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Lower haemoglobin level and subsequent decline in kidney function in type 2 diabetic adults without clinical albuminuria

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Abstract *Aims/hypothesis:* Anaemia has been suggested to be an independent risk factor for subsequent progression of advanced diabetic nephropathy; however, the relationship between haemoglobin levels and progression of nephropathy in patients without clinical albuminuria is unknown. *Methods:* We conducted this prospective hospital-based cohort study of 464 type 2 diabetic patients (149 women and 315 men, 55±13 [mean±SD] years of age) with serum creatinine <177 μmol/l (2.00 mg/dl) and urinary albumin : creatinine ratio <300 mg/g creatinine. GFR was estimated using the equation formulated by the Modification of Diet in Renal Disease Study group, refitted for Japanese individuals. Most patients had haemoglobin concentrations in the normal range (144±15 g/l), only modest renal impairment (GFR: 74.8±14.5 ml min^{-1} 1.73 m^{-2}), and normal urinary albumin levels (81.5/18.5% with normo-/microalbuminuria). The primary outcome measurement was the rate of change in GFR determined by regression analysis with GFR as a function of time. Patients were followed up for a mean observation period of 5.0±0.9 (range: 2.5 to 6.2) years. *Results:* Univariate and multiple regression analyses yielded a significant association between the rate of change in GFR and baseline haemoglobin concentration. After adjusting for covariates, the rate of decline in GFR was significantly greater in patients in the lowest haemoglobin quartile (−3.27 ml min^{-1} 1.73 m^{-2} $year^{-1}$) than in the third (−2.71 ml min^{-1} 1.73 m^{-2} $year^{-1}$, p=0.024) and highest quartiles (−2.78 ml min^{-1} 1.73 m^{-2} $year^{-1}$, p=0.046). *Conclusions/interpretation:* Lower haemoglobin concentrations in type 2 diabetic patients without clinical albuminuria may be a significant predictor of subsequent decline in GFR.

Keywords Anaemia · Diabetic nephropathy · GFR · Haemoglobin

Abbreviations ACR: albumin:creatinine ratio · ADA: American Diabetes Association · ANCOVA: analysis of covariance · LV: left ventricular · MDRD: Modification of Diet in Renal Disease

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Introduction

In chronic kidney diseases, anaemia commonly results from reduced erythropoietin production of failing kidneys; similarly, in diabetic patients, anaemia is probably associated with erythropoietin deficiency due to concomitant kidney disease [1, 2]. However, the precise mechanisms of anaemia in diabetic patients are unknown, and a decline in haemoglobin concentrations appears to occur earlier in diabetic patients with nephropathy than in patients with non-diabetic kidney diseases [2–7]. In addition to erythropoietin deficiency due to tubulointerstitial changes, which is an early morphological alteration of diabetic nephropathy [8], autonomic neuropathy [9, 10] and increased serum advanced glycation end-products [11] may participate in the pathogenesis of anaemia in diabetic kidney disease.

Recent studies suggest that anaemia may be an independent risk factor for cardiovascular morbidity and mortality in persons with or without diabetes [12, 13]. In addition, we [14] and others [15–17] have demonstrated that decreased haematocrit or haemoglobin levels may be useful in identifying type 2 diabetic individuals at

increased risk of progression of kidney disease. These studies have included diabetic patients with advanced stages of nephropathy, defined as an elevated serum creatinine concentration and/or clinical albuminuria; however, the relationship between haemoglobin levels and the progression of kidney disease in diabetic patients without clinical albuminuria is unknown. We therefore conducted a prospective hospital-based cohort study to examine the relationship between baseline haemoglobin concentrations and the rate of decline in GFR in type 2 diabetic patients with normo- and microalbuminuria.

Subjects and methods

Study population

This was an observational prospective cohort study consisting of consecutive patients referred to the outpatient clinic of the Department of Medicine, Diabetes Centre, Tokyo Women's Medical University Hospital in Tokyo, Japan during 1999. At the first visit, medical history was taken, and demographic, anthropometric, and routine laboratory data were collected. Patients were instructed to bring a first-morning urine specimen to the clinic for the determination of albuminuria. Routine laboratory data were regularly monitored during the follow-up visits.

