Anemia and Diabetes in the Absence of Nephropathy

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OBJECTIVE — Patients with diabetes commonly have a greater degree of anemia for their level of renal impairment than those presenting with other causes of renal failure. To clarify the contribution and differing roles of diabetes and nephropathy in the development of anemia in diabetic patients, we examined the hematologic and hematometric parameters of diabetic patients without nephropathy.

RESEARCH DESIGN AND METHODS — The study group was comprised of 62 patients with type 2 diabetes who had been followed for a median of 7 years. For the study, these patients had additional samples taken during their annual routine blood testing for the measurement of extra parameters, including serum ferritin, serum erythropoietin (Epo) levels, and the percentage of reticulocytes. These measurements were combined with the routine parameters Hb, hematocrit, HbA1c, and glomerular filtration rate.

RESULTS — In all, 8 of the 45 male patients (17.8%) and 2 of the 17 female patients (11.8%) were classified as anemic (Hb <13g/dl and <11.5g/dl, respectively). Although only a small number of the patients had anemia as defined by normal values, a retrospective analysis of individual patients over time revealed a sustained though small decrease in Hb from initial presentation. A statistically significant difference in Epo levels (P = 0.016 by Kruskal-Wallis test) was observed from the group with the lowest (Hb ≤11.5) to that with the highest (Hb ≥14.5) Hb values, with a median Epo value of 37 (interquartile range 24–42) vs. 13 (9–15) IU/l, respectively. In contrast, there was no evidence of an increased reticulocyte response to higher levels of Epo (r = 0.134 [Pearsons], P = 0.36). Reticulocyte counts ranged from 44 (38–57) to 14.5) to that with the highest (Hb ≥14.5) Hb values, with a median Epo value of 37 (interquartile range 24–42) vs. 13 (9–15) IU/l, respectively. In contrast, there was no evidence of an increased reticulocyte response to higher levels of Epo (r = 0.134 [Pearsons], P = 0.36). Reticulocyte counts ranged from 44 (38–57) to 76.5 (56–83) in the lowest and highest Hb groups, respectively.

CONCLUSIONS — Although only a small number of subjects in the group were overtly anemic, all subjects had an ongoing, small but significant decrease in Hb since presentation. This study of diabetic patients without nephropathy shows an expected increase in Epo production in response to lowering levels of Hb but without the expected reticulocyte response.

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In the U.K., as in the rest of the Western world, diabetes is the most prevalent cause of renal failure. Over the next 10 years, the number of patients with diabetes and end-stage renal disease is expected to double, causing a significant increase in the burden of care for this patient population (1). Although the prognosis with diabetic nephropathy has improved since early reports (2,3), there remains an excess mortality of 70–100 times that of an otherwise matched population (4). Survival rates on dialysis remain poor, with up to 33% of patients dying within a year of starting dialysis (4). Furthermore, for patients who require renal replacement therapy, morbidity as assessed by hospitalization is 2–3 times greater than for nondiabetic patients with end-stage renal failure (2). This excess of morbidity and mortality in part relates to the high incidence of cardiovascular disease in this patient group (5). The identification of mechanisms underlying modifiable factors that may prevent or slow progression or improve patient survival in diabetic nephropathy has therefore become increasingly important.

Patients presenting with diabetic nephropathy commonly have a greater degree of anemia for their degree of renal impairment than those presenting with other causes of renal failure, and anemia develops earlier in these patients than in those with renal impairment from other causes (6,7). Recent studies have identified anemia as a risk factor for the need for renal replacement therapy in diabetes (8); in addition, a lower Hb is significantly associated with a more rapid decline in the glomerular filtration rate (GFR) (9). Furthermore, treating anemia early in renal failure has been demonstrated to slow the rate of decline of renal function (10). Anemia also has a negative impact on patient survival, and is considered to be an important cardiovascular risk factor associated with renal disease. Understanding the pathogenesis of anemia associated with diabetes and nephropathy may therefore lead to opportunities for developing interventions to optimize outcomes in these patients.

Many factors have been suggested as the reason for the earlier onset of anemia in patients with diabetes, including severe symptomatic autonomic neuropathy, causing efferent sympathetic denervation of the kidney and loss of appropriate erythropoietin (Epo) production; damage to the renal interstitium; systemic inflammation; and inhibition of Epo release. It has also been shown that a normochromic, normocytic anemia can occur before...
evidence of renal impairment is present. To clarify the contribution of diabetes and nephropathy to anemia in patients with type 2 diabetes, we examined the hematologic and hematinic parameters of diabetic patients without nephropathy. A retrospective analysis was also performed in which we investigated the longitudinal changes in hematologic parameters of this patient group.

