by this study which holds true, from either perspective there is cause for concern in the use of ACEIs in HD patients on Epo. Hypertensive patients with CRF have an inherently activated renin-angiotensin-aldosterone system which is then further activated with HD, so the desire to use ACEI therapy is physiologically rational. However, the clinical decision becomes one of whether the risk of potential anemia via ACEI use (with or without Epo therapy) can be balanced against the need to treat HTN in this patient population in an effort to minimize the adverse effects of uncontrolled BP on other organ systems. ACEIs may ultimately prove to be a less desirable choice in HD patients if they lead to a predisposition for enhanced Hg support (i.e., transfusions, higher Epo doses, etc.). The use of new angiotensin-II receptor antagonists (e.g., losartan) may overcome the detrimental effects of ACEI on anemia and/or Epo-responsiveness without compromizing the BP control available through the manipulation of the renin-angiotensin-aldosterone system (91).

5.4 Multiple regression analysis

Stepwise multiple regression analysis revealed that both ACEI use and HD adequacy were predictors of 4-6m Epo dose requirements. The severity of Epo-induced HTN also came close to being an important predictor ($p=0.058$) in this model. One of the over-riding concerns throughout the analysis of the data for this project has been the impact of outliers. While most of the parametric analyses were not significantly impacted by the exclusion of these outliers, this same manipulation of the data changed the regression model such that only ACEI use was found to be a significant predictor.

Regardless of the impact of outliers, both models were able to explain only a small amount (approximately 11% or less) of the variability in the 4-6m Epo dose. Thus, the question of what the most important indicator of Epo response is for HD patients remains. Further studies should rule out the role of endogenous Epo levels, the absolute dose of ACEIs, the role of antiplatelet agents and other factors so as to develop a greater understanding of predictors of response to Epo therapy.

5.5 Limitations of the study

5.5.1 General limitatons

Since the purpose of the project was to determine plausible factors that

might contribute to the postulated HTN-Epo dose phenomenon, and considering the early stage of hypothesis development, an observational, descriptive study using a retrospective, longitudinal design was deemed to be the best approach. Another benefit of following such a design was the avoidance of bias in patient selection.[1] It was assumed that the proposed cohort would reflect the usual distribution of HTN status within this patient population pre-Epo; and, subsequently, that the 4 HTN groups evaluated at the end of 4-6m of therapy would be representative of the HD population subgroups which they exemplify.

However, the retrospective nature of the review and the reliance on information gleaned from the medical record inevitably resulted in difficulties related to data retrieval, especially for patients who had been receiving Epo for several years. In addition, the retrospective design decreased the probability of determining the exact nature and relative importance of any relationship between BP and Epo (although the short time frame allowed for the definition of Epo-induced HTN alleviated this problem to some extent), let alone determinining whether that relationship had any impact on QOL (92). An ideal study design would have involved prospective evaluation of outcomes in patients assigned to 4 mutually exclusive subgroups (based on some predetermined definition of

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[1] Had a single point in time been chosen for cohort selection, the investigator would not have been able to assess those patients not achieving 4-6 months of therapy due to adverse effects [including HTN], etc.

HTN). Under the circumstances, however, this approach was not justifiable. Given the very early hypothesis testing stage of investigation, a prospective protocol would have been premature and an inappropriate use of scarce research funds.

It had originally been anticipated that no more than 10% of the expected study population would be lost to follow-up, transfer out of U of O's dialysis network, or switch to CAPD. Lower than expected accrual rates (due to HD bed shortages[m] and, thereby, more CAPD use during that time) resulted in only 111 evaluable cases being available during the time frame defined. It was not felt to be reasonable to extend the data collection period into 1993 or earlier, as data became progressively more scarce and difficult to retrieve.

It was beyond the scope of this project to measure the endogenous Epo levels of patients involved in the study. As noted in the discussion above, this may be a key physiologic parameter that accounts for the variability in outcomes and absence of relationships between some parameters and the dependent variable.

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[m] Bed shortages occurred in spite of long known expectations of increasing need based on changes in population demographics and improved outcomes in other disease categories (e.g., improved cancer and post-myocardial infarction therapy, but increases in the number of compromised patients with end organ damage who will eventually need HD). An announcement was made by the Ontario government to commit additional HD beds for the region in the fall of 1998, but these addition facilitites still have yet to be realized as of the summer of 1999.

5.5.2 Sources of bias (93)

Selection bias might have affected the research project. It could be argued that, in fact, the group of HD patients that received Epo was not representative of the entire HD population that would be eligible for Epo therapy. Consequently, the data in Table 2 were evaluated for their consistency with dialysis-specific and/or population-based demographic information from the Canadian Organ Replacement Register (CORR; 94) and on-line data from Statistics Canada (95).

According to the CORR, the average age of new dialysis patients in 1996 (the most recent data available) was 60 years, only slightly higher than the mean age of patients starting dialysis in this study. The slightly higher proportion of male patients in the study population is also consistent with Canadian trends in the adult age group (i.e., 15 to 75 years of age); but differs from the overall population gender distribution of 49.5% males. In 1996, the total Canadian visible minority population made up 11.2% of the total population, whereas the study population was made up of 18% visible minority patients. It is not clear from the CORR database whether this difference in the racial backgrounds of HD patients is specific to the study group or consistent with other HD populations across the country.

Finally, the primary cause of renal disease in the study population was

similar in many instances to that reported via the CORR database, wherein the cause of disease was reported as follows (for new patients in 1996): diabetes 29%; HTN 10%, drug induced 1%; glomerulonephritis 16%; unknown 14%; pyelonephritis 5%, other 13%; polycystic kidney disease 4%; other renal vascular disease 8%. Thus, the study population appeared to be fairly representative of Canadian HD populations overall.

Observation or information bias may have been a source of bias, not so much in terms of loss to follow-up but rather related to loss to CAPD (either pre- or during HD). While misclassification is always a possibility in retrospective designs, it was not considered to seriously affect this study as the documentation of the key variables was fairly consistent and many sources were available to confirm patient BP classification(s). However, misclassification may have occured as a result of the means by which BP was described in the study. Rather than using absolute SBP or DBP values (and the resulting categories based on them) as the main measure of BP status, the mean arterial pressure (a calculated value that reflects the impact of both SBP and DBP) might have been tested for its relationship to Epo dosing requirements. This would required further analysis of the existing data.

5.5.3 Ethical issues

Due to the nature of the study, the research protocol was given a waiver of the requirement for review by the local hospital Ethics Committee (see Appendix XVI). Because of the chart review nature of the study, the fact that the study involved no direct contact with patients and will had no direct impact on ongoing patient care, patient consent was not considered necessary by this Committee. Nevertheless, every effort was made to keep the identity of patients confidential (i.e., a single investigator handled the data, and the data were reported in aggregate).