The study included Japanese individuals with type 2 diabetes, aged 20 years or older, with a baseline serum creatinine concentration <177 μmol/l (2.0 mg/dl) and urinary albumin:creatinine ratio (ACR) in the first-morning urine <300 mg/g creatinine, indicating normo- or microalbuminuria according to the American Diabetes Association (ADA) criteria [18]. Subjects were excluded from the study if they had been treated with recombinant human erythropoietin or if they had fewer than 2.5 years of follow-up observation since the first visit. This minimum observation period was selected on the basis of a previous recommendation for an observation period of at least 2 years for valid determination of the rate of decline in GFR [19].

The study protocol was designed in adherence to the Declaration of Helsinki and informed consent was obtained from the subjects.

Methods

Blood pressure was measured at each visit while the subject was seated, using an oscillometric device (HEM-707; Omron, Kyoto, Japan). Baseline systolic and diastolic blood pressures were defined using average values measured on at least three and up to five visits. Serum creatinine was initially measured by Jaffe's method in the hospital laboratory. From January 2003, the method was replaced by an enzymatic method; therefore, serum creatinine concentrations obtained after January 2003 were adjusted using the following regression equation, obtained from a correlational analysis between both measurements of serum creatinine analysis in 7,370 samples from diabetic patients: serum creatinine (Jaffe's method in μmol/l)=1.029×serum creatinine (enzymatic method in μmol/l)+20.292 (r=0.999, p<0.001). Urinary ACR was calculated from urinary albumin, determined using the latex agglutination method, and urinary creatinine concentration, the latter being initially determined using Jaffe's method (until January 2003) and thereafter by an enzymatic method. As the difference between the methods of measuring urine creatinine concentrations was considered negligible, urine creatinine concentrations were not adjusted.

The primary outcome measurement of this study was the rate of change in estimated GFR. GFR was estimated using the following equation, originating from the Modification of Diet in Renal Disease (MDRD) Study group [20], and refitted for Japanese individuals, as recently proposed by the Working Group of Japan Chronic Kidney Disease Initiative: $GFR=186\times SCr^{-1.154}\times Age^{-0.203}\times 0.742$ (if female)×0.881, where SCr=serum creatinine (unpublished). In a preliminary assessment of 9,312 Japanese diabetic individuals with serum creatinine <177 μmol/l and urinary ACR <300 mg/g creatinine, estimated GFR values using this equation were well-correlated with values obtained using a quadratic GFR equation [21] (r=0.865, p<0.001, Babazono et al., unpublished observations).

For each individual, the rate of change in GFR was determined by the parameter estimates using a simple regression analysis, with GFR as a function of time in years, applied to all estimates of GFRs obtained for that individual during the follow-up period. Because of considerable sex-related differences in haemoglobin concentrations, separate subgroup analyses were also conducted for women and men.

Statistical analysis

Data are expressed as arithmetic mean±SD, geometric mean and 95% CI, or median and interquartile range, as appropriate according to the data distribution. Continuous data between women and men were compared using Student's t-test or Wilcoxon's sum-rank test; categorical data were analysed with Fisher's exact probability test.

To test the effects of haemoglobin levels on the changes in GFR, univariate and multivariate regression analyses were performed. For univariate correlation analyses, Pearson's (r) and Spearman's (rs) correlation coefficients were calculated. For multiple regression analysis, the following were included as covariates: age, diabetes duration, medications for diabetes and hypertension, BMI, systolic and diastolic pressures, HbA_{1C}, logarithmically transformed urinary ACR, and estimated GFR at baseline. Diabetes duration was dichotomised at the median. To determine the potent factors associated with the rate of change in estimated GFR, a stepwise selection procedure was performed, specifying the significance levels, both for entering another covariate into the model, and for removing a covariate from the model, as 0.05.

Haemoglobin concentration was initially and always included in the model, regardless of whether it reached the significant level.

The rate of change in GFR was also compared among quartiles established using baseline haemoglobin concentrations, after adjusting for covariates by analysis of covariance (ANCOVA). Because of the differences in haemoglobin concentrations between the sexes, separate quartiles were obtained for women and men, and then the quartiles for both sexes were pooled.

All statistical analyses were performed using the Statistical Analysis System (SAS Institute, Cary, NC, USA) version 9.13. A p value of less than 0.05 was considered significant.

