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Original Article

ORIGINAL ARTICLE

New-onset anemia and associated risk of ESKD and death in non-dialysis CKD patients: a multicohort observational study

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ABSTRACT

Background. Anemia is a common complication of chronic kidney disease (CKD), but its incidence in nephrology settings is poorly investigated. Similarly, the risks of adverse outcomes associated with new-onset anemia are not known.
Methods. We performed a pooled analysis of three observational cohort studies including 1031 non-anemic CKD patients with eGFR <60 mL/min/1.73 m^2 regularly followed in renal clinics. We estimated the incidence of mild anemia (hemoglobin 11–12 g/dL in women and 11–13 g/dL in men) and severe anemia (hemoglobin <11 g/dL or use of erythropoiesis-stimulating agents) during a 3-year follow-up period. Thereafter we estimated the risk of end-stage kidney disease (ESKD) and all-cause death associated with new-onset mild and severe anemia.
Results. The mean age was 63 ± 14 years, 60% were men and 20% had diabetes. The mean estimated glomerular filtration rate (eGFR) was 37 ± 13 mL/min/1.73 m^2 and the median proteinuria was 0.4 g/day [interquartile range (IQR) 0.1–1.1]. The incidence of mild and severe anemia was 13.7/100 patients-year and 6.2/100 patients-year, respectively. Basal predictors of either mild or severe anemia were diabetes, lower hemoglobin, higher serum phosphate, eGFR <30 mL/min/1.73 m^2 and proteinuria >0.50 g/day. Male sex, moderate CKD (eGFR 30–44 mL/min/1.73 m^2) and moderate proteinuria (0.15–0.50 g/day) predicted only mild anemia. The incidence of anemia increased progressively with CKD stages (from 8.77 to 76.59/100 patients-year) and the proteinuria category (from 13.99 to 25.02/100 patients-year). During a median follow-up of 3.1 years, 232 patients reached ESKD and 135 died. Compared with non-anemic patients, mild anemia was associated with a higher adjusted risk of ESKD {hazard ratio [HR] 1.42 [95% confidence interval (CI) 1.02–1.98]} and all-cause death [HR 1.55 (95% CI 1.04–2.32)]. Severe anemia was associated with an even higher risk of ESKD [HR 1.73 (95% CI 1.20–2.51)] and death [HR 1.83 (95% CI 1.05–3.19)].

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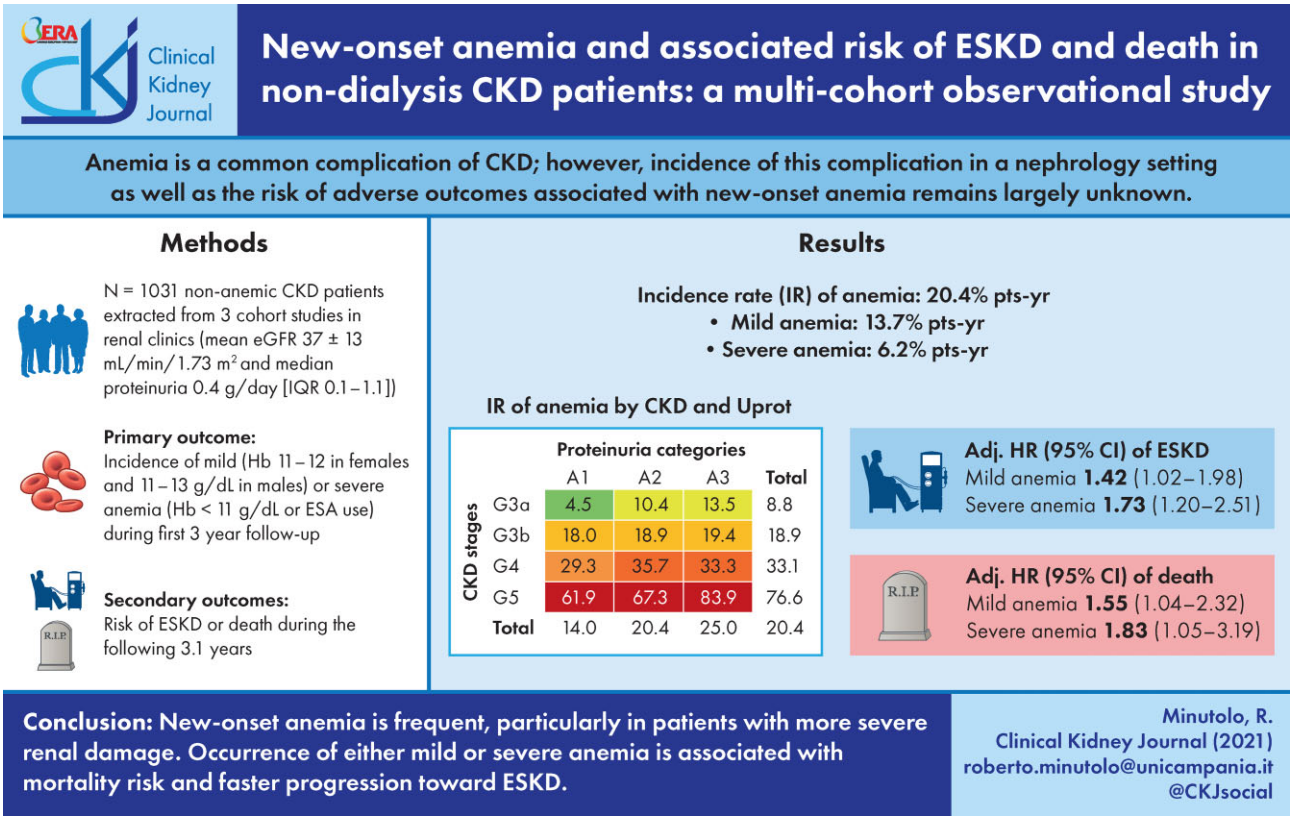
Conclusions. New-onset anemia is frequent, particularly in patients with more severe renal damage and in those with diabetes mellitus. The occurrence of anemia, even of a mild degree, is associated with mortality risk and faster progression towards ESKD.

