Lowering the Erithropoietin Requirement and Cost for Dialysis Patients with 'Functional Iron Deficiency' using Ascorbic Acid (Vitamin C)

Marie Philipneri, MD. St. Louis University

Abstract

Patients on maintenance dialysis often receive recombinant human erythropoietin (EPO) for management of anemia. A number of patients require excessive amounts of EPO in an attempt to overcome the EPO resistance secondary to functional iron deficiency, characterized by low transferrin saturation despite persistently elevated ferritin (indicating excessive body iron stores but ineffective utilization).

In a few small studies, ascorbic acid (AA) has been shown to reduce the EPO requirements, presumably by mobilizing the iron stores. Intravenous AA at 500 mg and 300 mg doses, given after each hemodialysis for eight weeks, has shown promising results. The goal of this study was to determine the lowest effective dose of AA for chronic hemodialysis patients with EPO resistance due to functional iron deficiency.

This was an open-labeled, prospective, multiphase study. Patients on chronic hemodialysis and receiving EPO for three months or longer with serum ferritin level > 500 ng/ml and transferrin saturation < 20 % were screened. Patients with C-reactive protein > 2 mg/dl were excluded. The study comprised of four consecutive treatment phases. Each phase consisted of an eight-week treatment period and a four-week follow-up (washout) period. During the treatment periods, the patients received IV AA 100 mg (phase I), 200 mg (phase II), and 300 mg (phase III) then oral AA 250 mg (phase IV). Oxalate and AA levels were obtained before and after each treatment phase.

Eight patients were enrolled and six patients completed the study. Positive impact of AA supplementation on lowering the EPO requirement was mainly seen with the 300mg dosage and maintained on oral supplementation. Neither the oxalate nor AA levels increased significantly during the study. Overall the patients tolerated both forms of ascorbic acid well.

The role of ascorbic acid in reducing the EPO requirement in hemodialysis patients with abnormal iron metabolism appears promising. Larger studies are needed to confirm the efficacy of the AA and examine the potential adverse events, particularly the higher risks of infection reported in the literature.

Introduction

EPO therapy has greatly minimized the need for blood transfusion (1), and thus the incidence of hemosiderosis. However, patients may develop a relative EPO resistance due to functional iron deficiency, characterized by low transferrin saturation despite persistently elevated serum ferritin level, indicating excessive body iron stores (2).

Iron therapy may improve this hyporesponsiveness but likely to worsen the hemosiderosis (3-6). Administration of IV ascorbic acid (AA) has been shown to facilitate iron mobilization from the tissue stores, improve iron utilization by the erythrocyte precursors, and thus effectively overcome the EPO resistance due to functional iron deficiency (7-13).

The basis for this improvement in iron utilization is as follows: Iron which is stored as a complex ferric compound (ferritin) in the reticuloendothelial system must be converted to the ferrous form in order to be mobilized from the crystalline ferritin core. AA (vitamin C) serves as an electron donor (or reducing agent) which likely aids in the reduction of iron from the ferric to the ferrous state (14, 15).

HD patients are at risk of developing vitamin C (MW 176 Daltons and water soluble) deficiency due to its losses during dialysis (16,17) and limited oral intake, since patients avoid many fruits and vegetables, which are rich in vitamin C but are also high in potassium. Most dialysis patients in United States receive 100 mg of oral vitamin C daily in the form of renal vitamin (18). The studies suggest that additional AA supplements may have a role in mobilizing the body iron stores (7-13). However, the lowest effective dose, the optimal duration of therapy, and the long-term health impact remain unsolved. AA deficiency also has been linked to higher rates of myocardial infarction (19), scurvy (20) and leg cramps (21).

AA doses up to 150 mg daily have been recommended in renal failure patients (22). There are concerns that higher doses of AA could potentially lead to oxalate accumulation and deposition of calcium oxalate in tissues (23, 24). Moreover, high serum levels of AA may lead to increased susceptibility to bacterial infections due to impaired phagocytic activity of the neutrophils (25, 26). Paradoxically, iron overload may also predispose patients to higher

incidence of bacterial infections, for the same reason. Therefore, lower doses of AA may prove to be safe and effective.

Material and Methods

Study Design:

This was an open-labeled, prospective, multiphase study (Appendix A). The patients who meet the selection criteria underwent four treatment phases consecutively (see Figure 1). Therefore, the duration of each patient's participation was up to 48 weeks. The study protocol was approved by the Institutional Review Board, at the Saint Louis University.

Study Objectives

- To determine the lowest dose of intravenous AA effective in lowering the EPO requirements in hemodialysis patients with EPO resistance due to functional iron deficiency.
- To assess the efficacy of oral ascorbic acid in lowering the EPO requirements in hemodialysis patients with EPO resistance due to functional iron deficiency.
- To evaluate the extent of oxalate accumulation in hemodialysis patients treated with low dose IV ascorbic acid or oral ascorbic acid.

Patients:

The patients were recruited from a total of about 140 outpatient hemodialysis patients at an outpatient dialysis unit in Saint Louis. The patients, who met the initial selection criteria and consented for the study, underwent the screening tests. If these screening tests further met the selection criteria, they were enrolled in the study.

