HbA1c reliability in patients with diabetes on regular hemodialysis before and after erythropoietin therapy

Mohamed M. Abdel-Aziz[a], Inas F. Shaltout[a], Ula M. Al-Jarhi[a], Yasmine T. Alshewi[b] and Mahmoud M. Elalfy[c]

Departments of [a]Medicine, [b]Chemical Pathology, Cairo University and [c]Eltalaba Hospital, Cairo, Egypt

Correspondence to Mohamed M. Abdel-Aziz, MD, Department of Internal Medicine, Faculty of Medicine, Cairo University, 11451 Cairo, Egypt
Tel: 002-01005587271;
e-mail: m_momtaz_m@hotmail.com

Received 18 January 2013
Accepted 30 January 2013

Egyptian Journal of Internal Medicine
2013, 25:70–74

Purpose
The purpose of this study was to determine the effect of erythropoietin (EPO) treatment on HbA1c levels in diabetic patients on regular hemodialysis and to assess the reliability of HbA1c as a marker for glycemic control in such patients.
Methods
The study included 41 patients on regular hemodialysis who were EPO naive: 31 with diabetes mellitus and 10 nondiabetic controls. Baseline HBA1c and fasting blood glucose levels were measured and repeated after a 3-month course of EPO.
Results
HbA1c decreased significantly after EPO therapy ($P=0.01$) and was associated with a significant decline in fasting blood glucose levels ($P=0.001$), with a significant negative correlation with hemoglobin ($r=-0.185$, $P=0.03$). HbA1c showed significant correlation with fasting blood glucose in diabetic patients before EPO therapy ($r=0.82$, $P<0.0001$). This correlation was found to be independent of other laboratory parameters. No correlation was found between HbA1c and fasting blood glucose levels after 3 months of EPO treatment.
Conclusion
HbA1c is not a reliable marker for glycemic control in hemodialysis patients, especially for those on EPO therapy.

Keywords:
diabetes mellitus, end-stage renal disease, erythropoietin, HbA1c

Egypt J Intern Med 25:70–74

1110-7782

Introduction and aim of the work

Monitoring glycemia is the cornerstone of diabetes care [1]. It is recommended by the ADA for prevention of microvascular complications [2]. Available techniques that have been approved for monitoring glycemia include self-monitoring of blood glucose, A1c measurement, and continuous glucose monitoring in selected cases [1].

A1c measures the amount of glycosylated hemoglobin in the blood. Glycosylated hemoglobin can be differentiated into three distinct fractions: A1a, A1b, and A1c; of these, A1c is the most abundant [3]. The process of glucose binding to hemoglobin occurs continuously during the life span of a red blood cell (RBC), which is ~120 days. Thus, it is commonly accepted that the level of A1c reflects the previous 2–3 months of glycemic control and is widely used as a measure of long-term control [4].

The direct correlation of A1c with mean plasma glucose levels has been well established, especially after the A1c-Derived Average Glucose Trial [5]. However, A1c is not a 'fair' measure of plasma glucose for the entire 3 months. It was shown to reflect the latter days in the erythrocyte life span to a greater extent. Fifty percent of the resultant A1c was attributed to the past 30 days only, 25% reflected the previous 30–60 days, whereas the remaining 25% reflected the rest of the 120 days [6].

A1c levels do not vary according to age and sex. Timing of A1c sampling in relation to meals is irrelevant as well [7]. Nevertheless, ethnic variation has been claimed to exist and needs further evaluation [8], especially as large studies on A1c reliability in the Egyptian population are lacking.

More importantly, A1c was found to be directly influenced by the turnover rate of RBCs. This is explained by the fact that the longer the RBCs circulate in the blood stream, the more extensively its hemoglobin becomes glycated (low turnover). The reverse is true if RBCs spend less time in the circulation (high turnover) [9].