GRAPHICAL ABSTRACT



Keywords: anemia, epidemiology, ESKD, non-dialysis CKD

INTRODUCTION

Anemia is a common complication of chronic kidney disease (CKD) and its prevalence increases progressively as renal function deteriorates, with almost all patients on dialysis receiving erythropoiesis-stimulating agents (ESAs) [1, 2]. It has been reported that anemia involves a large proportion of CKD patients followed in renal clinics, from 60% in Sweden [3] to 58% in Spain [4] and 45–62% in Italy [5, 6]. This finding has been recently confirmed by the Chronic Kidney Disease Outcomes and Practice Patterns Study (CKDopps), in which 28–52% of 6766 CKD patients followed in France, Germany, Brazil and the USA showed hemoglobin (Hb) <12 g/dL [7].

In contrast with anemia prevalence, the incidence of new-onset anemia remains poorly investigated in non-dialysis CKD patients, even in those under nephrology care. In a small prospective study involving 344 patients with CKD stage 3, Portolés *et al.* [8] reported that ~30% of patients developed renal anemia over 36 months of observation. A similar finding can be extrapolated from a study focusing on the relationship between fibroblast growth factor-23 and anemia, where new-onset anemia was reported in 36% of 1,834 non-anemic individuals with an estimated glomerular filtration rate (eGFR) of 20–70 mL/min/1.73 m^2 [9]. The incidence reported in these two studies is likely underestimated due to the exclusion of patients with more advanced CKD. Finally, by analyzing a subgroup of non-anemic CKD patients enrolled in the KoreaN cohort study for Outcome in patients With Chronic Kidney Disease (KNOW-CKD), Nam *et al.* [10] reported that 25% of patients developed anemia (Hb <13.0 g/dL for men and <12.0 g/dL for women) over a median follow-up of 21 months.

Of note, no study has evaluated the occurrence of different degrees of anemia, that is, mild anemia (Hb 11–12 g/dL in women or 11–13 g/dL in men) or severe anemia (Hb <11 g/dL or need of ESA supplementation), as well as determinants of incident anemia. This information is relevant when considering that therapeutic approaches differ in the two anemic conditions, with iron supplementation being the only option for mild anemia, while ESA therapy with or without iron is indicated for severe anemia.

