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Anaemia, independent of chronic kidney disease, predicts all-cause and cardiovascular mortality in type 2 diabetic patients

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ABSTRACT

Objective: There is limited and controversial information on whether anaemia is a risk factor for cardiovascular mortality in type 2 diabetes, and whether this risk is modified by the presence of chronic kidney disease (CKD). We assessed the predictive role of lower hemoglobin concentrations on all-cause and cardiovascular mortality in a cohort of type 2 diabetic individuals.
Methods: The cohort included 1153 type 2 diabetic outpatients, who were followed for a mean period of 4.9 years. The independent association of anaemia (i.e., hemoglobin <120 g/l in women and <130 g/l in men) with all-cause and cardiovascular mortality was evaluated by Cox proportional hazards regression models and adjusted for several potential confounders, including kidney function measures.
Results: During follow-up, 166 (14.4%) patients died, 42.2% ($n=70$) of them from cardiovascular causes. In univariate analysis, anaemia was associated with increased risk of all-cause (hazard ratio HR 2.62, 95% confidence intervals 1.90–3.60, $p<0.001$) and cardiovascular mortality (HR 2.70, 1.67–4.37, $p<0.001$). After adjustment for age, sex, body mass index, smoking, hypertension, dyslipidemia, diabetes duration, hemoglobin A1c, medication use (hypoglycemic, anti-hypertensive, lipid-lowering and anti-platelet drugs) and kidney function measures, the association of anaemia with all-cause (adjusted HR 2.11, 1.32–3.35, $p=0.002$) and cardiovascular mortality (adjusted HR 2.23, 1.12–4.39, $p=0.020$) remained statistically significant.
Conclusions: Anaemia is associated with increased risk of all-cause and cardiovascular mortality in type 2 diabetic individuals, independently of the presence of CKD and other potential confounders. The advantage to treat anaemia in type 2 diabetes for reducing the risk of adverse cardiovascular outcomes remains to be demonstrated.

1. Introduction

Several epidemiologic studies have consistently shown that anaemia and chronic kidney disease (CKD) are both important risk factors of all-cause and cardiovascular mortality in the general population and in some groups of high-risk patients [1–5]. However, the information on the relationship among anaemia, CKD and cardiovascular outcomes in people with type 2 diabetes is limited and controversial, even though anaemia is common in type 2 diabetic patients with CKD. In fact, anaemia is often more severe and occurs at an earlier stage of CKD in patients with type 2 diabetes compared with those with other causes of kidney disease [5].

In a recent study Vlagopoulos et al. [6] showed that anaemia, as defined by low levels of hematocrit (i.e., <36% in women and <39% in men), was a significant risk factor for cardiovascular disease and all-cause mortality primarily in diabetic patients who also had CKD (defined as an estimated glomerular filtration rate <60 ml/min/1.73 m^2), whereas anaemia was not a risk factor for any outcome in those with normal or near-normal kidney function [6]. In contrast, Tong et al. reported that in a cohort of Chinese type 2 diabetic patients, who were followed for ~3 years, the risk of future cardiovascular events increased progressively in presence of decreasing levels of hematocrit only in those without declining kidney function [7].

The aim of this study was to examine whether anaemia is associated with increased risk of all-cause and cardiovascular mortality

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in a cohort of type 2 diabetes patients, and whether this risk is modified by the presence of CKD.

2. Methods

The study was performed within the frame of the Verona Diabetes Study, an observational longitudinal study on chronic complications in type 2 diabetic outpatients attending the diabetes clinic at the University Hospital of Verona [8,9]. Data included in this analysis are based upon the cohort of Caucasian type 2 diabetic outpatients ($n = 1153$), who were recruited over the period of January 2000 to January 2002 and then followed-up until September 30, 2007, after excluding (i) patients who had a history of malignancy, chronic obstructive pulmonary disease, end-stage renal disease or cardiovascular disease (defined as angina, myocardial infarction, revascularization procedures and stroke), and (ii) those who had incomplete clinical and biochemical data for analysis. Type 2 diabetes was established when diagnosis was made after the age of 35 years, irrespective of treatment, or when the disease was treated with diet or oral hypoglycemic agents, irrespective of age at diagnosis. More details about the study design and recruitment methods have been reported elsewhere [10]. Briefly, the 1153 participants included in this analysis represented approximately 30% of the whole sample of type 2 diabetic outpatients ($n = 3727$), who regularly attended our diabetes clinic during years 2000–2002 and who had complete laboratory data for analysis after excluding patients with the above-mentioned comorbid conditions ($n = 989$, 26.5%). Notably, baseline demographics (~50% male; mean age: 67 years *vs.* 66 years; mean body mass index: 27.9 kg/m^2 *vs.* 28.2 kg/m^2; mean diabetes duration: 16.0 years *vs.* 15.4 years and mean hemoglobin A1c 7.5% *vs.* 7.6%, respectively) as well as the overall crude rates of all-cause (14.4% *vs.* 14%) and cardiovascular (6.1% *vs.* 6.5%) mortality were not significantly different between the 1153 participants of the study and those with missing data for hemoglobin or other laboratory variables ($n = 1585$). All participants were periodically seen (every 3–6 months) for routine medical examinations of glycemic control and chronic complications of diabetes. The local ethics committee approved the study protocol. All participants gave their informed consent.