5.5.4 Issues of reliability and validity

In the context of this study, the most pressing challenge to measurement reliability related to individual BP measurements as recorded in the charts. Because of the retrospective nature of the work, it was not possible to confirm the conditions under which BP measurement occurred (i.e., the degree of inter-rater reliability). The reliability of the data collection process was ensured by the use of a standardized data collection database, and by limiting the data collection to a single investigator.

A potential threat to the internal validity of this study arose as a result of the difficulty in separating out HTN as a side effect (commonly observed in CRF

patients treated with Epo) versus HTN as a disease state in and of itself (96). The study design went to great lengths to describe HTN status prior to each patient's exposure to Epo, as well as changes in BP status after initiation of the drug using a variety of tools (i.e., dichotomous, categorical, continuous variables). The issue of whether HTN in its role as a side effect of Epo was independent of HTN as a disease state was not the important issue at this stage of hypothesis generation. The purpose of this research was simply to determine whether a relationship existed between BP elevations and Epo dose requirements.

In terms of the validity of the measurement tool, the data collection databases were based on a prototype tool developed and used in previous funded research carried out by the investigator (16).

The complexity of the databases (see Appendix VII) demonstrates the lengths to which the research has gone in describing potential confounders and effect modifiers to account for potential challenges to the internal validity of the study. These variables were evaluated and controlled for statistically in the analysis phase of the study.

6.0 Conclusions:

This study was designed to clarify the role of HTN in determining Epo responsiveness in patients on HD. The hypothesis that the presence of HTN predicts the need for higher Epo doses to achieve a target Hg level was not supported, and no relationship was demonstrated between any of the measures of HTN status and Epo dosing requirements in this cohort of patients during their first four to six months of Epo therapy.

Angiotensin-converting enzyme inhibitor therapy was the only parameter that was found to consistently be related to the dependent variable. The primary regression analysis also indicated that HD adequacy was a predictor of the 4-6m Epo dose; but the significance of this variable was lost when extreme values of the dependent variable were excluded from the analysis. It was further demonstrated that patients who were treated with ACEIs received significantly less Epo and, independently, achieved significantly lower 4-6m Hg levels than those patients not on ACEI treatment. The expected physiologic response of higher Epo doses being required in those on ACEI therapy did not occur in this cohort of patients. The relevance of this phenomenon is unclear and may well be a consequence of subgroup analysis, as it contradicts both theoretical and empirical evidence of the interaction between ACEI therapy, hematologic outcomes and Epo therapy.

Appendices

Appendix I

Manitoba Epo Prescribing Criteria (1992)

The Section of Nephrology of the University of Manitoba, who comprise all the potential users of Epo for anemia of renal failure in Manitoba, have agreed:

1) that all patients being considered for Epo will be presented to, and considered by, the Section of Nephrology as a whole, or a subcommittee reporting to the Section, before being commenced on therapy;

2) no physician other that a member of the Section of Nephrology may prescribe Epo for renal failure patients, except by special agreement with the Section;

3) no hospital, other than a teaching hospital or a hospital related to the Local Centre Dialysis Program of the Manitoba Renal Failure Program, shall supply Epo;

4) that in descending order of importance, one or more of the following will be considered as reasons for commencing Epo therapy -

   i) transfusion dependency requiring one or more units of blood per month to remain free of anemia symptoms, such as angina, shortness of breath, severe weakness, and tiredness. This will normally mean a hemoglobin of no more than 75g/L.
   ii) awaiting transplant to ensure that no transfusions are given which may cause pre-sensitization.
   iii) failure of all other forms of anemia therapy (other than frequent transfusion). These include iron (serum ferritin at least 100), folate, B12, vitamins B and C, and androgens. Failure of these therapies may also occur due to side effects.
   iv) transfusion complications with iron overload (serum ferritin >1000).
   v) multiple red cell antibodies causing transfusion reactions.

vi) evidence of cardiovascular disease which can be ameliorated by improving anemia (ischemic heart disease, cardiomyopathy, heart failure, peripheral vascular disease).

vii) severe pulmonary disease.

5) Relative contraindications to Epo therapy will include:

i) moderate or severe hypertension;

ii) thrombotic tendency;

* iii) age > 70 years;

* iv) severe other disease with life expectancy < 3 years

(These patients [iii & iv] can be transfused to maintain comfort unless marked iron overload is already present.)

* age limitation has been liberalized Revised 1992

Appendix II

Ontario Epo Prescribing Criteria:[n]

"What is the indication for Erythropoietin?

a) on transplant list (and who would otherwise require transfusion)
b) transfusion reaction (How many transfusions have been administered in the last year?)
c) cross-match difficulties
d) severe symptomatic anemia (and who would otherwise required transfusion)
e) iron overload:serum ferritin? (and who would otherwise require transfusion)
f) severe anemia (patients with hemoglobin <55 g/L who would otherwise required transfusion)"

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[n] Recombinant Human Erythropoietin Funding Enrollment for E.S.R.D. Patients. Ontario Ministry of Health, Drug Programs Branch. (1996)

Appendix III

Theoretical model and conceptual framework

A model of the theory underlying this project is outlined in Figure XI. The theory states that when patients with CRF anemia are treated with Epo therapy, there are three possible scenarios related to their BP status: they may have exacerbation of their underlying HTN, develop new-onset HTN or may have no change in their BP status. Those patients who have normal baseline BP, or whose BP remains unaffected with Epo therapy, will tend to have a normal hematologic response to Epo therapy. On the other hand, those patients with underlying HTN or those who develop new-onset HTN appear to have an impaired hematologic response to Epo therapy (through some unknown mechanism defined by physiologic or other factors).

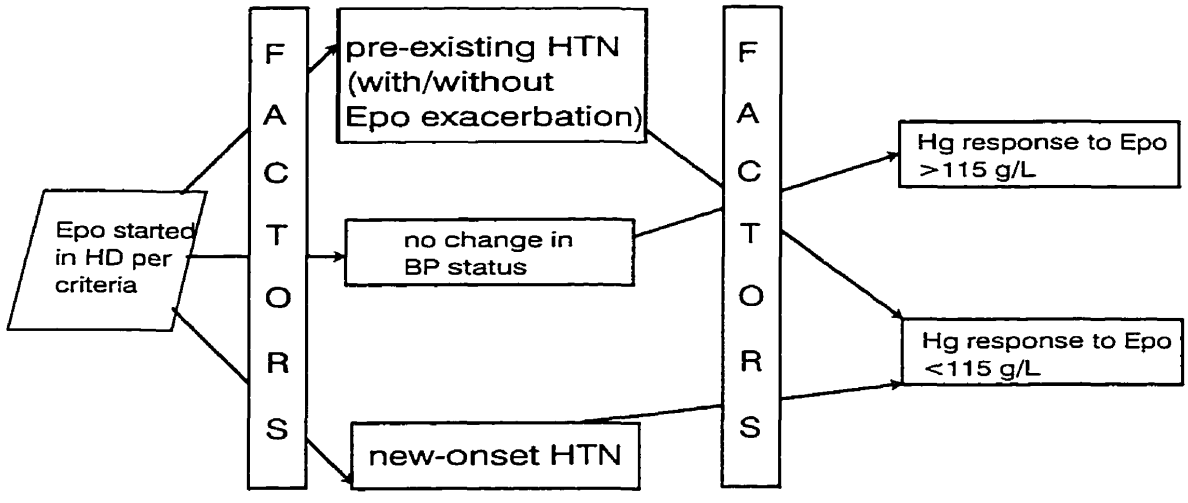

Figure XI. A conceptual model of the inter-relationship between HTN and Epo responsiveness.

