Haemoglobin variability in Chinese pre-dialysis CKD patients not receiving erythropoietin

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Abstract

Background. Although originally described in dialysis patients treated with recombinant human erythropoietin (rHuEPO), haemoglobin variability has recently also been noted to be increased in patients who have chronic kidney disease (CKD) without dialysis. In our country, pre-dialysis CKD patients generally would not receive rHuEPO treatment. We studied the degree of
haemoglobin variability and its prognostic implication in this group of patients.

**Methods.** We reviewed 332 patients with Stages 3 through 5 CKD; patients with overt iron deficiency or requiring blood transfusion were excluded. Patients were followed for up to 5 years. End points include all-cause mortality, progression to dialysis-dependent renal failure and hospitalization.

**Results.** The average haemoglobin level was 11.4 ± 2.8 g/dL; intra-individual SD of haemoglobin was 0.76 ± 0.61 g/dL. Haemoglobin variability, as represented by intra-individual SD of haemoglobin, was significantly associated with the average haemoglobin level (r = −0.130, P = 0.017), Charlson’s comorbidity score (r = 0.113, P = 0.040) and proteinuria (r = 0.151, P = 0.044). Univariate analysis showed that patients with high haemoglobin variability had a significantly higher all-cause mortality (log-rank test, P = 0.030) and risk of progression to end-stage renal disease (log-rank test, P = 0.021) and was associated with the adjusted duration of hospitalization (r = 0.134, P = 0.015). However, all associations become statistically insignificant after multivariate analysis to control for confounding factors.

**Conclusions.** Fluctuation of haemoglobin level is common and substantial in Chinese pre-dialysis CKD patients. Our results suggest that the observed clinical effect of haemoglobin variability in this patient population is an epiphenomenon secondary to the association between haemoglobin variability and other clinical factors.

**Keywords:** anaemia; cardiovascular disease; renal failure; survival

**Introduction**

Anaemia is a common complication in patients with end-stage renal disease (ESRD) treated with long-term dialysis and recombinant human erythropoietin (rHuEPO). It is now recognized that, in addition to a low haemoglobin level, substantial variability occurs in haemoglobin values in rHuEPO-treated a haemodialysis patients [1]. More importantly, greater haemoglobin variability has been suggested to be associated with comorbidity, intercurrent illness and a higher mortality in dialysis patients [2]. For example, Yang et al. [3] noted that each 1 g/dL increase in the residual SD was associated with a 33% increase in rate of death. Several factors have been found to affect haemoglobin variability, including those that are drug related, such as pharmacokinetic parameters, patient-related differences in demographic characteristics and factors affecting clinical status, as well as clinical practice guidelines, treatment protocols and reimbursement policies [4]. It is not clear whether adverse effects of haemoglobin variability are due to the therapy with rHuEPO and iron or despite such therapeutic agents [4].

Although originally described in dialysis patients, haemoglobin variability has recently also been noted to be increased in patients who have chronic kidney disease (CKD) without dialysis, and the phenomenon is again associated with an increased risk for death even in those who are not receiving rHuEPO [5]. However, most of the published studies on haemoglobin variability come from Western countries with a target haemoglobin level range of 11–12 g/dL; the clinical implication of haemoglobin variability in anaemic CKD patients without rHuEPO treatment remains unknown.

In Hong Kong, pre-dialysis CKD patients would not receive rHuEPO treatment unless they paid for the drug expense and had symptomatic anaemia (usually with haemoglobin level <7 g/dL). Although this practice may not be ideal, it provides an excellent opportunity to examine the degree of haemoglobin variability and its prognostic implication in a large unselected group of anaemic pre-dialysis CKD patients without rHuEPO treatment.

**Patients and methods**

**Case selection**

This was single-centre study of a university teaching hospital. We reviewed all patients who were seen in our nephrology out-patient clinic between January and December 2003 and had Stages 3 through 5 CKD [estimated glomerular filtration rate (GFR) < 60 ml/min/1.73m²]. As part of our internal quality assurance programme, all CKD patients had their haemoglobin level checked during every clinic visit within this period. All patients had a minimum of three haemoglobin measurements within a 6-month period, and they were excluded if they required dialysis or received a kidney transplant during this period. All subsequent laboratory data were collected, and patients were followed up until 30 June 2009. We excluded patients who had a history of overt iron deficiency or were being treated with any erythropoiesis-stimulating agents or those who had blood transfusions during the baseline period. Ethics approval was obtained from the Clinical Research Ethics Committee of our university.