Similarly, the association between new-onset anemia and adverse outcomes in CKD patients has been poorly investigated. Only two studies have addressed this issue, but they adopted different study designs, outcomes and anemia definitions [5, 8]. Indeed, in one study, the risks of end-stage kidney disease (ESKD) and all-cause mortality were assessed in the period following anemia occurrence [5], while in the other study,

the incidence of the outcome of the interest (hospitalization, mortality and progression to CKD stage 4–5) was captured in the same time period in which anemia onset was assessed [8]. More important, anemia was defined by using different Hb cut-off levels, without differentiating mild from severe anemia. Conversely, knowing the specific impact of mild or severe anemia on adverse outcomes as well as the clinical and demographic factors promoting a different anemia degree is relevant to optimize risk stratification of the CKD population.

In this study we estimated the incidence of mild and severe anemia in patients with moderate to advanced CKD by selecting, from a multicohort study, non-anemic CKD patients regularly followed in renal clinics in Italy. As secondary objectives, we explored the clinical characteristics predicting different degrees of anemia and the impact of development of this complication on the future risk of ESKD and all-cause mortality.

MATERIALS AND METHODS

This is a pooled analysis of three prospective cohorts carried out in 40 nephrology clinics in Italy [6, 11, 12] (Supplementary data, Figure S1). A detailed description of the cohorts is reported in Supplementary data, Items S1 and S2. The three cohorts shared similar selection criteria by including consecutive CKD patients steadily followed in nephrology clinics for at least 6 months before the study baseline. Common exclusion criteria were renal replacement therapy, acute kidney injury in the 6 months prior to the baseline visit, active malignancy and advanced liver or heart disease. For the purposes of the present study, additional exclusion criteria were duplicate patients, missing visits after baseline and the presence of anemia at the baseline visit (Hb <12 g/dL in women and <13 g/dL in men or use of ESA). Institutional review boards of the participating centers approved the three studies and patients gave written consent to use their clinical data. The research was conducted according to the principles of the Declaration of Helsinki.

Clinical covariates

Participating nephrologists collected demographic information and clinical history, performed a physical examination with assessment of height, body weight, blood pressure (BP), and medication profile and reviewed lab results [13, 14]. Information was reported in anonymous electronic case reports that were sent to the coordinating centers (Supplementary data, Item S1) for quality assessment, storage and analyses. All the participating nephrologists used the same criteria to define existing comorbidities, pathologies and cause of CKD [13, 14]. Laboratory protocols were standardized using in-house analytical measurements. eGFR was estimated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation; since creatinine was not standardized, we decreased creatinine values by 5% [15].

All available visits during the first 3 years of follow-up were reviewed to evaluate the incidence of anemia, classified as mild (Hb 11–12 g/dL in women and 11–13 g/dL in men) or severe (Hb <11 g/dL irrespective of sex or use of ESA).

Outcomes

Primary outcome was the occurrence of either mild or severe anemia in the 3 years after baseline visit. Secondary outcomes were the predictors of mild and severe anemia and the risk of ESKD, defined as chronic dialysis or kidney transplantation, and all-cause death associated with onset of mild and severe anemia. The follow-up for events (ESKD and death) started from the visit in which anemia (mild or severe) occurred, or from the last visit during the 3-year observation period only for those patients not developing anemia, and lasted until the last clinic visit before the end of follow-up period, death or ESKD, whichever occurred first.

Statistics

Continuous variables were reported as either mean ± standard deviation (SD), mean [95% confidence intervals (CIs)] or median and interquartile (IQR) range based on their distribution and compared by either one-way analysis of variance, Kruskall–Wallis test, paired Student's *t*-test or Wilcoxon test, as appropriate. Categorical variables (expressed as percentage) were analyzed using the chi-squared or McNemar test.

Because mild and severe anemia are competitive events, that is, the occurrence of severe anemia prevents mild anemia, we calculated during a period of 3 years after the baseline visit the cumulative incidence of mild anemia by using the cumulative incidence function method. Cumulative incidence curves by either eGFR categories (G3a–G5) or proteinuria categories (A1–A3) were compared by using the Gray test [16]. Predictors of mild and severe anemia were evaluated by using multinomial logistic regression analysis. This model was chosen instead of ordinal logistic regression under a proportional odds model because the proportionality assumption was not met. The model was built by including *a priori* the variables potentially associated with the occurrence of anemia: age, sex, body mass index (BMI), diabetes mellitus, prior cardiovascular disease, Hb, serum phosphate, transferrin saturation (TSAT), ferritin, use of renin–angiotensin system (RAS) inhibitors, eGFR categories (G3a–G5) and proteinuria categories (A1–A3).