Results

Demographic and clinical characteristics

Among adult individuals with type 2 diabetes who were referred to the outpatient clinic of the Department of Medicine, Diabetes Centre, Tokyo Women's Medical University Hospital in 1999, a total of 564 had sufficient baseline and follow-up data to qualify for inclusion in this study. Of these, 100 patients were excluded due to a serum creatinine level ≥177 μmol/l and/or urinary ACR ≥300 mg/g creatinine. After these exclusions, 464 individuals, including 149 women and 315 men, aged 20 to 85 (55±13 [mean±SD]) years were selected for analysis.

Demographic and clinical characteristics of the subjects included in this study are presented in Table 1. Men had lower systolic and diastolic blood pressure, lower incidence of hypertension, and higher levels of serum creatinine and haemoglobin than women. Estimated GFR and urinary ACR were comparable between the sexes. The presence of anaemia, defined as a haemoglobin concentration <120 g/l in women and <130 g/l in men (a sex-specific definition established by the WHO [22]), was observed in 19 (12.8%) women and 32 (10.2%) men. Using the sex- and age-specific anaemia criteria in Japanese adults proposed by the Japanese Society for Dialysis Therapy Guidelines [23], only 6 (4.0%) women and 12 (3.8%) men were diagnosed as having anaemia. Based on ADA-guideline criteria [18], 126 (84.6%) women and 252 (80.0%) men were classified as having normoalbuminuria, and 23 (15.4%) women and 63 (20.0%) men were classified as having microalbuminuria; 26 (17.4%)

Table 1 Clinical characteristics of study subjects

	Overall (N=464)	Women (N=149)	Men (N=315)
Age (years)	56±13	56±14	55±13
Known diabetes duration (years)[a]	2.9 (0.3–9.5)	2.7 (0.3–8.0)	3.0 (0.3–9.8)
BMI (kg/m^2)	24.2±4.1	24.1±4.3	24.2±4.0
SBP (mmHg)	135±19	140±22	133±18*
DBP (mmHg)	81±10	82±11	80±10*
Antihypertensive medications (%)			
ACEI and/or ARB	8.9	10.8	8.0
CCB	18.7	20.3	17.9
Others	7.8	8.1	7.7
Any	14.5	27.7	23.0
Hypertension (%)[c]	50.9	58.4	47.3*
Antidiabetic medications (%)			
Diet alone	52.6	49.0	54.3
Oral hypoglycaemic agents	35.3	38.9	33.7
Insulin	12.1	12.1	12.0
HbA_{1C} (%)	8.4±2.0	8.3±1.8	8.4±2.1
Haemoglobin (g/l)	144±15	135±14	148±14*
Anaemia (%)[d]	3.9	4.0	3.8
Serum creatinine (μmol/l)	82.2±15.9	69.8±12.4	88.4±14.1*
Estimated GFR (ml min^{-1} 1.73 m^{-2})	74.8±14.5	73.6±14.0	75.4±14.7
CKD Stage 1/2/3 (%)[e]	14.8/70.0/15.1	12.8/69.8/17.5	15.9/70.2/14.0
Urine ACR (mg/g creatinine)[b]	11.5 (10.5–12.8)	11.2 (9.4–13.3)	11.6 (10.2–13.3)
Normo-/microalbuminuria (%)	81.5/18.5	84.6/15.4	80.0/20.0

Data are expressed as mean±SD, percent of patients, [a]median (interquartile range), and [b]geometric mean (95% confidence interval)
SBP Systolic blood pressure, *DBP* diastolic blood pressure, *ACEI* ACE inhibitor, *ARB* angiotensin receptor antagonist, *CCB* calcium channel blocker, *CKD* chronic kidney disease, *ACR* albumin:creatinine ratio
[c]SBP ≥140 mmHg and/or DBP ≥90 mmHg or treated with antihypertensive medications
[d]defined by the Japanese Society for Dialysis therapy Guideline
[e]Stage 1: GFR ≥90 ml min^{-1} 1.73 m^{-2}; stage 2: GFR=60–89 ml min^{-1} 1.73 m^{-2}; stage 3: GFR=30–59 ml min^{-1} 1.73 m^{-2}
*p<0.05 vs women

Table 2 Univariate correlation analysis with changes in GFR as a dependent variable