Inclusion criteria:

- Patients on chronic hemodialysis three times a week
- Duration of hemodialysis for three months or longer
- Patients receiving EPO for three months or longer
- Serum ferritin level of 500 ng/ml or more
- Transferrin saturation < 20 %

Exclusion criteria:

- History of infection or inflammation within three months
- C-reactive protein level greater than 2 mg/dl
- Bleeding or hemolysis within three months
- Folate level less than 4 ng/ml
- Vitamin B_{12} level less than 200 pg/ml
- Simultaneous participation in another study
- Pregnant women
- Subjects under 18 years of age

Measurements:

The monthly blood draws routinely performed at the chronic dialysis unit were used for screening purposes and follow up evaluations. Additional tests that were obtained during the screening period include C-reactive protein, serum oxalate level, and serum ascorbic acid level as well as stool guaiac for occult bleeding. Plasma oxalate and plasma ascorbate levels were also measured at the beginning and end the of each treatment period.

Routine hemoglobin and hematocrit levels attained bimonthly as well as serum iron, total iron binding capacity (TIBC) and serum ferritin determined monthly, at the chronic dialysis unit, were used for analysis throughout the study period.

Procedures:

The EPO dosage adjustments and replacement of parenteral iron at maintenance doses (i.e., Infed 50-100 mg/week or Ferrlecit 62.5 – 125 mg/week) were continued, per routine dialysis unit protocol. Administration of the loading doses of parenteral iron was avoided during this study. However if the patients needed to receive the loading doses under unforeseen circumstances, they were excluded from the remainder of the study.

The patients served as their own controls. The laboratory values and the EPO dose of the patients in the eight weeks prior to participation in the study was used as the baseline parameters for comparison with the changes after the administration of different doses of IV ascorbic acid or oral ascorbic acid.

Data analysis

Data were recorded in hard copy and computerized forms and stored in a secure area within the Nephrology Division. The investigators performed all data analyses. Data are expressed as mean values +/- SD (or range). Paired Student's *t* test was used for the analysis of the changes in the laboratory parameters and the EPO dose at the end of each phase versus the beginning of that phase. A P value < 0.05 was considered statistically significant.

Results

Twenty-two patients who were eligible at screening and agreed to participate in the study signed the informed consent. Three of these patients were subsequently excluded, before any screening tests were performed, due to history of infection or inflammation within three months. CRP levels were obtained in nineteen patients. Six of these patients were excluded due to high CRP levels (greater than 2mg/dL).

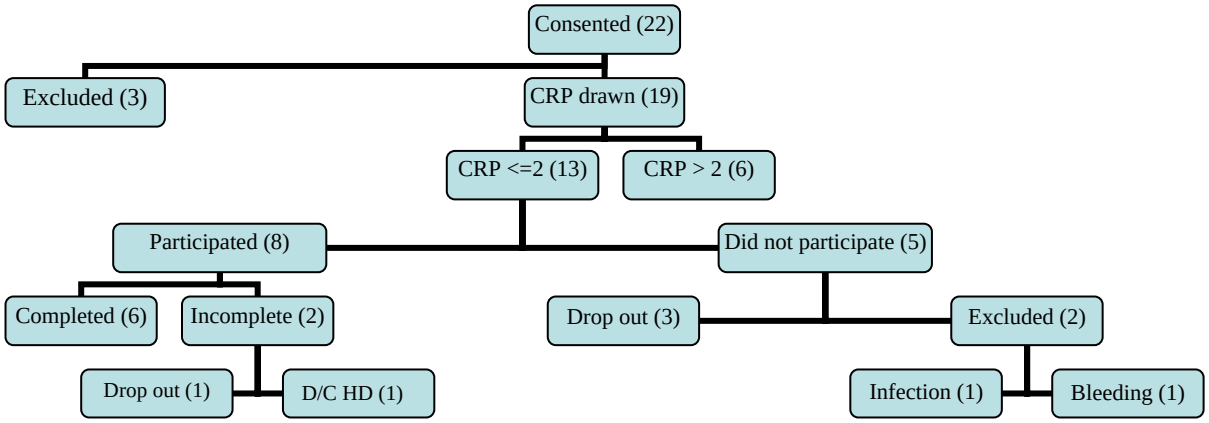

Figure 1: Study patient flow chart

Of the thirteen patients who had CRP levels less than or equal to 2mg/dL, three patients withdrew their consent prior to enrollment in the study. The main reason for their withdrawal was the long duration of the study. Two additional patients did not begin the study since they were noted to have had the exclusion criteria (one patient with infection within three months, at an outside hospital and whose results were unavailable at the time of screening and the

second patient with history of blood transfusion) after the CRP level was drawn. Eight patients who met the final study criteria were enrolled in the study. Six patients completed the study. One patient did not complete the study since he withdrew from dialysis and expired thereafter. Another patient withdrew from the study after the first 200mg dose of vitamin C during phase II of the study due to 'chills' during vitamin C infusions.

Baseline characteristics

Table 1 show the baseline characteristics of the patients who participated in the study. The average age was 46.8 years. Men and women were equally distributed. Majority (7/8) were of African American race. Half of the patients had Diabetes mellitus and majority (7/8) had hypertension. Interestingly their average hematocrit and EPO dose were 39% and 12,650 units/Kg/week respectively. Their mean intact PTH level was 581pg/mL.