We determined the ability of demographic and clinical characteristics to discriminate subgroups of patients with a different incidence of severe anemia by means of classification tree analysis based on chi-squared automatic interaction detection. The classification tree analysis is represented graphically as an inverted tree. In the model, we included the same factors used for the multinomial regression analysis. To avoid computer-generated cut-offs, all continuous variables were dichotomized before inclusion in the model as follows: age ≤ or >65 years, serum phosphate ≤ or >3.8 g/dL, TSAT<20% and/or ferritin <100 ng/mL. Hb was included as less than or greater than or equal to the sex-specific median value, because the absolute value of Hb is closer to the threshold of 11 g/dL (severe anemia) in females than in males.

Multivariable Cox proportional hazards models were used to estimate the risks of ESKD and all-cause death associated with the development of mild or severe anemia by using as a reference category the subgroup that never showed anemia. All Cox models were adjusted for potential confounders measured at the visit in which anemia was detected or at the 3-year visit for those not developing anemia. The following covariates were identified *a priori*: age, sex, diabetes mellitus, prior cardiovascular disease, BMI, serum phosphate, total cholesterol, eGFR, log-transformed 24 h proteinuria, systolic blood pressure and use of RAS inhibitors. Proportional hazards assumptions were evaluated using Schoenfeld residuals tests. We used Cox models because the cause-specific relative hazards are more appropriate for studying the cause of disease in the case of a competing event [17]. Data were analyzed using Stata, version 14 (Stata Corp, College Station, TX, USA) and SPSS statistics version 26 (IBM, Armonk, NY, USA).

Table 1. Demographic and clinical characteristics of patients at entry visit

Characteristics	Overall (N = 1031)	No anemia (n = 634)	Mild anemia (n = 266)	Severe anemia (n = 131)	P-value
Age (years), mean ± SD	62.8 ± 14.4	62.0 ± 14.2	65.0 ± 14.2	62.3 ± 15.3	0.015
Men (%)	59.9	61.5	63.5	45.0	<0.001
BMI (kg/m^2), mean ± SD	27.3 ± 4.7	27.7 ± 4.5	27.8 ± 4.9	26.3 ± 5.0	0.004
Smoking (%)[a]	13.5	13.9	12.7	13.3	0. 914
Diabetes mellitus (%)	20.3	16.4	27.1	25.2	<0.001
History of CVD (%)	27.8	26.8	31.2	26.0	0. 357
eGFR categories (%)					<0.001
Stage G3a	25.5	32.8	14.3	13.0	
Stage G3b	43.1	45.7	44.0	28.2	
Stage G4	26.7	19.7	35.7	42.0	
Stage G5	4.8	1.7	6.0	16.8	
Proteinuria categories (%)					<0.001
A1 (<0.15 g/day)	29.9	35.3	23.7	16.0	
A2 (0.15–0.50 g/day)	26.6	26.5	29.3	21.4	
A3 (>0.50 g/day)	43.5	38.2	47.0	62.6	

[a]Data available in 763 patients.

RESULTS

Differences in demographic and clinical characteristics between enrolled patients and those excluded due to the presence of anemia at baseline are depicted in Supplementary data, Tables S1 and S2. From the pooled cohort (N = 2483), we first extracted unique CKD patients with at least two visits and subsequently excluded patients with anemia at baseline (N = 1096); the remaining 1031 subjects that met inclusion criteria were analyzed (Supplementary data, Figure S1). Differences in demographic and clinical characteristics between enrolled patients and those excluded due to the presence of anemia at baseline are depicted in Supplementary data, Tables S1 and S2.

Overall, the mean eGFR was 37 ± 13 mL/min/1.73 m^2 and the median proteinuria was 0.4 g/day (IQR 0.1–1.1). At baseline, Hb was 14.0 ± 1.3 g/dL with a normal iron status (TSAT >20% and ferritin >100 ng/mL) in 51.2% of patients. Underlying causes of renal diseases were as follows: hypertensive nephropathy 24.6%, glomerulonephritis 16.8%, diabetic nephropathy 15.0%, tubulointerstial nephritis 8.1%, polycystic kidney disease 5.8% and other/unknown 29.6%.