Similarly, factors shortening the life span of RBCs will falsely reduce A1c level. The A1c level has been shown to be falsely decreased in patients with hemolysis, such as sickle cell anemia or thalassemia, and in those who have undergone blood transfusions [10].

Conditions that may cause the A1c level to be falsely elevated include uremia, chronic alcohol intake, splenectomy, chronic renal failure, iron deficiency anemia, and hypertriglyceridemia [11,12].

The aim of this study was to determine the effect of erythropoietin (EPO) treatment on HbA1c levels in diabetic

patients on regular hemodialysis and to assess the reliability of HbA1c as a marker for glycemic control in such patients.

Research design and methods

This is a prospective comparative cohort study.

Patients and methods

The study included 42 end-stage renal disease patients receiving regular hemodialysis in three different hemodialysis units in Cairo: Student Hospital (14 patients), Mostafa Mahmoud haemodialysis unit (15 patients), and Imbaba El Aam hospital (13 patients). All patients were EPO naive; 31 patients were known to be diabetic and 11 were not known to be diabetic. All patients were started on EPO treatment for 3 months. Exclusion criteria included factors that may alter hemoglobin status or known to influence A1c levels: prior EPO treatment, chronic alcohol intake, splenomegaly and splenectomy, hypertriglyceridemia, hyperbilirubinemia, chronic ingestion of salicylates, opiate addiction, hemolytic anemia, recent blood transfusions, chronic liver disease, and intake of vitamins C and E, dapsone, antiretrovirals, phenacetin, or nitrites.

The total number of patients who completed the study was 41. One patient dropped out and underwent renal transplantation. The medical records for all patients were reviewed and the available data were collected. Informed consent was obtained from all participants.

All patients were subjected to a thorough clinical examination including recording of their dry body weight, height and BMI, assessment of blood pressure, heart rate, signs of anemia, uremia, volume overload, and chest, heart, abdomen, and neurological examination.

Laboratory investigations included assessment of blood urea and serum creatinine levels before and after hemodialysis, creatinine reduction ratio, urea reduction ratio, serum sodium and serum potassium levels, complete blood count, and serum uric acid, serum albumin, fasting serum glucose level, and HbA1c levels.

Laboratory method for A1c measurement

A1c was measured by column chromatography using a quantitative colorimetric ion exchange chromatographic determination of glycohemoglobin in whole blood' assay kit, manufactured by Stanbio Laboratory (Boerne, Texas, USA, Cat. No. 0350). In this method, a preparation of hemolyzed whole blood is mixed with a weakly binding cation-exchange resin. The nonglycosylated hemoglobin (HbA0) binds to the resin, leaving HbA1 free to be removed by means of a resin separator in the supernate. The percentage of HbA1 is determined by measuring the absorbance values at 415 nm of the HbA1 fraction and of the total Hb fraction, calculating the ratio of absorbances (R), and comparing this ratio with that of a glycohemoglobin standard obtained through the same procedure. Results, expressed as HbA1, are converted to HbA1c using a conversion factor [13].

Statistical methods

The data were coded and entered in a Microsoft Excel spreadsheet. SPSS, version 15 (SPSS Inc., Chicago, Illinois, USA) was used for summarization and presentation of the data, including comparison of all laboratory parameters before and after EPO therapy using the paired t-test and two-tailed probability. Further, correlation of HBA1c with fasting blood glucose (FBS) and other parameters was determined by calculation of correlation coefficients.

MedCalc version 12.2.1.0 (MedCalc Software, Acacialaan, Ostend, Belgium) was used for design of scatter graphs and comparison diagrams.

Ethical consideration

The medical record profession has its own code of ethics, which applies to all medical record practitioners. Confidentiality of data, safe data storage, and privacy rights are respected by all who handle patient information. Data were coded and patient names or identity was concealed in data collection forms or during statistical analysis.

The study protocol was approved and verified by the faculty of medicine ethical committee, Cairo University.