Body mass index (BMI) was calculated by dividing weight in kilograms by the square of height in meters. Blood pressure was measured with a standard mercury manometer. Information on comorbid conditions, current use of medications and smoking history was obtained in all patients by interviews during medical examinations. In all participants venous blood was withdrawn in the morning after an overnight fast for standard biochemical work-up. Hemoglobin concentration was determined by a photometrical technique on the fully automated hematological analyzer ADVIA 120-TM (Bayer Diagnostics, Milan, Italy). Serum creatinine (measured by using the Jaffé method – kinetic alkaline picrate assay), lipids and other biochemical blood measurements were determined by automatic colorimetric methods (DAX 96, Bayer Diagnostics, Milan, Italy). LDL-cholesterol was calculated by Friedewald's equation, except in those with triglycerides exceeding 4.55 mmol/l ($n = 36$). Hemoglobin A1c was measured by a high-performance liquid chromatography analyzer (Bio-Rad Diamat, Milan, Italy) and the upper limit of normality was 5.6%.

Anaemia was defined as hemoglobin concentrations <120 g/l in women and <130 g/l in men, according to the World Health Organization criteria. Glomerular filtration rate was estimated (eGFR) from the four-variable Modification of Diet in Renal Disease (MDRD) equation [11] as follows: $\text{eGFR} = 186 \times (\text{serum creatinine}^{-1.154}) \times (\text{age}^{-0.203}) \times 1.212$ (if black) $\times$ 0.742 (if female). Urinary albumin excretion was measured from an early morning urine sample on at least three consecutive occasions, within a period of 4–6 months, as the albumin/creatinine ratio (ACR) by an immuno-nephelometric method. Microalbuminuria was defined as an ACR of 30–299 mg/g and macroalbuminuria as an ACR of ≥300 mg/g [12]. The presence of CKD was diagnosed when a patient had either eGFR <60 ml/min/1.73 m^2 and/or abnormal albuminuria (i.e., micro- or macroalbuminuria) irrespective of eGFR values [11,12]. Hypertension was defined as a systolic blood pressure ≥140 mmHg and/or a diastolic blood pressure ≥90 mmHg or current use of any treatment with anti-hypertensive medications. Information regarding specific classes of anti-hypertensive drugs (e.g., ACE-inhibitors and angiotensin receptor blockers) was not currently available. Atherogenic dyslipidemia was diagnosed when plasma triglycerides were ≥1.70 mmol/l and/or HDL-cholesterol was <1.04 mmol/l or when patients were taking lipid-lowering agents [12].

Vital status on September 30, 2007 was ascertained for all participants ($n = 1153$) by examining the database of the Social Health Unit of the Veneto Region, which includes all records of mortality occurred within the Veneto Region as well as the specific causes of death. Causes of death were identified in 100% of subjects. Trained nosologists coded death certificates using the International Classification of Diseases, Ninth Revision (ICD-9). Deaths were attributed to cardiovascular diseases when ICD-9 codes were 390–459. A selected sample of death certificates was reviewed manually to validate the process.

2.1. Statistical analysis

Data are presented as means ± SD or proportions. Skewed variables were logarithmically transformed to improve normality prior to analyses. The unpaired *t*-test and the chi-squared test with Yates's correction for continuity (for categorical variables) were used to compare the baseline clinical characteristics of participants stratified by the presence or absence of anaemia, defined as hemoglobin <120 g/l in women and <130 g/l in men. Univariate survival analysis stratified by anaemia and CKD status was performed by the Kaplan–Meier analysis, and the overall significance was calculated by the log-rank test. Cox regression analysis was used to study the effect of anaemia on the risk of all-cause and cardiovascular mortality after adjustment for potential confounders. Four forced entry multivariate regression models were performed. Hemoglobin was included as either continuous or dichotomous variable in these models. The first multivariate regression model was adjusted for age (years), gender (male *vs.* female) and BMI (kg/m^2); the second model for age (years), gender (male *vs.* female), BMI (kg/m^2), diabetes duration (years), hemoglobin A1c (%), hypertension (yes/no; see definition above), atherogenic dyslipidemia (yes/no; see definition above), LDL-cholesterol (mmol/l) and current use of medications (anti-platelet and hypoglycemic drugs) (yes/no); finally, the third and fourth regression models were further adjusted for eGFR and albuminuria, which were included as either categorical (i.e., eGFR <60 ml/min/1.73 m^2 *vs.* ≥60 ml/min/1.73 m^2 and ACR <30 mg/g *vs.* ≥30 mg/g, respectively) or continuous measures. The covariates included in multivariate regression models were chosen as potential confounders based on their biological plausibility or statistical association with mortality in univariate analysis. Results are presented as hazard ratios (HRs) with 95% confidence intervals (CI) and statistical significance was evaluated by the likelihood-ratio test. HRs for continuous variables were computed for each SD change. Statistical analyses were performed with statistical package SPSS 14.0. *p*-Values <0.05 were considered statistically significant.