**RESEARCH DESIGN AND METHODS** — Participants were recruited from the Research Clinic of the Academic Diabetes Research Unit, Llandough Hospital, Cardiff, where patients are under long-term follow-up care. All patients had been followed for a minimum of 5 years. Written informed consent was obtained from 62 patients. All recruited patients had type 2 diabetes and had undergone exhaustive yearly health checks, including formal isotope measurement of GFR and assessment of proteinuria. GFR was measured after a single intravenous injection of $^{51}$Cr-EDTA at $t = 0$, with blood samples being drawn at 44, 120, 180, and 240 min. GFR was calculated by biexponential analysis of the 240-min fractional curve for $^{51}$Cr-EDTA corrected to a standard body surface area of 1.73 m$^2$.

At the subjects’ annual diabetes review, serum ferritin, serum Epo levels, and reticulocytes were measured as well as routine parameters including Hb, hematocrit, HbA$_1c$, and GFR. In addition, retrospective hematologic results were collected from the patient records for the period before recruitment.

Statistical analysis was performed using SPSS for Windows, Vers. 11.5. All parameters were tested for distribution, and nonparametric analysis was undertaken where appropriate. Kruskal-Wallis tests were undertaken for $k$ independent variables and Mann-Whitney tests were used for two independent variables. All values are given as medians (interquartile ranges). Correlation coefficients were measured using Pearson’s correlation coefficient. The criterion for statistical significance was $P < 0.05$.

**RESULTS** — The median patient age was 62 (55–69) years with a 45:17 ratio of male-to-female patients. All patients had good glycemic control and diabetes duration of 7 (5–11) years. No patient tested positive for microalbuminuria or proteinuria at any time during the follow-up period. The GFR for all patients reflected normal renal function throughout the study. However, there was a statistically significant drop in GFR from presentation (from $127 \pm 3.6$ to $104 \pm 3.1$ ml/min; means ± SE; $P < 0.001$) that reflected a decrease from a hyperfiltration state in most patients. Therefore, the absence of microalbuminuria and normal GFR excluded the development of incipient diabetic nephropathy over the course of the follow-up period.

The study subjects were divided into anemic and nonanemic groups using normal ranges for male (>13g/l) and female (>11.5g/l) patients. At the time of the study, 8 of 45 male and 2 of 17 female (11.8%) patients were classified as anemic (17.8%). In contrast, at presentation, only 4 of the 62 patients were anemic (7%). Of the anemic patients, only one had an underlying chronic disorder (rheumatic disease) that explained the anemia. Although only a small number of the patients had anemia as defined by normal values at time of study, the retrospective analysis of individual patients...
since presentation revealed a sustained though small decrease in Hb from initial presentation (Fig. 1); analysis of individual changes in Hb over time indicated that 36 of the 62 individuals (58.1%) had sustained a drop in Hb (Fig. 2).

Serum Epo levels in relation to Hb levels at the time of follow-up are illustrated in Fig. 3. A significant negative correlation (Pearson’s correlation coefficient = -0.612) between Epo levels and Hb was demonstrated (P = 0.01). These data suggest an appropriate Epo response to the fall in Hb, as Epo levels were higher in the lowest Hb group. Statistical analyses confirmed a statistically significant difference (P = 0.016 by Kruskal-Wallis) between the group with the lowest (Hb ≤11.5) to group with the highest (Hb ≥15.5) Hb values.

Despite the increase in Epo in those patients with a fall in Hb, there was no reticulocyte response to the higher levels of Epo (Pearson’s correlation coefficient = −0.134; NS). The reticulocyte count was no different between the patient groups with the lowest and highest Hb levels (Fig. 4), and there was no relation between reticulocyte count and Epo concentration (Fig. 5).

To ensure that the observed changes were not the result of iron deficiency, the ferritin concentration was determined at the time of the study for all patients (Fig. 6). There was no difference in the ferritin concentration between the group with the lowest and that with the highest Hb levels, with none of the patient groups demonstrating iron deficiency as assessed by this parameter.