Results

In diabetic patients ($n = 31$) before EPO treatment mean (± SE) A1c was 6.57 ± 0.25 and mean (± SE) FBS was 121.52 ± 7.02. After EPO treatment for 3 months the mean value (± SE) of A1c and FBS was 5.87 ± 0.18 and 105.16 ± 4.2, respectively. There was significant decline in HbA1c after 3 months of EPO therapy ($t = -2.639$, $P = 0.0131$), which was associated with significant decline in FBS ($t = -3.545$, $P = 0.0013$) (Fig. 1).

Among patients who were not known to be diabetic ($n = 10$), three met the recent ADA criteria for diagnosis of diabetes according to the basal A1c level [14,15]. No significant decline in A1c or FBS was demonstrated statistically in this group of patients; however, the three patients who were found to be diabetic experienced a significant decline in their A1c levels, and in their FBS to a lesser extent (Fig. 2).

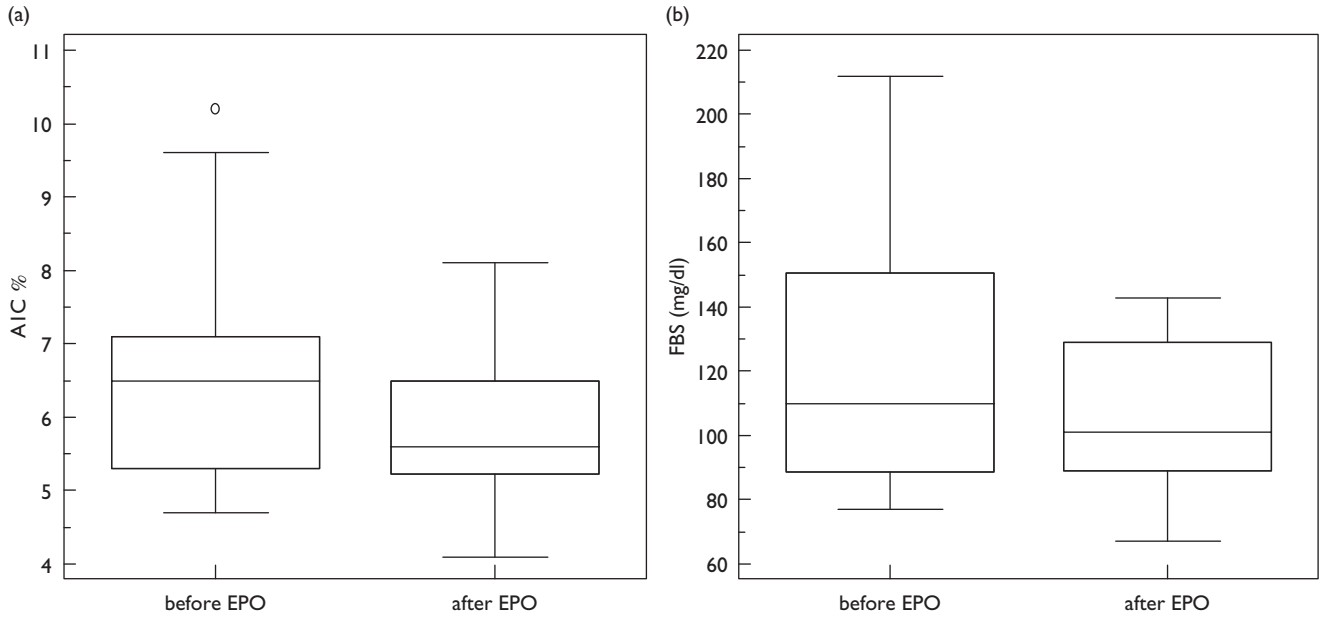
HbA1c showed significance correlation with FBS in diabetic patients before EPO therapy ($r = 0.82$, $P < 0.0001$). This correlation was found to be independent of other laboratory parameters. However, this positive correlation was lost after 3 months of EPO therapy. No correlation was found between A1c and FBS in nondiabetic controls (Fig. 3).