Table 1: Baseline characteristics of the study participants, listing their demographics and pertinent laboratory test results.

Demographics:	
Age (mean+/-SD) years	46.8+/-16.7
Gender: male	4/8 (50.0%)
Race: African American	7/8 (87.5%)
Diabetes Mellitus: Yes	4/8 (50.0%)
Hypertension: Yes	7/8 (87.5%)
Measurements:	
Hematocrit (mean+/-SD) %	39.0+/-4.3
Ferritin (mean+/-SD) ng/ml	911.0+/-469.3
% Transferrin Saturation	16.6+/-2.0
EPO dose (mean+/-SD) (Units/Kg/week)	12,650+/-5896
CRP level (mean+/-SD) mg/dl	0.6+/-0.6
Intact PTH (mean+/-SD) pg/mL	581+/-497
Aluminum (mean+/-SD) mcg/L	10.8+/-2.5
Serum Vitamin B12 (mean+/-SD) (pg/mL)	863.6+/-418.4
Serum Folate (mean+/-SD) ng/mL	12.5+/-6.6

Hematocrit levels and Epo doses

Figure 1 depicts the overall trends of the hematocrit levels for the eight patients who took part in the study. The hematocrit levels ranged between 25.3 and 49.5% during the entire study period. The EPO doses were adjusted per the dialysis unit protocol throughout the study. The patients frequently achieved their target hematocrit levels with the EPO dose adjustments. There were no specific trends in the hematocrit levels among the four phases of the study.

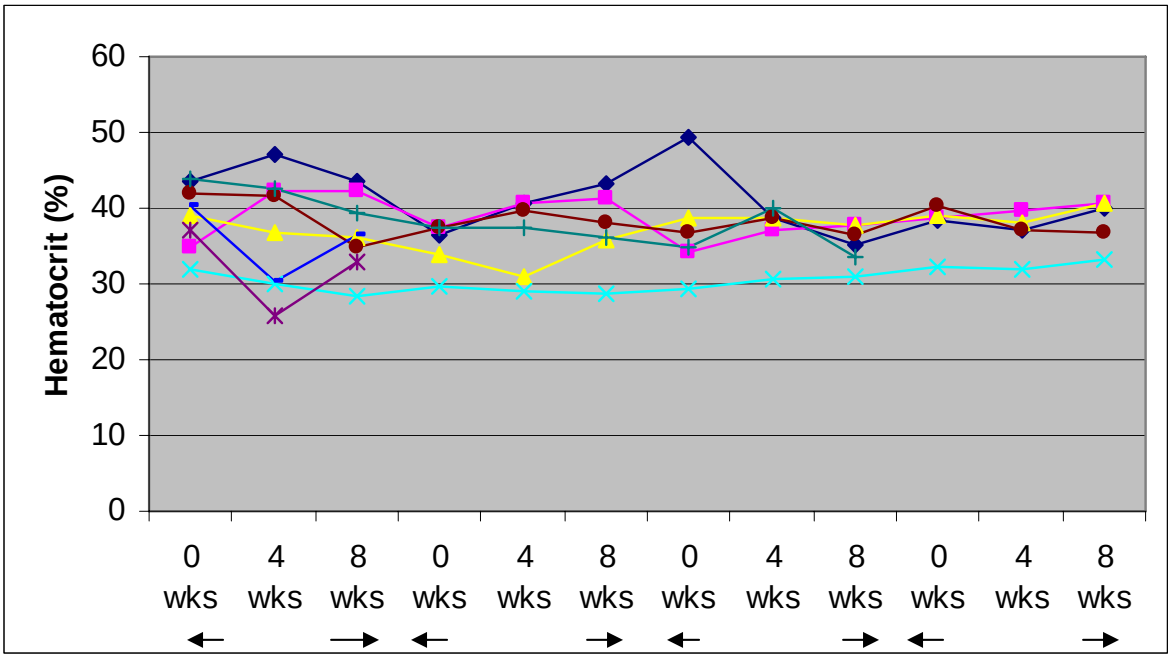

Figure 2: Hematocrit levels for the study

In contrast, the EPO requirements decreased in three out of the six patients who completed the study. In one patient, the EPO dose decreased by 83% and this effect was seen at the end of phase II. In the second patient, 75% decrease in EPO dose was noted but the decrease was gradual and seen throughout all phases. In the third patient, the EPO dose was higher during phase II compared to phase I but then there was a 20% decrease in EPO dose.