CONCLUSIONS — Anemia is a common complication of chronic kidney disease. It is often more severe and occurs at an earlier stage in patients with diabetic nephropathy than in patients with chronic kidney disease of other causes. Numerous studies have addressed the interaction between diabetes and renal failure in its pathogenesis. The anemia associated with nephropathy results from Epo deficiency, which seems to develop in patients with type 1 diabetes who have even relatively normal levels of serum creatinine. Early Epo-deficiency anemia occurs in both type 1 and type 2 diabetes, although the prevalence may be higher in type 1 diabetes (6); however, most diabetic patients with Epo-deficiency have
type 2 diabetes because type 2 is more common than type 1 diabetes. There is also a greater prevalence of Epo-deficiency anemia in women than in men, but this is not related to iron stores. In addition, Epo-deficiency anemia is associated with the presence of autonomic neuropathy in diabetic patients. In most studies to date, the predominant risk factor for the development of anemia in a diabetic population has been found to be the presence of renal disease, manifested as impaired renal function or albuminuria (11).

Although a small number of patients in the study group were overtly anemic, it is interesting to note that 60% had an ongoing, small but significant decrease in Hb since presentation. This may be partly accounted for by the effect of age, which is known to affect Hb levels, but the prevalence differs markedly from that of a similarly aged Caucasian population by 2–6% (12). The levels of anemia found in this group of type 2 diabetic patients are similar to the levels described in a cohort of type 1 diabetic patients in the recently published study by Thomas et al. (11), where 14% of patients were found to be anemic. It is of note, however, that the study cohort of Thomas et al. contained patients with nephropathy of varying degrees, whereas the group studied within the current study had normal GFRs and no microalbuminuria.

This study attempted to add to our understanding of the mechanisms behind the early onset of anemia in diabetic patients by separating the impact of diabetes from that of nephropathy. The group of patients studied had undergone intensive follow-up in a multidisciplinary diabetic clinic. Furthermore, as a cohort that was self-selected to attend the research clinic, they were a well-motivated and educated group of patients. In contrast to studies performed in patients with nephropathy, this study of diabetic patients without nephropathy showed a different picture in terms of Epo response. We demonstrated the expected normal increase in Epo production in response to lowering levels of Hb in our cohort of diabetic patients in the absence of nephropathy. This is in contrast to the characteristics of anemia associated with diabetic nephropathy, in which impaired function of Epo-producing fibroblasts associated with interstitial fibrosis (13) and a defect of “anemia-sensing” mechanisms associated with autonomic neuropathy (14) may both contribute to EPO deficiency. An analysis of the relation between Epo levels and the reticulocyte response in patients

![Figure 4](image-url)

**Figure 4**—Relation between reticulocyte count and Hb levels at the time of patient recruitment into the study. Data represent medians and interquartile ranges, with open circles representing outliers (>2 SDs from the population mean).

![Figure 5](image-url)

**Figure 5**—Relation between reticulocyte count and Epo levels at the time of patient recruitment into the study. Data represent medians and interquartile ranges, with open circles representing outliers (>2 SDs from the population mean).
EPO resistance in diabetes

with chronic mild anemia of nondiabetic origin conducted by Souweine et al. (15) demonstrated a positive correlation \( P = 0.958 \) in patients with no or mild anemia. In our patient cohort, however, the rise in Epo was not accompanied by the expected normal reticulocyte response, suggesting a state of relative Epo resistance. Our data also suggest that diabetic patients, in the absence of renal disease, are able to mount an appropriate Epo response, consistent with the demonstration of an appropriate response to hypoxia previously reported (16). One of the most potent causes of suboptimal response to Epo is chronic and overt inflammation (17) associated with an increased production of cytokines, such as tumor necrosis factor-\( \alpha \), interleukin-1, or interferon-\( \gamma \) (18), which might suppress erythocyte stem cell proliferation (19). It is therefore interesting to speculate that overt inflammation associated with the diabetic state may contribute to Epo unresponsiveness before the onset of nephropathy.

Although ferritin levels are not always an accurate reflection of iron status, levels in this patient group were within the normal range and no patient showed evidence of overt iron depletion. This finding is consistent with previous studies that failed to demonstrate iron deficiency in a mixed cohort of type 1 and 2 diabetic patients (20). These studies demonstrated elevated iron indexes to be more common in diabetic patients, suggesting that excess iron may have a role in the diabetes development.

Recent studies have highlighted an association between anemia and the development and progression of diabetic nephropathy. There is also a high cardiovascular risk in patients with diabetic nephropathy and a clear association between anemia and abnormal cardiac function. It is therefore an important observation that the development of anemia in diabetes may predate any abnormality in renal function. Furthermore, understanding the mechanism by which this occurs may provide the opportunity to develop therapeutic options that may improve patient outcomes.

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References


Figure 6—Relation between ferritin concentration and Hb levels at the time of patient recruitment into the study, confirming that all patients were iron replete. Data represent medians and inter-quartile ranges, with open circles representing outliers (\( \geq 2 \) SDs from the population mean).