Appendix IV

Model for establishing causation

In the vernacular of Bradford Hill's criteria (97) as a model for establishing causation, the following points summarize the theory underlying the objectives and hypothesis of this study:

- the purpose of the study was to determine the **strength of association** between the independent and dependent variables
- while the study represented an early stage of hypothesis development, its results were expected to contribute to the level of **consistency** of the association
- by identifying and measuring known confounding and effect modifying variables, the proposal attempted to maximize the **specificity** of the association between the principle variables
- cause and effect could not confirmed absolutely from a **temporality** point of view because of the retrospective nature of the study (although the short time frame allowed for the definition of Epo-induced HTN alleviated this problem to some extent)
- due to the continuous nature of the two main variables, the possibility of a gradient or **dose-response** relationship between them was to be evaluated as part of the analysis

- the rationale described for the HTN-Epo dose relationship (i.e., Epo receptor down-regulation via elevated sympathetic tone) was **coherent, biologically plausible, and analogous** to established relationships, as demonstrated by a similar effect of HTN on another hormone (insulin) (51)
- the purpose of this research was to begin the process of gathering a body of **experimental research** to support the hypothesis

Appendix V

Sample Size Calculation (98,99,100)

The sample size calculated for this study was based on the use of stepwise multiple regression analysis as one of the analytic methods to be used. Based on the F test for significance and several key assumptions (i.e., a 2-tailed alpha of <0.05, a power of 0.8, a variance of 343.5 [from Epo pilot study] and a mean difference in Epo dose of 10u/kg 3x/w), the following sample size was calculated:

1) $$N = \frac{L\,(1 - R^2_{Y.B})}{R^2_{Y.B}} + u + 1,$$

where N is the number of cases necessary to have the specified probabilty of rejecting the null hypothesis (i.e., power) at the α level of significance, with u degrees of freedom when the effect size in the population is at a given level.

2) assuming that the population effect size, f^2, is of moderate value, (i.e., $f^2 = 0.15$; i.e., that the amount of variance in Epo dosing explained by the effect of BP is of moderate importance, per Cohen [98-100]), then:

$$R^2_{Y.B} = \frac{f^2}{1 + f^2},$$

where $R^2_{Y.B}$ is the proportion of Y variation accounted for by the B set of variables in the sample. Substituting the value for f^2, we calculate:

$$R^2_{Y.B} = \frac{0.15}{1 + 0.15}$$

$$= 0.13$$

3) If we limit the number of independent variables in the multiple regression model to 10 (i.e., degrees of freedom, u = 9) and we desire a power of 0.80 and $\alpha = 0.05$, then Table E.2 of Cohen and Cohen (100) indicates that L (the noncentrality parameter, a function of effect size and the number of independent variables in the multiple regression model) should have a value of 16.24 and, therefore:

$$N = \frac{16.24\ (1 - 0.13)}{0.13} + 9 + 1,$$

$$= 118$$

In order to account for subject attrition (estimated at 10%), to achieve precision in defining the magnitude of the effect size (i.e., the precision of the confidence intervals), and to maximize the power of the analysis, approximately 130 cases of Epo therapy need to be reviewed. (At the time that the thesis proposal was approved, it had been estimated that there were at least 260 patients on HD in the entire Ottawa region at any one time. With an Epo adoption rate of approximately 60%°, accruing 130 cases of Epo use between 1994 and 1998 was thought to be a reasonable expectation.)

This sample size is a **conservative** estimate of the number of cases points required to determine the presence or absence of the postulated phenomenon. If fewer variables are included in the multiple regression model, then the power of the analysis would be increased. This sample size also

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° Canadian Organ Replacement Register/Ortho Biotech Erythropoietin Project. June 5, 1996.

provides sufficient power (0.80) for other parametric analyses that might be used in the course of evaluating the data (e.g., t-tests, ANOVA, ANCOVA, etc.). (98-100)

Appendix VI

Potential variables to be included in the multiple regression model

Variable	Description
dependent variable	Epo dose (u/kg/w)*
independent variables	BP* Hg level* Hct level* age* gender# pre-dialysis weight* body mass index (BMI)* serum albumin level* number of months on HD* number of months on Epo* time between HD and Epo* dialysis frequency (per week)# time on dialysis (per session)# dialysis efficiency (Kt/V)* type of dialyzer# presence of residual renal function# HTN status on initation of Epo# antihypertensive medications used on Epo initiation# worsening HTN# HTN status after 6 months of Epo# antihypertensive medications used after 6 months of Epo# HTN control measures# occurrence of adverse events# use of antiplatelet therapy# other underlying disease states# smoker# ferritin/transferrin saturation on initiation of Epo* ferritin/transferrin saturation after 6 months on Epo* iron administration# route of iron administration# transfusions in first 6 months of Epo* use of anabolic steroids# presence of hyperparathyroidism# presence of aluminum intoxication#