The details on three patients who completed the study but whose EPO doses did not decrease throughout the study are as follows: In one patient, the EPO dose was stable until the last four weeks of the phase IV when the dose was increased by 25%. In the second patient, the EPO dose was increased by 36% until the beginning of phase III but then unchanged. In the third

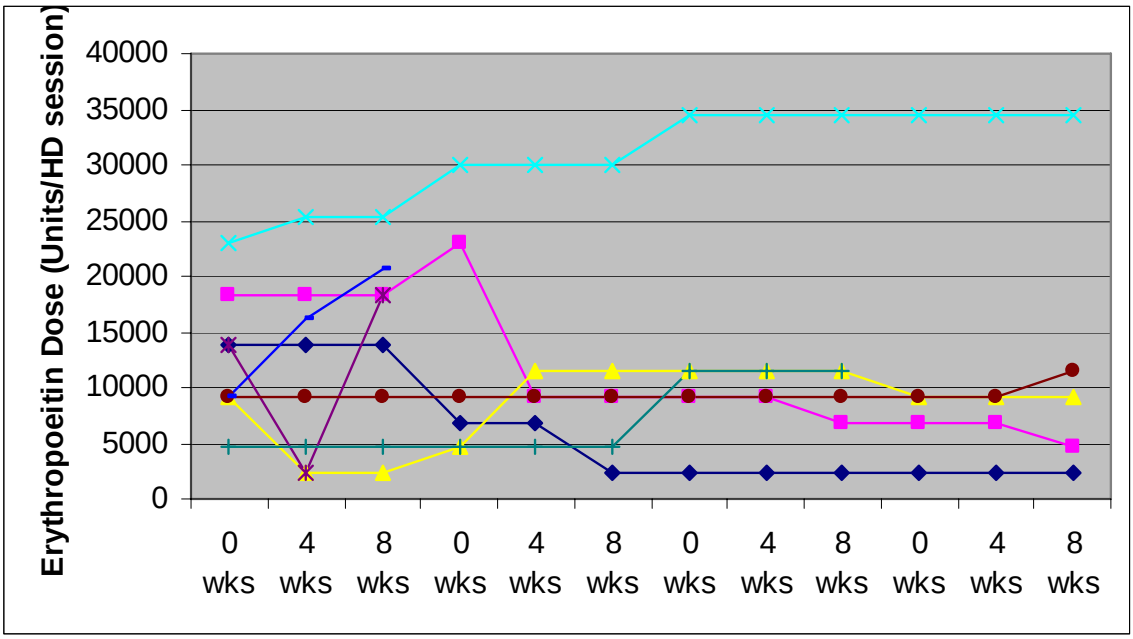

Figure 3: EPO doses for the study participants

patient, the EPO dose was increased at the beginning of phase II but then maintained during phase III and the dose was lower during phase IV. Overall, the EPO doses tended to be steady if not lower during phase III and appeared to be maintained during phase IV.

Ferititn and Transferrin saturation levels

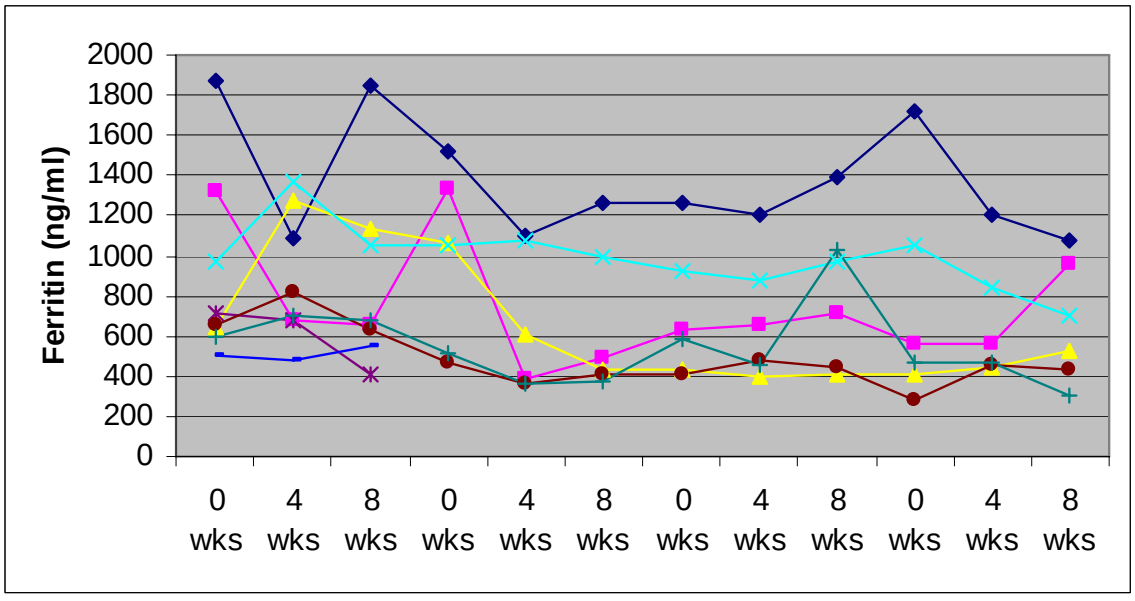

Figure 4: Ferritin levels for the study participants

The ferritin levels ranged from 276 to 1874 ng/mL. The transferritin saturation levels ranged between 8% and 41%. However there were no significant trends or patterns in ferritin or the transferrin saturation levels among the study participants throughout the study (Figure 2).