Incidence of anemia

Overall, we analyzed 3566 visits during a median follow-up of 2.7 years (IQR 1.9–3.0); the median number of visits per patient was 4 (IQR 3–4). The majority of patients (61.5%) remained free of anemia, while 25.8% and 12.7% developed mild anemia and severe anemia, respectively. Patients not developing anemia contributed to the follow-up with 1360 patient-years, while those developing mild and severe anemia contributed with 414 and 174 patient-years, respectively. As expected, follow-up was longer in patients not developing anemia [2.4 years (IQR 1.5–2.9)] than in those developing mild [1.5 years (IQR 1.0–2.2)] or severe anemia [1.2 years (IQR 0.6–1.8)]. Among the 131 patients developing severe anemia, 31 had previously experienced mild anemia; in these subjects, the median time from mild to severe anemia was 9.8 months (IQR 5.5–15.1). At baseline, compared with patients who remained non-anemic, those developing mild and severe anemia more frequently had diabetes mellitus, advanced CKD and more severe proteinuria (Table 1). No difference was detected for causes of renal diseases among the three groups (P = 0.113).

The incidence rate of anemia was 20.4/100 patient-years (13.7/100 patient-years for mild anemia and 6.2/100 patient-years for severe anemia). The cumulative incidence of anemia significantly varied by CKD stage, with severe anemia occurring predominantly in stage 5 (Figure 1A), while the incidence of mild anemia became evident in stage 3A and 3B (Figure 1B). A higher incidence of severe anemia was detected in patients with proteinuria in the category A3 (Figure 1C), while mild anemia similarly occurred in patients with A2–A3 proteinuria categories (Figure 1D). The incidence of anemia (either mild or severe) increased with increasing proteinuria and in each category with increasing CKD stage (Supplementary data, Table S3).

Predictors of mild and severe anemia

Baseline eGFR was higher in non-anemic patients than in those developing mild or severe anemia. Accordingly, serum phosphate levels were lower and Hb was higher (Table 2). During the observation period, eGFR declined less in non-anemic patients (P < 0.001); indeed, their median slope of eGFR was significantly lower [−0.79 mL/min/year (IQR −3.17–1.82)] compared with patients developing mild anemia [−2.20 mL/min/year (IQR −5.26–1.23)] and severe anemia [−3.56 mL/min/year (IQR −9.01 to −0.27)] (P = 0.001 versus mild anemia). At the end of the observation period, proteinuria and blood pressure were lower compared with baseline, while in patients developing mild or severe anemia these parameters remained unchanged (Table 2). Iron supplementation was infrequent at baseline and its use increased during the observation period only in patients developing anemia, thus contributing to maintain constant TSAT and ferritin levels; however, these parameters remained suboptimal in relation to the prevalence of iron deficiency (Table 2).

Table 3 reports the results of multinomial regression analysis. The likelihood of having mild anemia progressively increased from stage G3b to G5 of CKD as well as in the presence of proteinuria (categories A2–A3). Conversely, a higher probability of severe anemia was detected only in patients with more severe renal damage at baseline, that is, in patients with eGFR <30 mL/min/1.73 m^2 as well as in those with proteinuria >0.5 g/day. Diabetes mellitus was associated with a higher risk of mild and severe anemia (72% and 87%, respectively); this occurred in the presence of similar eGFRs and proteinuria in patients with