	Overall (*N*=464)		Women (*N*=149)		Men (*N*=315)	
	r (*rs*)	*p* value	*r* (*rs*)	*p* value	*r* (*rs*)	*p* value
Age (years)	0.099	0.033	0.193	0.018	0.057	0.315
Known diabetes duration (years)*	−0.081	0.082	−0.083	0.315	−0.090	0.112
BMI (kg/m^2)	0.151	0.001	0.035	0.676	0.204	<0.001
SBP (mmHg)	0.012	0.803	−0.059	0.473	0.074	0.190
DBP (mmHg)	0.112	0.016	0.101	0.220	0.127	0.024
HbA_{1C} (%)	−0.083	0.077	−0.086	0.296	−0.086	0.129
Serum creatinine (μmol/l)	0.372	<0.001	0.403	<0.001	0.380	<0.001
Estimated GFR (ml min^{-1} 1.73 m^{-2})	−0.409	<0.001	−0.460	<0.001	−0.399	<0.001
ACR (mg/g creatinine)[a]	−0.264	<0.001	−0.250	0.002	−0.274	<0.001
Haemoglobin (g/l)	0.152	0.001	0.111	0.177	0.134	0.017

[a]Because of skewed distribution Spearman's correlation coefficient (*rs*) was calculated. In other variables, Pearson's coefficient (*r*) was calculated

SBP Systolic blood pressure, *DBP* diastolic blood pressure, *ACR* albumin:creatinine ratio

women and 44 (14.0%) men had an estimated GFR <60 ml min^{-1} 1.73 m^{-2}, defined as stage 3 chronic kidney disease, according to Kidney Disease Outcomes Quality Initiative guideline [24].

Follow-up data

The mean and median observation periods were 5.0±0.9 and 5.3 years, respectively, ranging from 2.5 to 6.2 years. The median number of follow-up measurements of serum creatinine concentrations, with valid information for estimation of GFR, was 11 measurements per subject and 2.3 measurements per subject per year. No patients were treated with recombinant human erythropoietin at baseline or during the follow-up period, and no subjects reached end-stage renal disease or required dialysis or kidney transplantation. The mean rate of change in GFR change was −2.94±2.12 ml min^{-1} 1.73 m^{-2} year^{-1} for overall subjects, −3.21±1.95 ml min^{-1} 1.73 m^{-2} year^{-1} for women, and −2.81±2.19 ml min^{-1} 1.73 m^{-2} year^{-1} for men. The difference between the sexes was of marginal statistical significance (*p*=0.056).

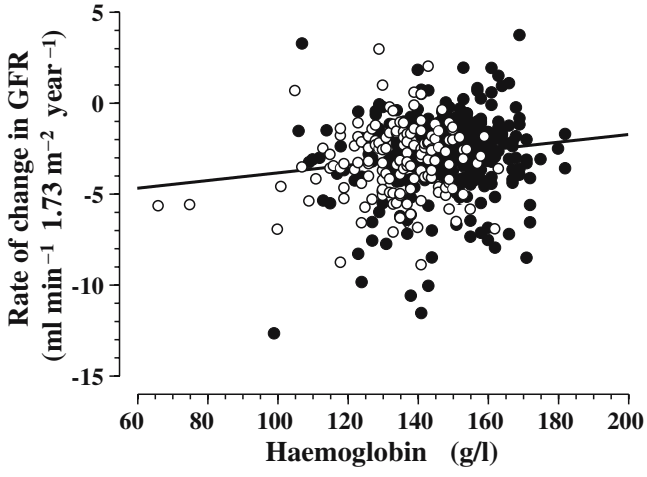

Fig. 1 Correlational analysis between baseline haemoglobin concentration and the rate of change in GFR (*r*=0.152, *p*=0.001). *Open circles*, women; *closed circles*, men

Association between haemoglobin levels and changes in GFR

In the univariate regression analyses, baseline haemoglobin concentration was significantly associated with the rate of change in GFR for study subjects overall (*r*=0.152, *p*=0.001; Table 2, Fig. 1) and in men (*r*=0.134, *p*=0.017), but not in women (*r*=0.111, *p*=0.117). Baseline urinary ACR and GFR were associated with the rate of change in GFR in both women and men. Based on the correlation coefficients, a lower baseline haemoglobin concentration, higher baseline ACR, and higher baseline GFR were predictive of a faster decline in GFR. Correlations of other variables with the rate of change in GFR are listed in Table 2.