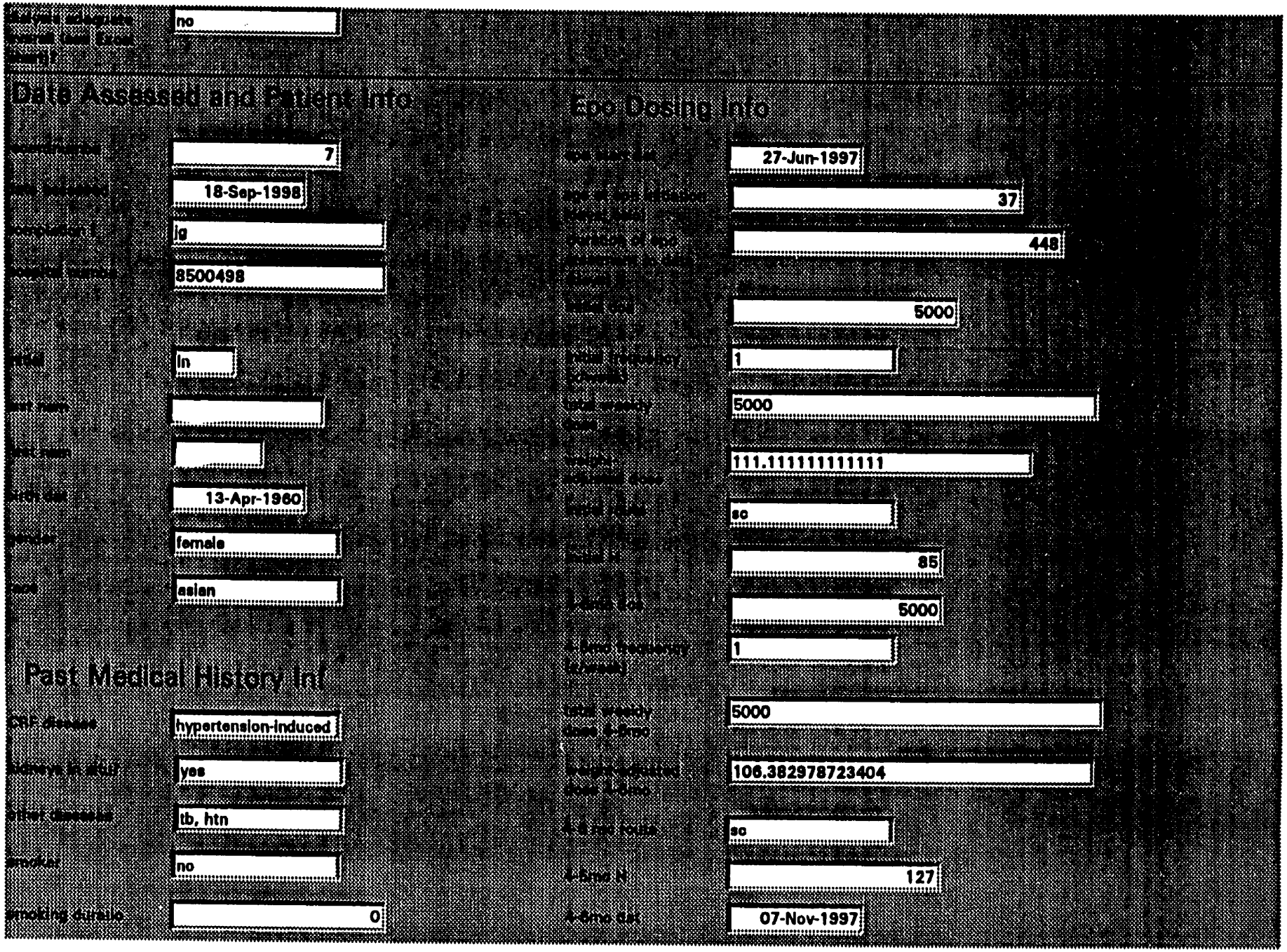
* continuous data
\# categorical data

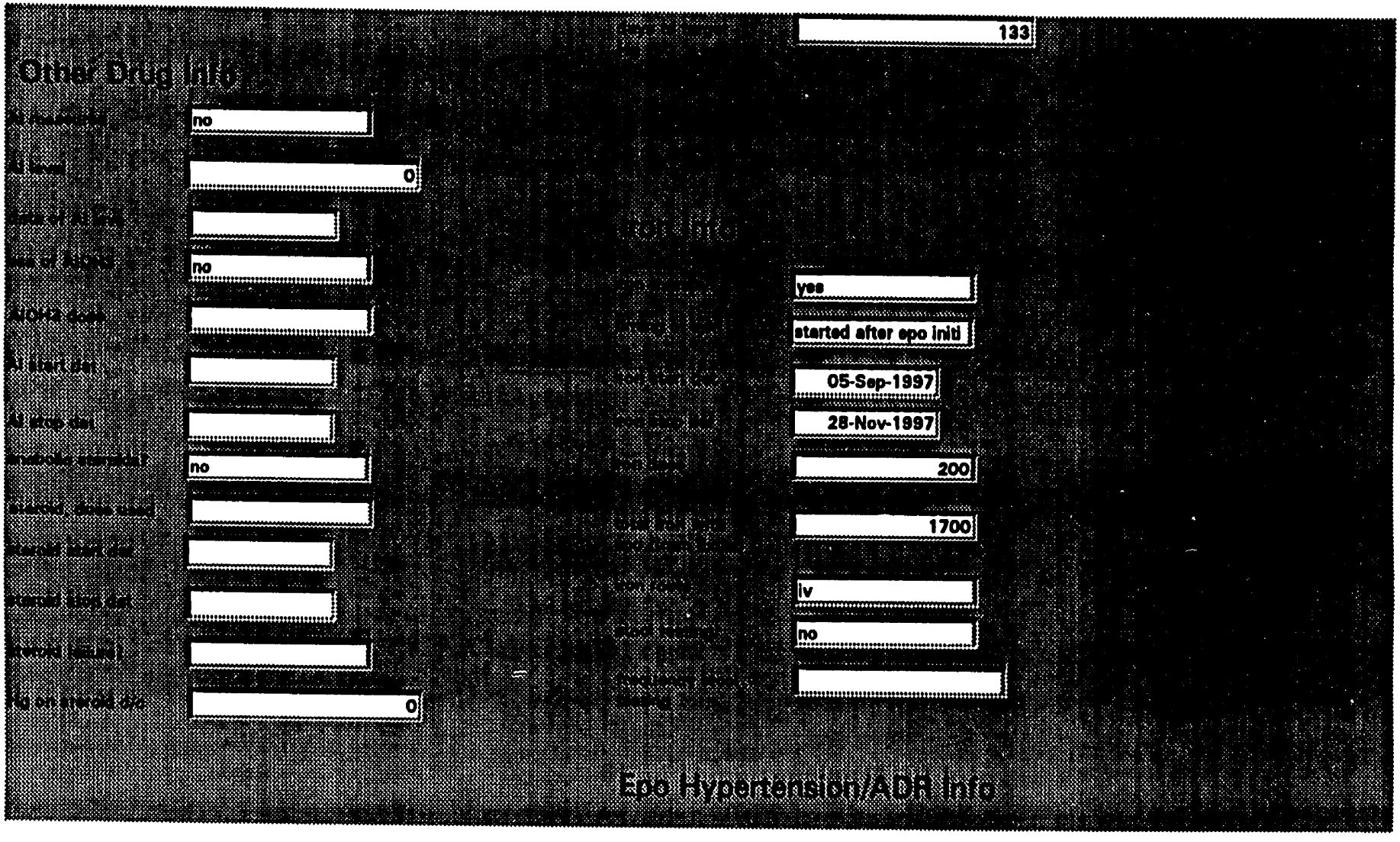
Appendix VII

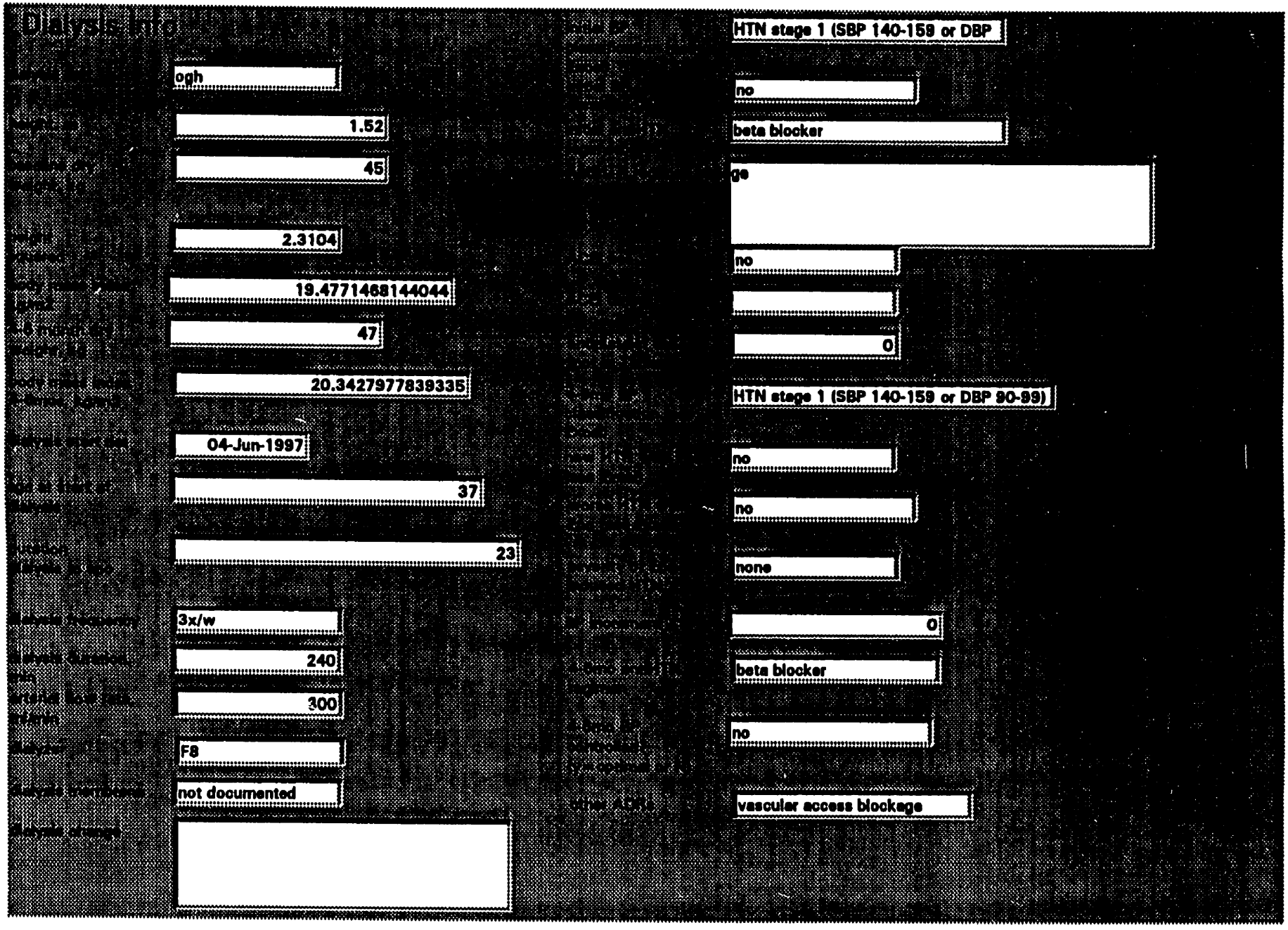
i) Structure of Access® database (attached)

ii) Structure of Excel® spreadsheet (attached)

Access


no
Date Assessed and Patient Info
7
18-Sep-1998
lg
8500498
ln
13-Apr-1960
female
asian
Past Medical History Inf
hypertension-induced
yes
tb, htn
no
0
Epo Dosing Info
27-Jun-1997
37
448
5000
1
5000
111.111111111111
sc
85
5000
1
5000
106.382978723404
sc
127
07-Nov-1997


Other Drug Info
no
0
no
no
0
133
yes
started after epo initi
05-Sep-1997
28-Nov-1997
200
1700
iv
no
Epo Hypertension/ADR Info


Dialysis Info
ogh
1.52
45
2.3104
19.4771468144044
47
20.3427977839335
04-Jun-1997
37
23
3x/w
240
300
F8
not documented
HTN stage 1 (SBP 140-159 or DBP
no
beta blocker
ge
no
0
HTN stage 1 (SBP 140-159 or DBP 90-99)
no
no
none
0
beta blocker
no
vascular access blockage

Excel

month #	week #	observation #	Patient record #	Date	Epo dose	Frequency per week	Dry Weight (target)	(calculate dose per kg)	Pre-dose Hg	Pre-dose Hct
	pre-epo week	1	9500496	04/06/97	0			#DIV/0!		85