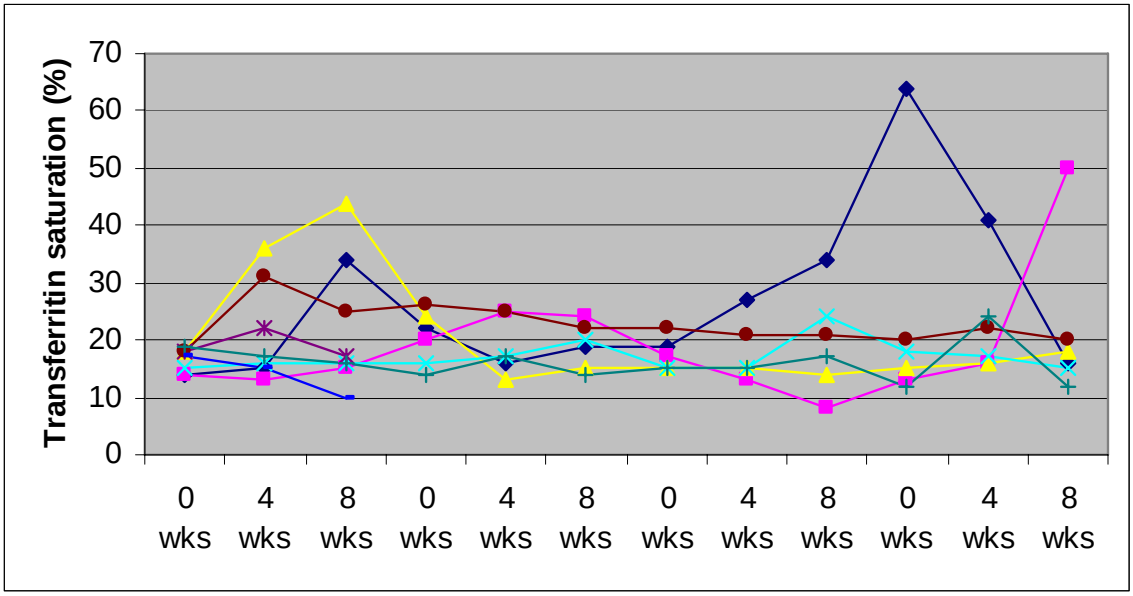

Figure 5: Transferrin saturation levels for the study participants

Ascorbate and Oxalate levels

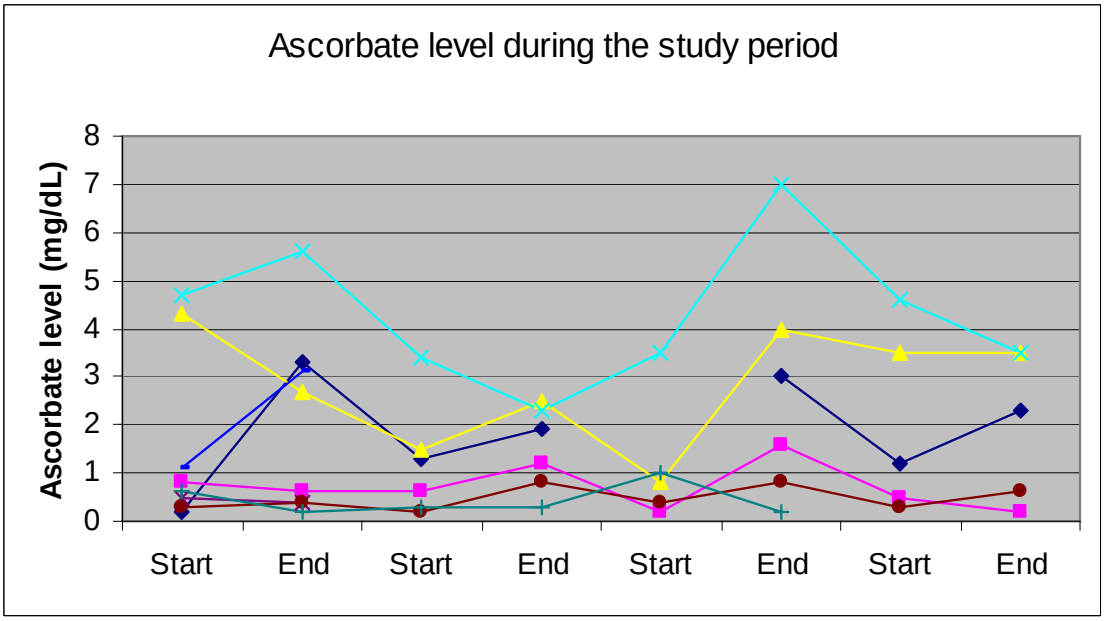

Figure 5: Ascorbate levels for the study participants

There were no significant increases in the Ascorbate levels throughout the study period. The oxalate levels at the end of phase I were higher than the baseline levels in six out of the eight patients, two patients had further increase in the Oxalate levels but they were lower over the subsequent phases. In other words, there were no consistent increases in Ascorbate and the oxalate levels across the four phases of the study.