A comparison of basal parameters with those after 3 months of EPO treatment revealed a nonsignificant increase in Hb% ($P = 0.085$). The decline in A1c after EPO therapy showed a significant negative correlation with Hb% increase ($r = -0.185$, $P = 0.032$).

Discussion

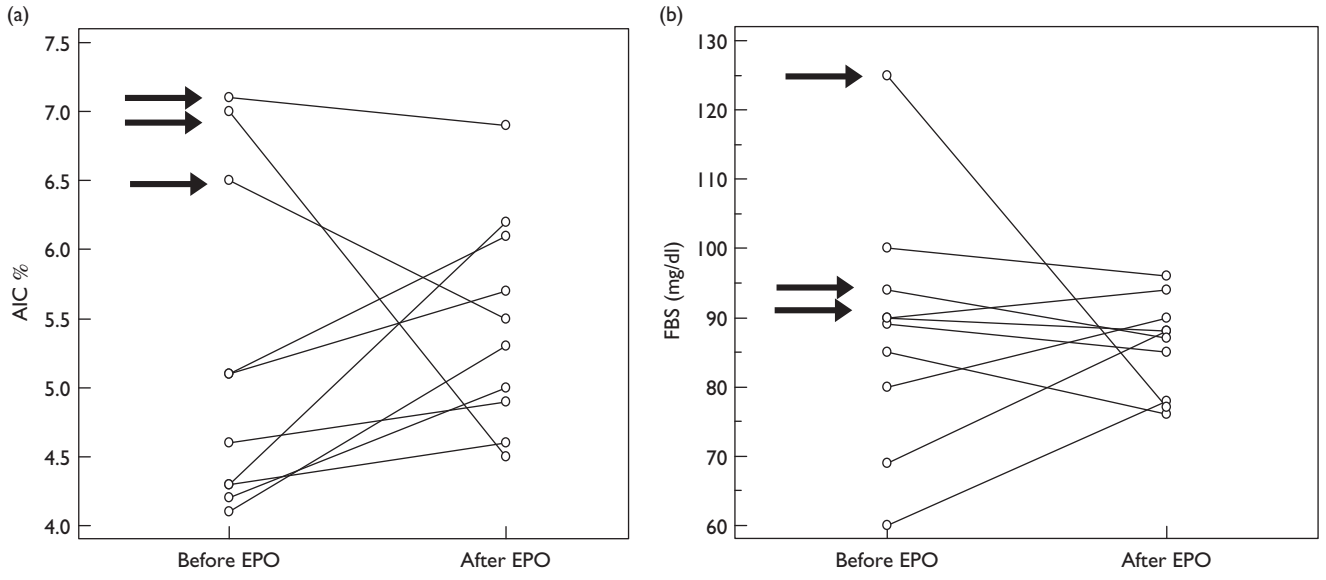
The study demonstrated a significant decline in both A1c and FBS levels after 3 months of EPO treatment. The reason for the improvement in FBS levels in patients who

Figure 1



Comparison of A1c (a) and fasting blood glucose (FBS) (b) levels before and after EPO treatment for 3 months in diabetic hemodialysis patients ($n=31$). Before EPO, mean (±SE) A1c was 6.57±0.25 and mean (±SE) FBS was 121.52±7.02. After EPO treatment for 3 months the mean value (±SE) of A1c and FBS was 5.87±0.18 and 105.16±4.2, respectively. The paired sample *t*-test showed a significant decrease after EPO treatment in both (a) and (b) [(a) $t=-2.639$, $P=0.0131$, 95% CI: −1.24 to −0.16; (b) $t=-3.545$, $P=0.0013$, 95% CI: −25.78 to −6.93]. CI, confidence interval; EPO, erythropoietin.