**Definitions of haemoglobin variability**

The intra-individual haemoglobin variability was defined using the earliest 6-month baseline period that included at least three haemoglobin measurements for each patient. Haemoglobin measurements included in the analysis were a minimum of 30 days apart in order to avoid the potential for multiple measurements as a result of intercurrent illness. An individual’s haemoglobin variability was subsequently quantified according to the following two definitions:

(A) the intra-individual SD of haemoglobin values during the 6-month period [6];

(B) the haemoglobin amplitude, defined as the difference between the highest and lowest haemoglobin level during the baseline period [7].

The slope of change in haemoglobin was also computed for each patient and analysed.

**Data collection and outcome measures**

Data were obtained through our computerized Clinical Management System databases or by manual chart reviews. Patient demographics, laboratory results, iron studies, CKD status, presence of thalassaemia trait and commencement of dialysis were recorded. The primary outcome was all-cause mortality. In this part, we performed the analysis both with and without considering the initiation of dialysis as a censoring event. Secondary outcome included time to ESRD (defined as the need for long-term dialysis or transplantation), death or ESRD, number of hospital admissions and duration of hospitalization before ESRD. For the time to ESRD analysis, death as a result of uraemia was counted as an event, while all other causes of death were counted as censored observations.

**Statistical analysis**

Statistical analysis was performed by SPSS for Windows software version 15.0 (SPSS Inc., Chicago, IL). Data are expressed as means ± SDs unless otherwise specified. Data were compared by Student’s t-test, chi-square test or Pearson’s correlation coefficient as appropriate. Kaplan–Meier analysis and log-rank test were used to analyse the effects of haemoglobin variability on all-cause mortality and time to ESRD.

Since log-rank test showed significant difference in the actuarial survival and dialysis-free survival between groups, Cox proportional hazard model was used to exclude confounding effects of other clinical parameters. In addition to the haemoglobin variability, we added age, sex, diabetic status, Charlson’s comorbidity score, baseline glomerular factor rate...
(GFR), proteinuria and average haemoglobin levels as independent variables to adjust for potential confounding variables. Backward stepwise elimination was applied to remove insignificant variables.

For hospitalization, the log-linear model was used for analysis because the data were significantly skewed [8]. The dependent variables were the number of hospital admissions as well as days hospitalized per year of follow-up. The baseline variables used for analysis were similar to those for survival analysis. A value of P < 0.05 was considered statistically significant. All probabilities were two tailed.

Results

During the study period, we identified 363 Stage 3–5 predialysis CKD patients in our clinic; 31 were excluded because of the need for intermittent transfusion or presence of overt iron deficiency. We studied the other 332 patients. The demographic and baseline clinical information are summarized in Table 1. Of the 332 patients, 32 (9.6%) had the thalassaemia trait and 149 (44.9%) had angiotensin-converting enzyme (ACE) inhibitor or angiotensin receptor blocker (ARB) treatment. The average haemoglobin level was 11.4 ± 2.8 g/dL; 96 patients (28.9%) had a baseline haemoglobin level <10 g/dL, while 51 (15.4%) were <9 g/dL. The intra-individual SD of haemoglobin was 0.76 ± 0.61 g/dL; haemoglobin amplitude was 1.46 ± 1.15 g/dL.

Factors affecting haemoglobin variability

There were modest but statistically significant correlations between intra-individual SD of haemoglobin and average haemoglobin level (r = −0.130, P = 0.017), Charlson’s comorbidity score (r = 0.113, P = 0.040) and proteinuria (r = 0.151, P = 0.044). Similarly, haemoglobin amplitude correlated with average haemoglobin level (r = −0.129, P = 0.019) and proteinuria (r = 0.153, P = 0.041). As compared to non-diabetic patients, diabetic ones had a higher intra-individual SD of haemoglobin (0.95 ± 0.75 versus 0.68 ± 0.52 g/dL, P = 0.002) and haemoglobin amplitude (1.80 ± 1.41 versus 1.31 ± 0.99 g/dL, P = 0.002). In contrast, patients with thalassaemic trait had a lower intra-individual SD of haemoglobin (0.57 ± 0.52 versus 0.78 ± 0.61 g/dL, P = 0.039) and haemoglobin amplitude (1.10 ± 0.99 versus 1.49 ± 1.16 g/dL, P = 0.041). Treatment with ACE inhibitor or ARB, however, did not affect the intra-individual SD of haemoglobin or haemoglobin amplitude (details not shown).