		2		07/06/97	0			#DIV/0!		98
		3		18/06/97	0			0		85
1	1	1		27/06/97	5000	1	45	111.1111111		85
	2	2		04/07/97				#DIV/0!		85
	3	3		11/07/97				#DIV/0!		94
	4	4		18/07/97	5000	1	45	111.1111111		98
2	5	5		25/07/97				#VALUE!		
	6	6		01/08/97				#DIV/0!		
	7	7		08/08/97				#DIV/0!		105
	8	8		15/08/97				#DIV/0!		105
3	9	9		22/08/97	5000	1	45	111.1111111		103
	10	10		29/08/97				#DIV/0!		
	11	11		05/09/97				#REF!		113
	12	12		12/09/97				#DIV/0!		105
4	13	13		19/09/97	5000	1	45	111.1111111		
	14	14		26/09/97				#DIV/0!		112
	15	15		03/10/97				#DIV/0!		
	16	16		10/10/97				#DIV/0!		113
5	17	17		17/10/97	5000	1	45	111.1111111		113
	18	18		24/10/97				#DIV/0!		124
	19	19		31/10/97				#DIV/0!		124
	20	20	x 07/11/97		5000	1	47	106.3829787		127
6	21	21		14/11/97	5000	1	44.8	111.6071429		127
	22	22		21/11/97				#DIV/0!		125
	23	23		28/11/97				#DIV/0!		125
	24	24		05/12/97	5000	1	45	111.1111111		
	equilibration	25		10/12/97	5000	1	45	111.1111111		112
	period	26		26/12/97				#DIV/0!		108
end of data	end of data	end of data	end of data	end of data	end of data	end of data	end of data	end of data	end of data	