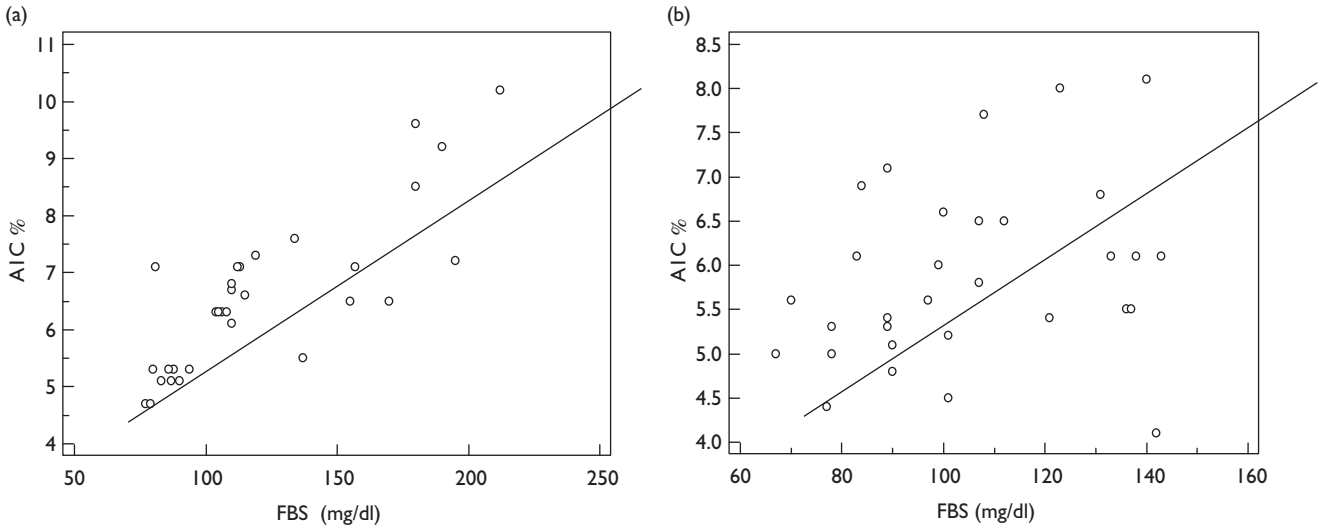
Figure 2



Comparison of A1c (a) and fasting blood glucose (FBS) (b) levels before and after EPO treatment for 3 months in nondiabetic hemodialysis patients ($n=10$). Before EPO, mean (±SE) A1c was 5.23±0.38 and mean (±SE) FBS was 88.2±5.55. After EPO treatment for 3 months, the mean value (±SE) of A1c and FBS was 5.47±0.24 and 85.9±2.20, respectively. The paired sample *t*-test showed no significant differences before and after treatment; however, arrowheads denote patients first discovered to be diabetic by A1c criteria. All three patients experienced a drop in A1c and FBS levels after EPO treatment. EPO, erythropoietin.

were known to be diabetic as well as in those diagnosed as diabetic during the study is as yet unclear. It is known that presence of anemia will result in falsely higher glucose levels when whole blood is tested. This is because RBCs are relatively glucopenic, and hence whole blood applied to glucose test strips has 15% less glucose compared with

Figure 3



Correlation between A1c and fasting blood glucose (FBS) in diabetic hemodialysis patients before (a) and after (b) EPO treatment for 3 months ($n=31$). (a) Significant positive correlation between A1c and FBS: correlation coefficient $r=0.8182$, $P<0.0001$, 95% CI 0.65–0.91. (b) Loss of correlation after EPO treatment. CI, confidence interval; EPO, erythropoietin.

plasma [4]. This 'artifact' does not apply to our study, which measured serum glucose, thus nullifying the effect of hematocrite on glucose levels. The decline in FBS therefore reflects a true improvement in glycemic control. Thus, presence of anemia may prove to have a deleterious effect on glycemic control, and its correction can gain additional importance in achieving tighter control levels.