As shown in Table 3, multiple regression analyses demonstrated a significant relationship between haemoglobin concentrations and the rate of change in GFR for all study subjects and, when analysed separately, in men and women. Significant associations of urinary ACR and baseline GFR with the rate of change in GFR were also confirmed in the multiple regression analyses for women and for men (Table 3).

Quartile ranges for baseline haemoglobin concentration for women and men are given in Table 4. Those factors found to be significantly associated with change in GFR in the multiple regression analysis (BMI, logarithmically transformed ACR, and baseline GFR, as well as sex), were incorporated as covariates in the ANCOVA. As shown in Table 4, the rate of change in GFR was significantly greater for the lowest haemoglobin quartile than for the third (*p*=0.024) and highest quartiles (*p*=0.046).

Table 3 Results of multiple regression analyses with stepwise variable selection to determine potent factors associated with changes of estimated GFR per year in patients with type 2 diabetes

Independent variable	Parameter estimate	Standard error	*p* value	Standardised estimate
Overall				
Intercept	−2.027	0.950	0.033	0.000
Haemoglobin (g/l)	0.021	0.006	<0.001	0.153
BMI (kg/m^2)	0.068	0.022	0.002	0.130
Log [ACR (mg/g Cr)]	−1.094	0.170	<0.001	−0.257
GFR (ml min^{-1} 1.73 m^{-2})	−0.060	0.006	<0.001	−0.403
Women				
Intercept	0.199	1.487	0.894	0.000
Haemoglobin (g/l)	0.022	0.010	0.022	0.163
ACEI or ARB (yes/no)	−1.094	0.453	0.017	−0.176
Log [ACR (mg/g Cr)]	−0.944	0.297	<0.001	−0.228
GFR (ml min^{-1} 1.73 m^{-2})	−0.072	0.010	<0.001	−0.506
Men				
Intercept	−2.476	1.307	0.059	0.000
Haemoglobin (g/l)	0.018	0.008	0.023	0.117
BMI (kg/m^2)	0.093	0.028	<0.001	0.167
Log [ACR (mg/g Cr)]	−1.080	0.210	<0.001	−0.253
GFR (ml min^{-1} 1.73 m^{-2})	−0.055	0.007	<0.001	−0.371

ACR Albumin:creatinine ratio

Discussion

In this hospital-based prospective cohort study in Japanese individuals with type 2 diabetes but without clinical albuminuria, we have demonstrated, for the first time, a significant association between baseline haemoglobin concentrations, most of which were in the normal range, and the rate of decline in GFR. This association was confirmed by treating haemoglobin concentration both continuously in the univariate and multivariate regression analyses and categorically in the ANCOVA. These results are consistent with our earlier observation [14] and other hospital-based cohort studies from Japan [15] and Denmark [17] in diabetic subjects with overt nephropathy, which indicated that decreased haemoglobin concentration or lower haematocrit may be an independent risk factor for the progression of kidney disease in diabetic patients, regardless of the presence of albuminuria. A sub-analysis of the Reduction in Endpoints in NIDDM with the Angiotensin II Antagonist Losartan study [16] also identified anaemia as a predictor of further progression of advanced kidney disease in patients with type 2 diabetes. The present study therefore extends these findings to diabetic patients with modest renal impairment and normo- and microalbuminuria.

In diabetes, anaemia, associated with erythropoietin deficiency, appears to occur earlier in the course of progression of kidney disease than in non-diabetic patients with kidney diseases [2–7]. Haemoglobin concentrations were found to be significantly decreased in patients with microalbuminuria compared to patients with normoalbuminuria [3]. Autonomic neuropathy has been postulated to play a role in erythropoietin dysregulation in diabetic patients [9, 10]. In addition, nutrient deficiencies including iron, folate, and vitamin B12, or undetected malignant diseases or anaemia of chronic inflammation (formally termed anaemia of chronic diseases) [25] may contribute to anaemia in diabetic subjects. Although a majority of

Table 4 Comparison of adjusted rate of change in GFR by quartiles based on baseline haemoglobin concentration