All-cause mortality

The patients were followed for an average of 62.6 ± 24.3 months. During this period, 115 patients (34.6%) died (86 before the initiation of dialysis). The causes of death were infection (32 patients), cardiovascular disease (24 patients), cerebrovascular disease (8 patients), uraemia (10 patients), cancer (2 patients), other specific reasons (11 patients) or unknown (28 patients).

When dialysis was censored, univariate analysis showed that patients with high intra-individual SD of haemoglobin had a significantly higher all-cause mortality (log-rank test, P = 0.030) (Figure 1A). In this analysis, each 0.1 g/dL higher of intra-individual SD of haemoglobin was associated with 1.8% excess in the odds of death. The same phenomenon was observed for patients with high haemoglobin amplitude (details not shown). However, the prognostic effect of intra-individual SD of haemoglobin disappeared with multivariate Cox regression analysis to adjust for confounding factors. The final Cox regression model is summarized in Table 2A. In this model, only age and baseline haemoglobin level were independent predictors of all-cause mortality. The result remained similar when patients with thalassaemia trait were excluded, when the slope of change in haemoglobin was included for the analysis or when the survival status was censored at 3 years (details not shown).

When dialysis was not censored, univariate analysis showed that patients with high intra-individual SD of haemoglobin had a significantly higher all-cause mortality (log-rank test, P = 0.010) (Figure 1B). In this analysis, each 0.1 g/dL higher intra-individual SD of haemoglobin was associated with 2.3% excess in the odds of death. The same phenomenon was observed for patients with high haemoglobin amplitude (details not shown). However, the prognostic effect of intra-individual SD of haemoglobin disappeared with multivariate Cox regression analysis to adjust for confounding factors. The final Cox regression model is summarized in Table 2B. In this model, only Charlson’s comorbidity index and baseline haemoglobin level were independent predictors of all-cause mortality. The results remained similar when patients with thalassaemia trait were excluded, when the slope of change in haemoglobin was included for the analysis or when the survival status was censored at 3 years (details not shown).

Progression of ESRD

During the study period, 86 patients (25.9%) progressed to dialysis-dependent ESRD. Univariate analysis showed that patients with high- and low-intra-individual SD of haemoglobin had a similar risk of progression to ESRD (log-rank test, P = 0.206), while patients with high intra-individual SD of haemoglobin had a significantly higher risk of progression to the combined end point of death or ESRD.
The same phenomenon was observed for patients with a high haemoglobin amplitude, but the result did not reach statistical significance ($P = 0.081$, details not shown). However, the effect of intra-individual SD of haemoglobin on the combined end point of death or ESRD disappeared with multivariate Cox regression analysis to adjust for confounding factors. In this model, only baseline-estimated GFR [adjusted hazard ratio (AHR) 0.978, $P = 0.008$] and baseline haemoglobin level (AHR 0.790, $P = 0.001$) were independent predictors of progression to ESRD. The results remained similar when the slope of change in haemoglobin was included for the analysis (details not shown). During the follow-up period, 10 patients were treated with erythropoiesis-stimulating agents, 3 of them died and 2 progressed to ESRD; the results remained similar when these patients were excluded from the analysis (details not shown).

### Hospitalization

During the study period, there were 1340 hospital admissions for a total of 8485 days. Only 83 patients (25.0%) did not require hospital admission. With univariate analysis, the intra-individual SD of haemoglobin had a modest but statistically significant association with the adjusted number of hospital admission ($r = 0.116$, $P = 0.034$) and adjusted duration of hospitalization ($r = 0.134$, $P = 0.015$). Similarly, haemoglobin amplitude was associated with the adjusted number of hospital admission ($r = 0.109$, $P = 0.047$) and adjusted duration of hospitalization ($r = 0.128$, $P = 0.020$). However, the effect of intra-individual SD of haemoglobin and haemoglobin amplitude disappeared with multivariate log-linear regression analysis to adjust for confounding factors. In these models, only Charlson’s comorbidity score and

#### Table 2. Cox proportional hazards model for all-cause mortality

<table>
<thead>
<tr>
<th>Variable</th>
<th>AHR (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A) Dialysis censored</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>1.102 (1.063–1.142)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Average haemoglobin (g/dL)</td>
<td>0.745 (0.600–0.925)</td>
<td>0.008</td>
</tr>
<tr>
<td>(B) Dialysis not censored</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Charlson’s score</td>
<td>1.523 (1.174–1.974)</td>
<td>0.002</td>
</tr>
<tr>
<td>Average haemoglobin (g/dL)</td>
<td>0.764 (0.651–0.895)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

CI, confidence interval.
baseline haemoglobin level were independently associated with the number of hospital admission and duration of hospitalization (details not shown). The result remained similar when the slope of change in haemoglobin was included for the analysis (details not shown).