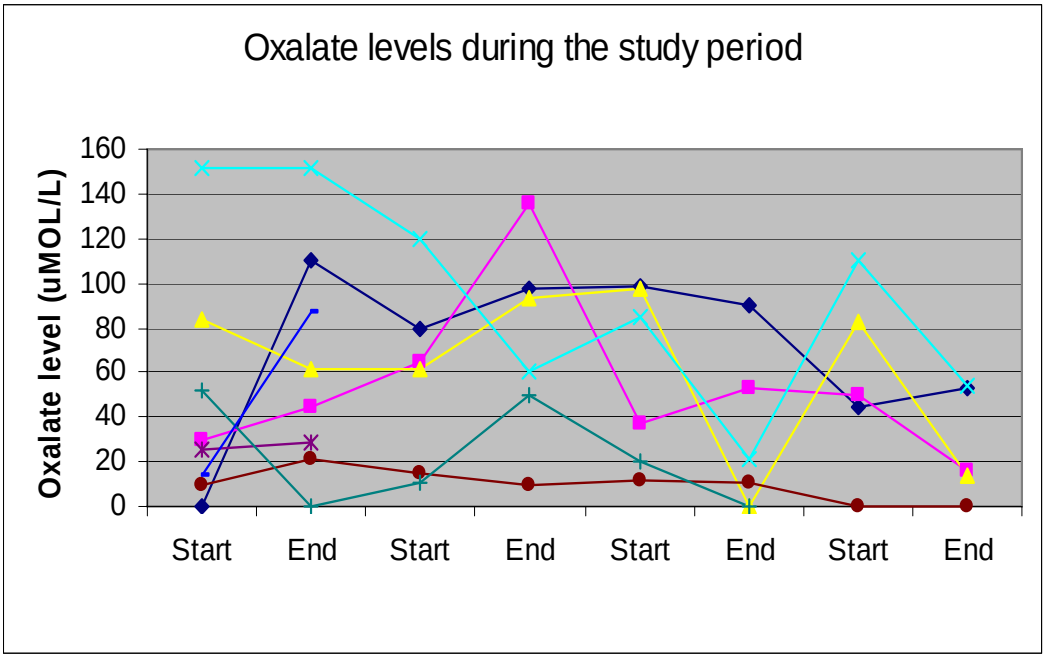

Figure 5: Oxalate levels for the study participants

Blood Pressure

The number, types and doses of antihypertensive medications used by the study participants were followed during the study. One patient who was using two antihypertensive medications one of the medications reduced to half the previous dose during phase II of the study and both agents were discontinued shortly after the completion of the study. One of the agents was resumed three months after the completion of the study. There was a corresponding decrease in the EPO dose requiring beginning during phase II. However, the EPO requirement did not increase up to six months after the completion of the study. There were significant trends noted in the antihypertensive medication use in rest of the patients.

Adverse Events

There were eight hospitalizations during the study period and their presumed diagnoses are as follows: abdominal pain, later diagnosed with acute myocardial infarction (1), chest pain without evidence for acute myocardial injury (1), pulmonary edema after missing two dialysis sessions (1), fall identified with defective wheel chair (1), nose bleed that resolved spontaneously (1), arteriovenous graft infection (1) and mental status change (2), secondary to cerebrovascular accident in one patient (and subsequent withdrawal from dialysis per family's request) and hypoxia secondary to pulmonary edema that promptly resolved following emergent dialysis in the second patient.

One patient reported chills during the intravenous AA administration (200mg dose) and withdrew from the study shortly thereafter. Other symptoms and signs noted during the study period include (the number of patients who experienced the symptoms or signs are given in parenthesis): intradialytic hypotension (7), cramps (7), dizziness (4), 'feeling bad' (4) nausea (4), shortness of breath (3), extremity pain (3), lower back pain (2), tachycardia (2), sweating (2), abdominal pain (2), clotted access (1), wound infection (1), blurred vision (1), generalized weakness (1), cough (1), numbness (1), vomiting (1), constipation (1), headache (1), low blood sugar (1) and pruritus (1)

Discussion

Though this study is small and has several limitations it provides additional insights into the possible role of AA for patients with EPO resistance due to functional iron deficiency. Once again, AA supplementation appears to lower EPO requirement in at least half of the patients. Determination of the lowest effective dose of AA was one the objectives of this study and 300mg dose appeared to be that dose. However it should be noted that due to the small size of the study, it was perhaps not powerful enough to demonstrate a significant benefit at the lower doses. Moreover, as in many previous studies, each dose was administered for a maximum period of eight weeks only. Therefore any foreseeable benefit of longer term (greater than eight weeks) exposure with each dose was not addressed by this study.

The study was designed with a four-week washout period in between each dosage increment. Once again this was based on prior studies reported in the literature and it should be noted that the optimal washout period is uncertain. A randomized, controlled design would be ideal but may not be practical and this study helps to illustrate the limitations of such a design.

Despite our meticulous efforts at screening patients, based on their monthly laboratory test results, and drawing CRP levels in a total of nineteen patients, we were unable to enroll the twenty patients as originally planned. We faced several challenges during our attempts to recruit additional patients for our study. Firstly, the patients were reluctant to commit to a long-term (forty-eight months) study. As addressed earlier, longer exposure to the agent of interest is crucial in order to determine its long-term benefits and risks. This study was specifically designed as a longitudinal study, involving four phases, in the same group of patients, in order to minimize interpersonal variation. However, based on the above limitation learned from this study, it might be prudent to design future studies involving different groups of subjects with comparable baseline characteristics for administration of different dosages.

Several patients also found participation in other studies, particularly those that are sponsored by drug &/or research companies who provide financial or other incentives to the participants, more attractive. Therefore, another lesson learned from this study is that incentives to study participants, particularly for a longer study such as this, need to be entertained during design of similar studies in the future.