Pre-dialysis SBP	Pre-dialysis DBP	(calculate mean arterial pressure)	Pre-dialysis transferrin saturation	Ferritin	Pre-dialysis TIBC	Pre-dialysis serum Fe	Pre-dialysis albumin
127	67	87.6					39
152	89	109.79					
159	87	110.79	23	177	44	10	37
		0					
		0					
148	84	105.12					
184	102	132.39	19	70	47	9	39
		0					
		0					
		0					
		0					
161	90	113.45	16	24	55	9	41
		0					40
		0					
		0	14	31	51	7	40
123	71	88.16					
		0					
		0					
		0					
123	87	99.49	28	225	36	10	39
		0					
		0					
		0					
136	94	107.66	31	248	39	12	42
		0					
		0					
138	78	97.6					
140	75	96.45	24	402	41	10	39
		0					
end of data	end of data	end of data	end of data		end of data	end of data	end of data

Pre-dialysis BUN	Post-dialysis BUN	(calculate dialysis efficiency)	K/V (from chart)	IV Fe dose	Dialysis changes (check if yes)	RBCs transfused	Proteinuria (check if yes)
43.6	23.8	-0.609848237				2	
		#DIV/0!					
27.8	5.8	-1.547178103					
		#DIV/0!					
		#DIV/0!					
		#DIV/0!					
20.6	5.2	-1.57863245					
		#DIV/0!					
		#DIV/0!					
		#DIV/0!					
35.3		#NUM!					
31.3	6.6	-1.580540741					
		#DIV/0!					
		#DIV/0!					
28	5.9	-1.557253152		200			
		#DIV/0!		300			
		#DIV/0!		600			
		#DIV/0!		200			
28.2	5.1	-1.740061439					
		#DIV/0!					
		#DIV/0!					
		#DIV/0!		200			
		#DIV/0!					
28.1	6.1	-1.717071253					
		#DIV/0!					
		#DIV/0!		200			
		#DIV/0!					
25.4	4.2	-1.796554545					
		#DIV/0!					
end of data	end of data	end of data		end of data	end of data	end of data	end of data

AntiHTN regimen changes (specify in Access)

on atenolol 50 bid

end of data

Appendix VIII

Defining blood pressure parameters

Blood pressure was documented as a continuous variable (in millimetres of mercury [mm Hg]), as well as on the basis of categorical variables describing BP changes and BP status at various points within the 4-6m time frame of interest (see Figure XII).

Baseline BP control (Yes/No)
Baseline BP category (based on JNC-VI grading; also with optimal and normal categories combined)
4-6m BP control (Yes/No)
4-6m BP category (based on JNC-VI grading; also with optimal and normal categories combined)
New onset HTN at 4-6m, Epo-induced (Yes/No)
Worsening of baseline HTN at 4-6m, Epo-induced (Yes/No)
Overall BP outcome (linkage of baseline HTN status with development of Epo-induced HTN)
Development of Epo-induced HTN (Yes/No)
BP outcome based on HTN conceptual model (4 categories)
Severity of Epo-induced HTN
Relative change in BP category, from baseline to 4-6m

Figure XII. Summary of BP variables.

Blood pressure readings from the day of Epo inititation formed the basis of the initial BP classification for each patient. Thereafter, pre-dialysis BP measures were recorded for one dialysis session per week of Epo therapy (for a minimum of 4 months) until the patient reached his or her target Hg level (or to a maximum of 6 months). The endpoint of data collection for each subject was a spot BP measurement on the day that the Hg level reached target within the 4-

6m observation period. Hemoglobin levels were recorded to coincide with each BP measure collected.

In addition to absolute BP values, categorical descriptions of the degree of BP control were defined for the baseline and the 4-6 month outcome period. These formed the basis of additional categorical variables in the statistical analysis. A systematic method was developed for classifying patients into subgroups at these different stages of Epo therapy. Patients were categorized on the presence and degree of pre-existing HTN according to the classification system defined by the Joint National Committee on Detection, Evaluation and Treatment of High Blood Pressure (JNC VI) (101), as outlined in Table 10. If the HTN was being treated, then the type of medication prescribed was also recorded.

Table 10. JNC VI Classification of blood pressure (adapted for patients with CRF [101,102,103,104]).

Category	Systolic Blood Pressure (SBP), mm Hg	Diastolic Blood Pressure (DBP), mm Hg
1) Optimal	< 120, and	< 80
2) Normal	< 130, and	< 85
3) High Normal	130 - 139, or	85 - 89
4) Hypertension		
a) Stage 1 (mild)	140 - 159, or	90 - 99
b) Stage 2 (moderate)	160 - 179, or	100 - 109
c) Stage 3 (severe)	180 - 209, or	110 - 119
d) Stage 4 (very severe)	≥ 210, or	≥ 120

Once therapy with Epo had begun, an assessment was made of whether the BP increased within the first 4-6 months of treatment (i.e., dichotomous variable, Yes/No). For the purposes of this study, the cut-off between these two options was set at a SBP of 130 and/or a DBP of 85 based on HTN treatment goals defined for patients with renal disease (101-104). Blood pressure status and antihypertensive medication requirements were reassessed using the categorization methods described above.

In those patients who developed HTN or whose HTN was exacerbated, an evaluation was made of the effort required to control BP after Epo initiation, as a proxy for defining the degree of severity of BP increase. Table 11 outlines an adaptation of the scoring system developed by Caravaca *et al.* (30) to categorize the severity of HTN (if any) which developed due to Epo therapy.

Table 11. Severity of Epo-induced HTN as defined by blood pressure control measures.

Category	Definition
1) good control (i.e., normal BP)	no change in BP from therapeutic goal (i.e., SBP < 130, DBP < 85)
2) mild HTN	BP easily controlled by one antihypertensive at conventional doses (if previously normotensive); or by increasing the dose of the same antihypertensive (if pre-existing HTN)
3) moderate HTN	BP increase controlled by adding one or two antihypertensives (excluding minoxidil) to previous treatment
4) severe HTN	unsuccessful control of HTN with three drugs, with signs and symptoms of hypertensive encephalopathy, requiring hospitalization to control BP and/or withdrawal of Epo therapy

Appendix IX

Dialysis information

Parameter	Description
dialysis frequency	2% two times per week 96% three times per week 2% "as required"
mean dialysis duration	229 minutes (range: 180-300)
mean arterial blood flow	318 mL/min (range: 150-450)
dialyzer model	88% F8 9% Toray 2% F80
dialyzer membrane*	not documented
dialysis adequate (i.e., Kt/V ≥ 1.3, assuming ≥3.5 hour dialysis session, 3 times per week)	67% no 28% yes 5% unable to assess
changes to dialysis	13 (12%) patients switched to CAPD at some point during epo therapy (short term)

* The literature has documented some relationship between the use of synthetic dialyzer membranes and higher Hct levels (105), but this study was unable to access information to support or refute this aspect of care.