In contrast, the decline seen in A1c levels after EPO therapy is a false decline. It is due to the addition of new RBCs to the existing pool, with less circulating time and hence lower glycation rates. The proportion of new RBCs to old ones after EPO therapy is deemed to increase, thus falsely decreasing A1c levels. This hypothesis can be extrapolated to expect an increase in A1c levels if EPO doses are decreased or stopped. Therefore, titration of EPO dose administration, which is a regular practice in hemodialysis patients, will cause continuous variability in A1c levels every time EPO is started, stopped or dose-adjusted.

A1c showed significant correlation with FBS in diabetic hemodialysis patients who were EPO naive. The loss of this correlation after 3 months of EPO therapy suggests that EPO may have an impact on HbA1c that is independent of blood glucose levels. Moreover, there was no correlation between changes in HbA1c levels and trends present in other laboratory data, such as serum albumin, creatinine clearance, and electrolytes. This shows that the significant decline in HbA1c after EPO treatment is unlikely to be attributed to the effect of other variables. It is because of an independent direct or indirect effect of EPO.

The results of the study suggest that the effect of EPO on HbA1c is probably mediated through the former's effect on erythroid lineage in the bone marrow and subsequent change in RBC turnover and life span. This is evidenced by the significant negative correlation between HbA1c decline and Hb% increase. Indeed, newly formed RBCs, which are stimulated by EPO and are less glycated, both decrease A1c and elevate Hb%.

Monitoring glycemia in diabetic hemodialysis patients is important in this subgroup with a higher risk of developing microvascular and macrovascular complications of diabetes. Recent studies have shown that identifying a reliable marker for glycemic control in such patients is challenging. However, it seems that higher A1c level is associated with decreased survival in these patients, whereas lower A1c level if not related to malnutrition or anemia is associated with better survival rates [16].

EPO is widely used in the treatment against renal anemia, and ~75% of patients on hemodialysis are candidates for EPO. A fall in HbA1c levels following EPO therapy has been attributed to the addition of new RBCs to the existing pool. This leads to an alteration in the proportion of young to old RBCs and also changes in rates of glycation [17].

Variability in the total number of RBCs as drug doses are titrated is also to be expected. Both the age of RBCs and their total number are known determinants of HbA1c levels; therefore, diabetic EPO receivers are likely to experience limitations in A1c reliability [18].

A study published in 2007 by Inaba and colleagues showed that HbA1c measurement in diabetic hemodialysis patients on weekly erythropoeitin might lead to underestimation of glycemic levels. The study concluded that this effect is likely due to the increasing proportion of young RBCs by the use of EPO [8].

Similarly, Ng *et al.* [19] in 2010 illustrated a significant fall in A1c levels following EPO therapy without changes in

glycemic control in patients with type 2 diabetes and chronic kidney disease.

The authors conclude that HbA1c is unreliable as a marker for glycemic control in diabetic patients on regular hemodialysis. Its value is even weaker in those receiving EPO therapy, as EPO was found to cause a significant decrease in HBA1c independent of serum glucose level. This results in 'falsely low' HBA1c values under such circumstances.

Good glycemic control is the recommended practice in diabetic patients, including those on hemodialysis. HBA1c is classically viewed as a gold standard biomarker for glycemic control; therefore, it is frequently used as a primary endpoint in major trials on diabetes. However, its significance in renal patients is beginning to be reconsidered. Primary and secondary endpoints in diabetic patients should be revised in favor of true morbidity and mortality, which are in fact our real targets in testing preventive and therapeutic interventions.

Acknowledgements

M.A. wrote the study design, data research, and contributed to discussion. U.M.J. wrote the manuscript and contributed to statistical analyses.

The authors thank Mahmoud Mohammed ElAlfy, MBBCh, Faculty of Medicine, Cairo University, for assistance in data collection.

They also thank Prof. Inas Fahim Shaltout, Professor of Internal Medicine and Endocrinology, Faculty of Medicine, Cairo University, for her support in providing expert assistance throughout the research.

Conflicts of interest

There are no conflicts of interest.