Overall, the dialysis patients have higher co morbidities and their rates of infections, especially dialysis access-related, hospitalizations and mortality are higher compared to their non-dialysis counterparts. Therefore, these patients fail to qualify for the study based on the inclusion/exclusion criteria. For instance, in our study, even after initial screening by review of laboratory data and existing medical information, half of the patients who consented to the study (11/22) were excluded based on eligibility criteria.

Moreover, in long-term studies such as this, the clinical practice is constantly updated based on the newer literature available. For instance, at the beginning of this study, iron

supplementation was held if ferritin exceeded 800 ng/mL. However, the protocol was modified during the study period by the dialysis unit to hold iron supplementation if ferritin was greater than 500 ng/mL. Since our study design allowed supplementation of maintenance iron per protocol, the above change in the protocol was continued in our study population. Once again, it may be gathered from this study that when designing long-term studies in the future, any potential changes in policies and protocols need to be addressed.

Baseline AA levels were found to be low (less than 2 mg/dL) in majority (6/8) of the patients which is in keeping with the notion that the dialysis patients are at risk for developing AA deficiency (16, 17). Vitamin C intake of dialysis patients is quite variable from day to day therefore the assessment of the vitamin C intake of the participants is quite challenging and not performed in this study. Determination of vitamin C losses during dialysis is also difficult to assess due to individual and inter dialysis variation in delivered dialysis dose to these patients. These patients were placed on a daily renal vitamin though the accurate assessment of their compliance is once again difficult. Nevertheless, if this trend is confirmed in larger studies, the 100mg dose of oral vitamin C daily, in the renal vitamin is worth revisiting, and perhaps considers 200-250mg oral vitamin C daily based on this study and other studies that have addressed the oral vitamin C.

In contrast to prior study findings, the ferritin levels were not significantly lower with AA administration, (7-9). This might be partially explained by the continuation of weekly (low-dose maintenance) iron administration in this study. However the hematocrit levels were generally well maintained without administration of iron boluses and overall lower EPO requirement as discussed above.

It is reassuring to note that there were no significant increases in the oxalate levels with the administration of incremental doses of AA. However, I would advocate monitoring the oxalate levels in future studies involving a greater number of patients before advocating a higher vitamin C intake at large among the dialysis patients. Measurement of the total body pool of vitamin C and oxalate might be clinically more relevant than simply their levels. A series of investigations were planned using isotopically-labeled vitamin C supplements in order to determine the magnitude of the total body pool in dialysis patients (27).

Both intravenous and oral AA forms were generally well tolerated in this group of patients. One patient reported chills during the intravenous AA administration (200mg dose) and withdrew from the study. None of the other patients from this study reported this symptom and there are established link of chills with AA in the literature. The other symptoms noted by the patients in this study are also common to dialysis patients. Therefore it was difficult to identify any increased incidence of these adverse effects with the use of AA without a control group in this study. Therefore future tailored studies to address the potential adverse effects would be crucial before advocating broader use of higher doses of AA in dialysis patients.

In summary, Erythropoietin (EPO) requirements decreased in half of the patients, who completed the study involving the Ascorbic Acid (AA) supplementation. Positive impact of AA supplementation on lowering the EPO requirement was seen with the 300 mg intravenous dosage, and appeared to be maintained on the oral supplementation (250mg). Neither the oxalate nor AA levels increased significantly during the study.

The role of Ascorbic Acid in reducing the EPO requirement, in hemodialysis patients with abnormal iron metabolism, appears promising. Larger studies are needed to confirm the efficacy and safety of the 300mg intravenous dose and the oral form of the Ascorbic Acid (Vitamin C). Lower doses of erythropoietin administration to maintenance hemodialysis patients could potentially minimize the dose-related adverse effects with the chronic use of erythropoietin as well as lead to huge cost savings long term.

Acknowledgement: I wish to thank the Missouri Kidney Foundation for their support in making this important cost containment project feasible. I would also like to thank all the nephrologists, nurses, particularly Michelle Brynda, R.N. and Valerie Jenkins, R.N. and the dialysis staff who assisted us during the study.

Bibliography

1. Goodnough LT. Strasburg D. Riddell J 4th. Verbrugge D. Wish J. Has recombinant human erythropoietin therapy minimized red-cell transfusions in hemodialysis patients?. Clinical Nephrology. 41(5):303-7, 1994

2. Tarng DC. Huang TP. Chen TW. Yang WC. Erythropoietin hyporesponsiveness: from iron deficiency to iron overload. Kidney International - Supplement. 69:S107-18, 1999

3. Hudson JQ. Comstock TJ. Considerations for optimal iron use for anemia due to chronic kidney disease. Clinical Therapeutics. 23(10):1637-71, 2001

4. Sakiewicz P. Paganini E. The use of iron in patients on chronic dialysis: mistake and misconceptions. Journal of Nephrology. 11(1):5-15, 1998

5. Drueke TB. Barany P. Cazzola M. Eschbach JW. Grutzmacher P. Kaltwasser JP. Macdougall IC. Pippard MJ. Shaldon S. van Wyck D. Management of iron deficiency in renal anemia: guidelines for the optimal therapeutic approach in erythropoietin-treated patients. Clinical Nephrology. 48(1):1-8, 1997

6. Kirschbaum B. Serial ferritin concentrations in hemodialysis patients receiving intravenous iron. Clinical Nephrology. 57(6):452-6, 2002

7. Gastaldello K, Vereerstreten A., et al: Resistance to EPO in iron overloaded hemodialysis patients can be overcome by ascorbic acid administration. *Nephrol Dial Transplant* 10 (suppl 6): 44-47, 1995.