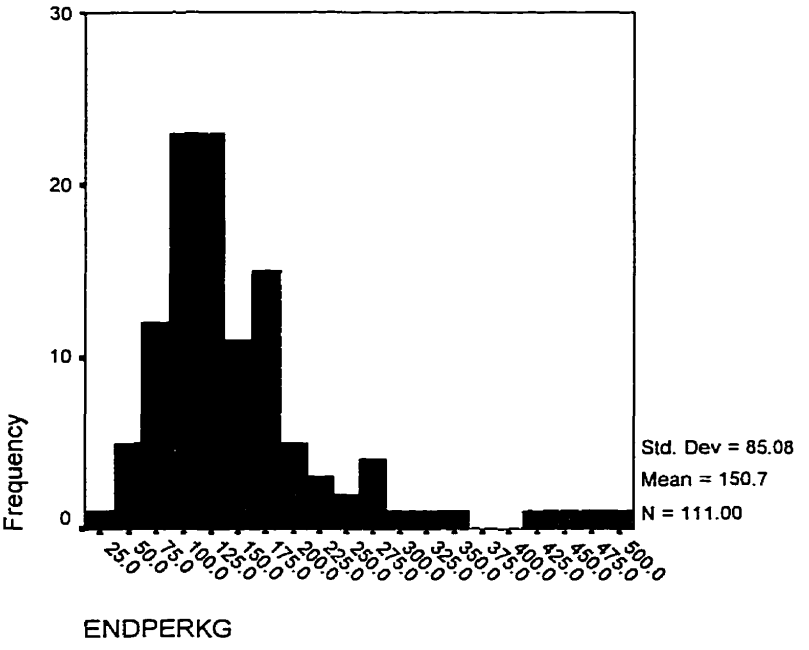
Appendix X

Iron therapy information

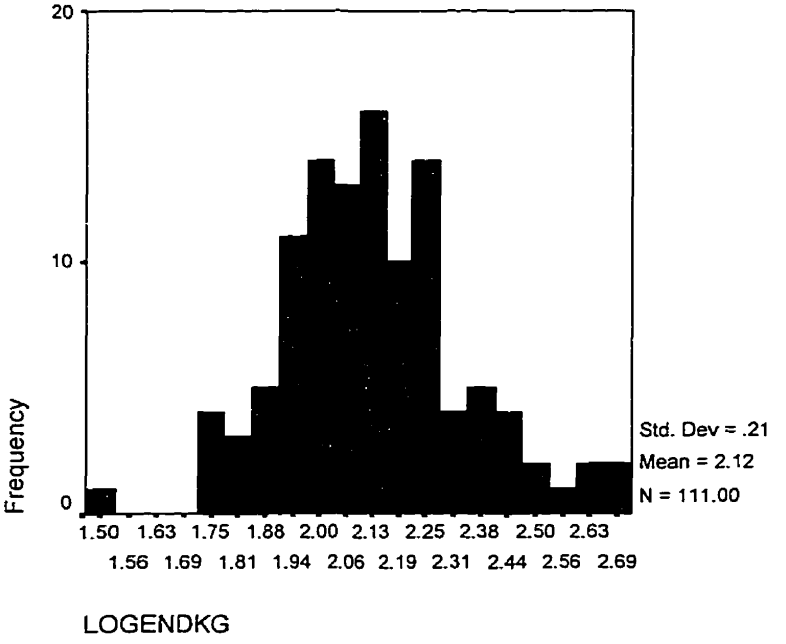
Parameter	Description
patients receiving some form of iron therapy	75% yes
timing of iron therapy	24% started prior to Epo 20% started concurrent with Epo 55% started after Epo
route of iron administration	24% oral only 55% IV only 20% oral and IV
oral iron dosing	19 900 mg/d 13 600 mg/d 4 300 mg/d 1 200 mg/d
IV iron dosing	- average IV iron per dose 193 mg (range: 100-500) - average total iron administered while trying to reach target Hg 1424 mg (range: 200-2900) - average IV iron administered per month 649 mg (range: 124-2000)
transfusions	- a total of 132 PRBC transfusions were administered to patients within the sample over the study period
stool testing	carried out in a total of 7 patients while on iron therapy

Appendix XI

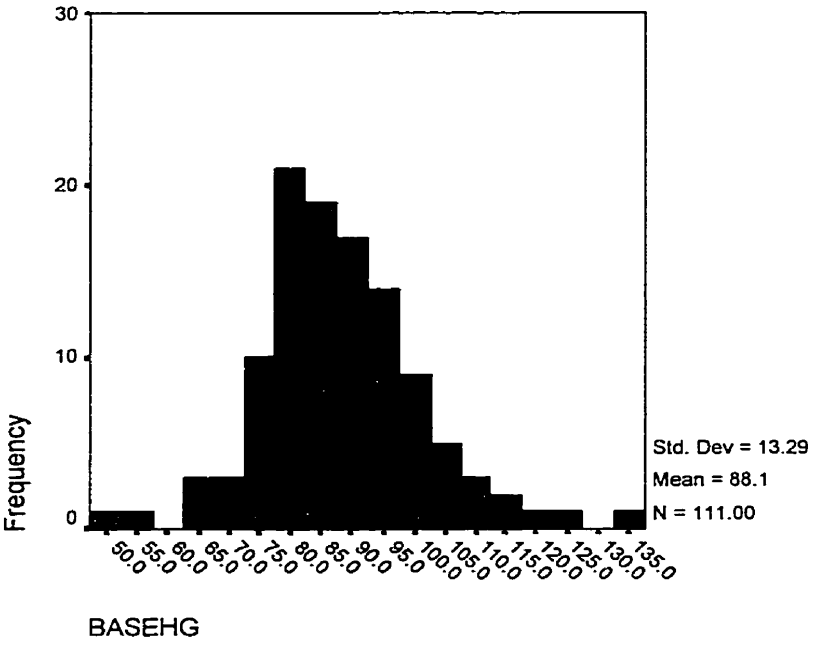
Frequency distribution of 4-6m Epo dose (absolute values)



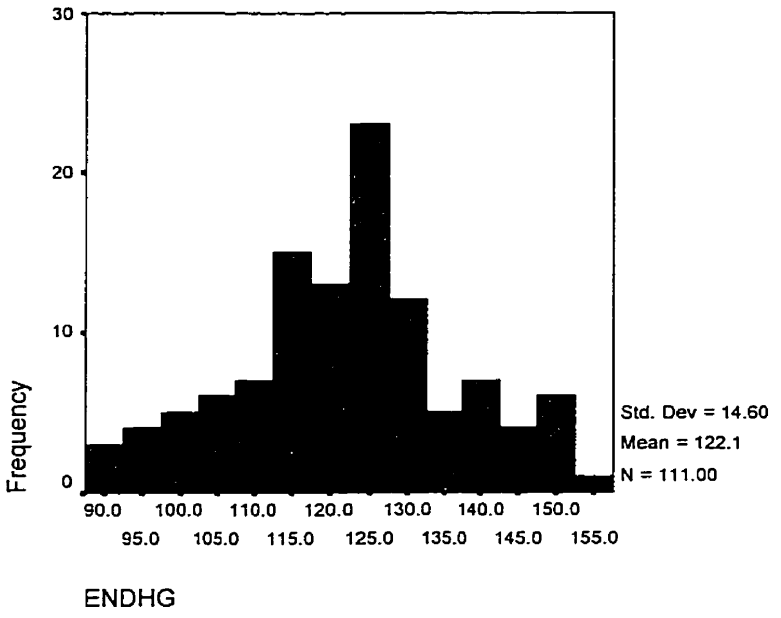
Frequency distribution of 4-6m Epo dose (log transformed values)