References

1 American Diabetes Association. Clinical practice recommendations: V. Diabetes care. Diabetes Care 2012; 35 (Suppl 1):S16–S28.

2 American Diabetes Association. Clinical practice recommendations: VI. Prevention, management of complications. Diabetes Care 2012; 35 (Suppl 1): S28–S38.

3 Bacigalupo G, Langner K, Saling E. Glycosylated hemoglobin (HbA1), glucose tolerance and neonatal outcome in gestational diabetic and non-diabetic mothers. J Perinat Med 1984; 12:137–145.

4 Saudek CD, Derr RL, Kalyani RR. Assessing glycemia in diabetes using self-monitoring blood glucose and hemoglobin A1c. JAMA 2006; 295: 1688–1697.

5 Nathan DM, Kuenen J, Borg R, Zheng H, Schoenfeld D, Heine RJ. Translating the A1C assay into estimated average glucose values. Diabetes Care 2008; 31:1473–1478.

6 Tahara Y, Shima K. The response of GHb to stepwise plasma glucose change over time in diabetic patients. Diabetes Care 1993; 16:1313–1314.

7 Chachou A, Randoux C, Millart H, Chanard J, Gillery P. Influence of in vivo hemoglobin carbamylation on HbA(1c) measurements by various methods. Clin Chem Lab Med 2000; 38:321–326.

8 Kahn R, Fonseca V. Translating the A1C assay. Diabetes Care 2008; 31:1704–1707.

9 Inaba M, Okuno S, Kumeda Y, Yamada S, Imanishi Y, Tabata T, *et al*. Glycated albumin is a better glycemic indicator than glycated hemoglobin values in hemodialysis patients with diabetes: effect of anemia and erythropoietin injection. J Am Soc Nephrol 2007; 18:896–903.

10 Smaldone A. Evidence-based clinical decision making – glycemic control and hemoglobinopathy: when A1C may not be reliable. Diabetes Spectrum 2008; 21:46–49.

11 Wallach J. *Interpretation of diagnostic tests*. 7th ed. Philadelphia: Lippincott Williams & Wilkins; 2000. pp. 610–611.

12 Sinha N, Mishra TK, Singh T, Gupta N. Effect of iron deficiency anemia on hemoglobin A1c levels. Ann Lab Med 2012; 32:17–22.

13 Abraham EC, Huff TA, Cope ND, Wilson JBJ, Bransome EDJ, Huisman TH. Determination of the glycosylated hemoglobins (HB AI) with a new microcolumn procedure. Suitability of the technique for assessing the clinical management of diabetes mellitus. Diabetes 1978; 27:931–937.

14 American Diabetes Association. Executive summary: standards of medical care in diabetes – 2012. Diabetes Care 2012; 35 (Suppl 1): S4–S10.

15 Selvin E, Steffes MW, Gregg E, Brancati FL, Coresh J. Performance of A1C for the classification and prediction of diabetes. Diabetes Care 2011; 34:84–89.

16 Kalantar Zadeh K, Kopple JD, Regidor DL, Jing J, Shinaberger CS, Aronovitz J, *et al*. A1C and survival in maintenance hemodialysis patients. Diabetes Care 2007; 30:1049–1055.

17 Okada T, Nakao T, Matsumoto H, Nagaoka Y, Wada T. Influence of erythropoietin-stimulating agent treatment on glycated hemoglobin values in diabetic patients with chronic kidney disease. Clin Exp Nephrol 2011; 15:316–317.

18 Sacks DB, Bruns DE, Goldstein DE, Maclaren NK, McDonald JM, Parrott M. Guidelines and recommendations for laboratory analysis in the diagnosis and management of diabetes mellitus. Clin Chem 2002; 48: 436–472.

19 Ng JM, Cooke M, Bhandari S, Atkin SL, Kilpatrick ES. The effect of iron and erythropoietin treatment on the A1C of patients with diabetes and chronic kidney disease. Diabetes Care 2010; 33:2310–2313.