Quartile	Range of haemoglobin (g/l)		Number of subjects		Rate of change in GFR (ml min^{-1} 1.73 m^{-2} year^{-1})[a]	*p* value[b]
	Women	Men	Women	Men		
First	<126	<141	38	82	−3.27±0.17	–
Second	129–135	141–149	35	78	−2.99±0.17	0.251
Third	136–143	150–157	38	77	−2.71±0.17	0.024
Fourth	≥144	≥158	38	78	−2.78±0.17	0.046

Haemoglobin quartiles were determined separately for women and men, and then pooled
[a]Adjusted mean (±SEM) for BMI, logarithmically transformed ACR, baseline GFR, and sex
[b]*versus* the first quartile

diabetic patients in our study were normoalbuminuric and not overtly anaemic, our data suggest lower haemoglobin levels predict a more rapid rate of decline in GFR. However, the difference in the rate of decline in GFR between the lowest and highest haemoglobin concentration quartile (−3.27±0.17 vs −2.78±0.17 ml min^{-1} 1.73 m^{-2} $year^{-1}$, p=0.046) was relatively modest for patients in this study.

The mechanisms by which lower haemoglobin levels may impact on the progression of chronic kidney disease in type 2 diabetes are unknown. Some of the explanations may be provided by in vitro and in vivo studies examining the effects of anaemia-induced renal hypoxia and oxidative stress. In culture cells, hypoxia has been found to increase mesangial/tubular cell extracellular matrix synthesis, leading to fibrogenic changes in the kidney by activating several gene transcripts including transforming growth factor-β (TGF-β), osteopontin, and the recently discovered hypoxia inducing factor-1 (HIF-1) [26, 27]. Hypoxia-induced altered renal sympathetic nerve activity may differentially regulate glomerular haemodynamics [28]. Close associations between anaemia and cardiac disorders, including left ventricular (LV) hypertrophy and heart failure [29, 30], may also provide a basis for explaining the anaemia-induced loss of kidney function in diabetes, as these conditions are associated with decreased effective renal blood flow. Indeed, an increase in LV mass has been reported to occur early in the course of diabetic nephropathy. However, no significant association was observed between haemoglobin concentrations and LV mass index [31] in type 1 diabetic patients with nephropathy.

Several retrospective trials have suggested that correction of anaemia with erythropoietin may delay the onset of dialysis in predialysis patients [32, 33]; however, the benefits of treating anaemia associated with early diabetic nephropathy are still uncertain. This observational study did not attempt to determine whether correction of anaemia in early stages of diabetic kidney disease attenuates the decline rate of kidney function. Ongoing prospective multicentre studies such as the Anaemia Correction in Diabetes trial [34] and Effect of early Correction of Anaemia on the Progression of Chronic Kidney Disease (ECAP) trial [35] may provide important information in this regard.

Our study has several limitations. It was carried out in an urban university hospital in an ethnically homogenous population, which may not be representative of the entire type 2 diabetic patient population. The generalisability of these findings will need to be confirmed in future studies. The original and refitted MDRD equations may have limitations for estimating GFR in individuals with normal or near-normal kidney function [36, 37]. Our preliminary observations suggest that estimated GFR using the refitted MDRD equation in Japanese diabetic patients is well correlated with values obtained using a quadratic GFR equation, which is reported to perform better than the MDRD equation in healthy individuals [21]. Although the performance of creatinine-based estimation of GFR remains at issue, the disputed factors would affect absolute values most, and are of less importance when assessing individual change in GFR over a long period, which was the main focus of the present study. Finally, urinary ACR was measured on a single occasion, possibly leading to overestimation of albuminuria because of marked day-to-day variability in albumin excretion [38]. The ADA guideline recommends repeating measurements of urinary albumin for the classification of abnormalities in albumin excretion [18]. Although we did not obtain multiple measurements of urinary ACR, we restricted the timing of urine collection to first morning urine to minimise the variation of albumin excretion due to exercise and diurnal fluctuations [38, 39].

In conclusion, this hospital-based prospective cohort study in Japanese patients with type 2 diabetes indicates that lower haemoglobin concentration may be a significant predictor of subsequent decline in GFR even in patients without clinical albuminuria. Results of ongoing trials on the effect of correcting anaemia on the progression of early stages of diabetic kidney disease are eagerly awaited.

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