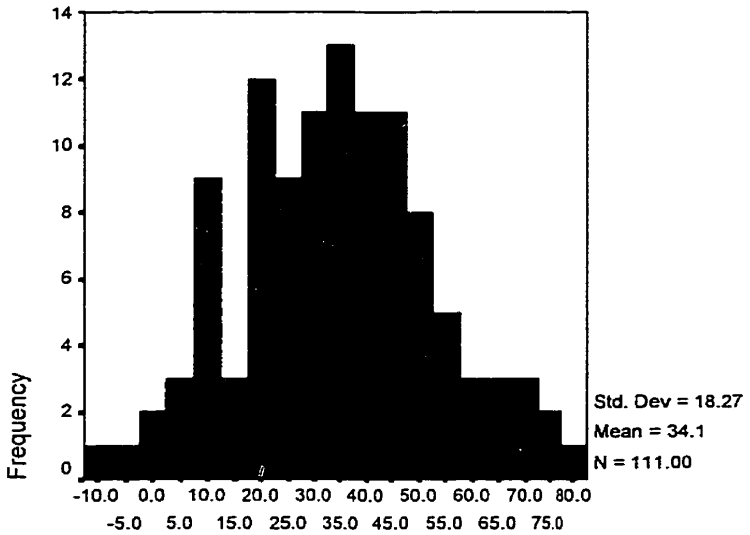
Frequency distribution of initial Hg levels



Frequency distribution of 4-6m Hg levels



Frequency distribution of change in Hg



Appendix XII

Overall BP control (Yes/No)

Time frame	BP controlled	BP not controlled	Unable to assess
baseline	22 (20%)	84 (76%)	5 (4%)
4-6m	25 (23%)	78 (70%)	8 (7%)

Initial BP classification (based on JNC-VI) and impact of Epo

Initial BP classification*	Number
optimal BP - worsened with Epo	10 6/10 (60%)
normal BP - worsened with Epo	12 10/12 (83%)
high-normal BP - worsened with Epo	18 10/18 (55%)
stage 1 HTN - worsened with Epo	35 20/35 (57%)
stage 2 HTN - worsened with Epo	17 8/17 (47%)
stage 3 HTN - worsened with Epo	11 5/11 (45%)
stage 4 HTN - worsened with Epo	2 0/2

* unable to identify initial BP classification for 6 patients in sample

Appendix XIII

Severity of Epo-induced HTN

Action in response to Epo-induced HTN	Number
no new or worsened HTN with Epo	43/111 (39%)
mild Epo-induced HTN	13/111 (12%)
moderate Epo-induced HTN	15/111 (14%)
no change in antihypertensive regimen, despite new/worsened HTN with Epo	31/111 (28%)
unable to assess severity of Epo-induced HTN	9/111 (8%)

Absolute BP increments (based on various categories of BP status)*

BP subgroup	BP increment (mean ± SD [range])
overall	15.5 ± 19.1 (0-73)
initial BP controlled	28.5 ± 22.2 (0-63)**
initial BP not controlled	13.0 ± 17.1 (0-73)**
optimal initial BP	27.8 ± 25.8 (0-63)
normal initial BP	29.1 ± 19.8 (0-55)
high-normal initial BP	19.6 ± 22.7 (0-73)
initial stage 1 HTN	13.9 ± 16.7 (0-52)
initial stage 2 HTN	8.6 ± 13.8 (0-42)
initial stage 3 HTN	7.9 ± 9.4 (0-21)
4-6m BP controlled	7.52 ± 12.5 (0-42)***
4-6m BP uncontrolled	17.8 ± 20.1(0-73)***

* NOTE: For purposes of consistency, the BP increment recorded was the change in SBP as SBP is more closely linked to acute adverse cardiovascular events (e.g., stroke).

** The BP increment for patients whose baseline BP was under control was significantly higher than for those whose baseline BP was not under control ($p<0.001$, 95% CI 6.9 to 24.2).

*** The BP increment for patients whose 4-6m BP was under control was significantly less than for those whose 4-6m BP was not under control ($p=0.17$, 95% CI 1.9 to 18.7).

Appendix XIV

4-6m Epo dose based on overall BP outcomes (including the presence/absence of Epo-induced HTN)

BP outcome category	Number of patients	4-6m Epo dose u/kg/w (mean)
baseline normotensive, no Epo-induced HTN	5	176.4
baseline normotensive, Epo-induced HTN	6	242.6
baseline HTN controlled, no Epo-induced HTN	1	125
baseline HTN controlled, Epo-induced HTN	10	124.1
baseline HTN not controlled, no Epo-induced HTN	37	139
baseline HTN not controlled, Epo-induced HTN	43	155.8

- no significant differences in dependent variable (p=0.097)

Appendix XV

4-6m Epo dose based on BP status and JNC-VI categories (basline and 4-6m measures)

Parameter	4-6m Epo dose u/kg/w (mean)	Significance
baseline BP control		
- yes	168.4	NS (p=0.278)
- no	146.3	
baseline BP categories		
- optimal*	153.9	NS (p=0.874)
- normal*	180.4	
- high-normal	162.1	
- stage 1	147.6	
- stage 2	141.1	
- stage 3	148.2	
- stage 4	105.3	
4-6m BP control		
- yes	163.3	NS (p=0.401)
- no	147.0	
4-6m BP categories		
- optimal*	145.6	NS (p=0.798)
- normal*	185.8	
- high-normal	154.4	
- stage 1	147.9	
- stage 2	149.1	
- stage 3	(none)	
- stage 4	152.3	

* even when these two categories were combined (due to small numbers), there was no statistically significant impact on 4-6m Epo dose based on baseline or 4-6m BP classification.

Appendix XVI

Ethics Committee Waiver Form (see attached)

HÔPITAL GÉNÉRAL D'OTTAWA OTTAWA GENERAL HOSPITAL

October 7, 1996

Dr. J. Glennie
Pharmaceutical Outcome Research Unit
Ottawa General Hospital

RE: Research Proposal

I have reviewed your research proposal "Determinants of Erythropoietin Requirements in Hemodialysis". In as much as this project will not involve intervention with patients nor their care and will be accomplished through a retrospective chart review, this study does not require review by the Research Ethics Board. Should there be any subsequent changes in your protocol that would require patient intervention or contact, please submit to the Board.

All the best with your research.

Sincerely

Mike Tierney, M.Sc.
Chair, Research Ethics Board

MT/ac

501 Smyth. Ottawa. Ontario K1H 8L6 (613) 737-7777

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