8. Tarng D, Huang TP, et al: A parallel, comparative study of intravenous iron versus intravenous ascorbic acid for EPO-hyporesponsiveness anemia in hemodialysis patients with iron overload. *Nephrol Dial Transplant* 13: 2867-2872, 1998.

9. Tarng D, Wei Y, Huang TP, et al: Intravenous ascorbic acid as an adjuvant therapy for recombinant EPO in hemodialysis patients with hyperferritinemia. *Kidney Int* 55:2477-86, 1999.

10. Petrarulo F, Giancaspro V. Intravenous ascorbic acid in haemodialysis patients with functional iron deficiency. Nephrol Dial Transplant 15: 1717-1718, 2000.

11. Melendez O. Intravenous vitamin C for erythropoietin resistance. Seminars in Dialysis. 13(5):335-6, 2000

12. Sezer S. Ozdemir FN. Yakupoglu U. Arat Z. Turan M. Haberal M. Intravenous ascorbic acid administration for erythropoietin-hyporesponsive anemia in iron loaded hemodialysis patients. Artificial Organs. 26(4):366-70, 2002

13. Lin CL. Hsu PY. Yang HY. Huang CC. Low dose intravenous ascorbic acid for erythropoietin-hyporesponsive anemia in diabetic hemodialysis patients with iron overload. Renal Failure. 25(3):445-53, 2003

14. Bridges KR, Hoffman KE: The effects of ascorbic acid on the intracellular metabolism of iron and ferritin. *J Biol Chem* 261:14273-277, 1986.

15. Lipschitz DA, Bothwell TH et al: The role of ascorbic acid in the metabolism of storage iron. *Br J Haematol* 20:155-163, 1971.

16. Bohm V. Tiroke K. Schneider S. Sperschneider H. Stein G. Bitsch R. Vitamin C status of patients with chronic renal failure, dialysis patients and patients after renal transplantation. International Journal for Vitamin & Nutrition Research. 67(4):262-6, 1997

17. Morena M. Cristol JP. Bosc JY. Tetta C. Forret G. Leger CL. Delcourt C. Papoz L. Descomps B. Canaud B. Convective and diffusive losses of vitamin C during haemodiafiltration session: a contributive factor to oxidative stress in haemodialysis patients. Nephrology Dialysis Transplantation. 17(3):422-7, 2002.

18. Fissell RB. Bragg-Gresham JL. Gillespie BW. Goodkin DA. Bommer J. Saito A. Akiba T. Port FK. Young EW. International variation in vitamin prescription and association with mortality in the Dialysis Outcomes and Practice Patterns Study (DOPPS). American Journal of Kidney Diseases. 44(2):293-9, 2004 Aug

19. Nyyssonen K. Parviainen MT. Salonen R. Tuomilehto J. Salonen JT. Vitamin C deficiency and risk of myocardial infarction: prospective population study of men from eastern Finland. BMJ. 314(7081):634-8, 1997.

20. Leggott PJ. Robertson PB. Jacob RA. Zambon JJ. Walsh M. Armitage GC. Effects of ascorbic acid depletion and supplementation on periodontal health and subgingival microflora in humans. Journal of Dental Research. 70(12):1531-6, 1991.

21. Khajehdehi P. Mojerlou M. Behzadi S. Rais-Jalali GA. A randomized, double-blind, placebo-controlled trial of supplementary vitamins E, C and their combination for treatment of haemodialysis cramps. Nephrology Dialysis Transplantation. 16(7):1448-51, 2001.

22. Descombes E. Hanck AB. Fellay G. Water soluble vitamins in chronic hemodialysis patients and need for supplementation. Kidney International. 43(6):1319-28, 1993

23. Shah GM, Ross EA, Sabo A, Pichon M, Reynolds RD, Bhagavan H: Effects of ascorbic acid and pyridoxine supplementation on oxalate metabolism in peritoneal dialysis patients. Am J Kidney Diseases. 20 (1):42-49, 1992

24. Rolton HA, McConnell KM, Modi-KS, Macdougall AI. The effect of vitamin C intake on plasma oxalate in patients on regular haemodialysis. Nephrol Dial Transplant 6: 440–443, 1991

25. Ritchey E, Wallin JD et al: Chemiluminescence and superoxide anion production by leukocytes from chronic dialysis patients. *Kidney Int* 19:341-358, 1981.

26. Dziubinski, JE: The effects of mega doses of ascorbic acid on bacterial activity and chemiluminescence production by human polymorphonuclear leukocytes; thesis University of Minnesota, Minneapolis, Minnesota, 1979.

27. Handelman G, Levine M. Vitamin C deficiency may be undertreated in hemodialysis. Dialysis Times: News & views from RRI 9(4):1 & 6, 2002