Discussion

In the present study, we found that in pre-dialysis Chinese CKD patients who were not treated with rHuEPO, haemoglobin variability has a modest association with all-cause mortality, risk of progression to dialysis-dependent renal failure and need of hospitalization. However, all associations become statistically insignificant after multivariate analysis to control for confounding factors. Our result suggests that the observed clinical effect of haemoglobin variability in this patient population is an epiphenomenon secondary to the association between haemoglobin variability and other clinical factors.

Similar to previous studies in erythropoietin-treated dialysis patients [1], we found that substantial variability occurs in haemoglobin values of in pre-dialysis Chinese CKD patients who were not treated with rHuEPO. The magnitude of haemoglobin variability that we observed is also similar to previous reports [2, 5, 9, 10]. Although it has been suggested that haemoglobin variability has a prognostic implication in CKD patients [3], our observation is in line with most of the published studies [2–4, 8, 9]. For example, Gilbertson et al. [2] found that the number of months with haemoglobin values below the target range, rather than haemoglobin variability itself, may be the primary driver of increased risk of death. Brunelli et al. [10] found that haemoglobin variability was not associated with all-cause mortality in incident haemodialysis patients. Although a recent study suggests that haemoglobin variability is associated with an increased risk for death in CKD patients who are not receiving rHuEPO [5], the possible effect of confounding clinical factors could not be excluded.

Although the result is similar, the patient population that we studied is slightly different from published reports [2, 10], which focused mainly on dialysis patients. In the report of pre-dialysis CKD patients by Boudville et al. [5], a substantial proportion of the study population did not receive rHuEPO. However, the threshold of prescribing rHuEPO to anaemic CKD patients in the United States is much lower than that in our country, and nearly one-third of our patients had a baseline haemoglobin <10 g/dL. Furthermore, Chinese patients have a high prevalence of chronic haemolytic condition, such as thalassaemia trait and glucose-6-phosphate dehydrogenase (G6PD) deficiency, which potentially affects haemoglobin variability. In our present study, we did not find any effect of thalassaemia trait; because of the retrospective design, we do not have data on the G6PD status of our patients. Taken together, our results indicate that in the context of low target haemoglobin and high prevalence of chronic hemolytic conditions, the prognostic implication of haemoglobin variability is similar to the Western population.

The mechanism of haemoglobin variability remains uncertain. In patients treated with rHuEPO, pharmacologic features and dosing of erythropoiesis-stimulating agents are generally believed to lead to a cyclical pattern of haemoglobin levels within the recommended range [4]. In patients without rHuEPO treatment, a number of actors related to patient demographics and clinical status have been found to associate with haemoglobin variability [11–14]. More recently, aberrant red cell kinetics and reduced erythrocyte life span in uraemia have been proposed to account for haemoglobin variability in non-rHuEPO-treated CKD patients [15]. Further research is necessary to clarify the physiological mechanism of haemoglobin variability, as well as the benefit of therapeutic intervention that aims to reduce the variability.

There are a few inadequacies in our study. First, our sample size was small and our study may not be powered to detect that haemoglobin variability is indeed an independent predictor of clinical outcomes. Secondly, the prevalence of patients with diabetic nephropathy was also low in this study, which may not be representative of our entire CKD population. In fact, the low prevalence of diabetic nephropathy may reflect a certain degree of selection bias in our study. For example, some patients with diabetic nephropathy may be under the care of endocrinologists until dialysis is needed. Furthermore, diabetic CKD patients tend to have more severe anaemia [16, 17] and blood transfusion (an exclusion criteria of our study) is more likely. For the same reason, our study did not include patients with very low baseline haemoglobin levels.

Because of the retrospective nature, iron status of our patients could not be ascertained beyond doubt. Although most of the anaemic patients had their iron profile checked (and replaced if deficient), occult iron deficiency was not screened in those with relatively normal haemoglobin levels. In other words, we could not exclude the possibility that the haemoglobin variability that we observed was a result of occult iron deficiency, or iron deficiency was a major prognostic indicator of CKD patients. Theoretically, fluctuation in haemoglobin level would precipitate cardiovascular disease, and analysis of its effect on hospitalization should focus on admissions due to acute coronary syndrome and congestive heart failure. However, because of the retrospective design, we could not ascertain the cause of hospitalization for each individual admission.

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Conflict of interest statement. None declared.

References


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