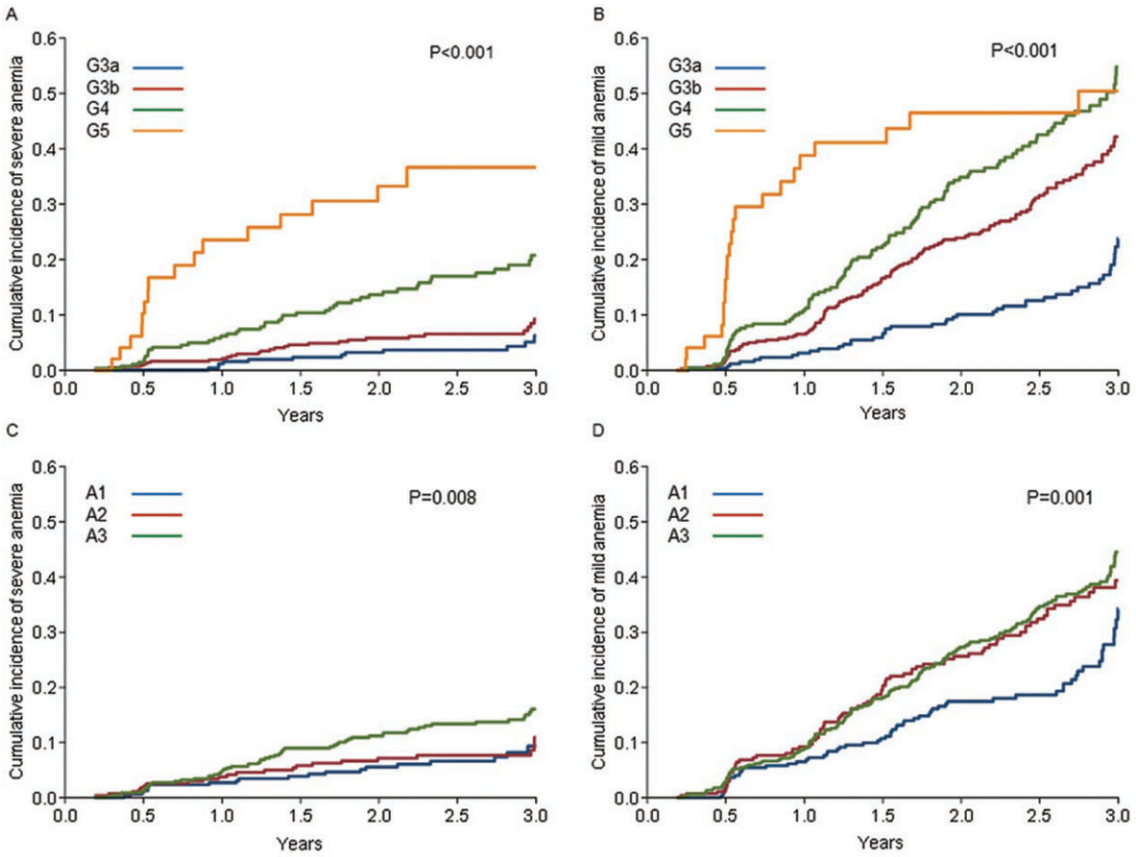


FIGURE 1: Cumulative incidence of severe anemia by (**A**) CKD stage or (**C**) proteinuria category and of mild anemia by (**B**) CKD stage or (**D**) proteinuria category.

Table 2. Laboratory and therapeutic features of patients developing anemia or not within 3 years of observation

Characteristics	No anemia ($n = 634$)		Mild anemia ($n = 266$)		Severe anemia ($n = 131$)		P-value for baseline visit	P-value for final visit
	Baseline	Final	Baseline	Final	Baseline	Final		
Serum phosphorus (mg/dL)	3.50 ± 0.68	3.50 ± 0.69	3.75 ± 0.75	3.83 ± 0.72	3.91 ± 0.67	4.06 ± 0.86	<0.001	<0.001
Total cholesterol (mg/dL)	202 ± 40	193 ± 38*	202 ± 41	188 ± 39*	207 ± 40	192 ± 46*	0.401	0.264
Hb (g/dL)	14.3 ± 1.3	14.2 ± 1.2	13.5 ± 1.0	12.3 ± 0.8	13.4 ± 1.1	11.1 ± 1.4	<0.001	-
TSAT (%)	25.3 ± 9.2	24.6 ± 9.0	24.6 ± 9.8	23.6 ± 10.1	23.5 ± 11.0	23.5 ± 11.4	0.135	0.198
Ferritin (ng/mL), median (IQR)	117 (70–149)	119 (70–146)	113 (61–151)	112 (59–152)	110 (70–143)	116 (75–158)	0.638	0.660
Iron deficiency (%)	47.0	47.6	51.5	54.1	51.9	51.9	0.349	0.181
eGFR (mL/min/1.73 m^2)	40 ± 12	39 ± 15*	33 ± 12	28 ± 15*	29 ± 13	22 ± 14*	<0.001	<0.001
Proteinuria (g/24 h), median (IQR)	0.3 (0.1–0.9)	0.2 (0.1–0.8)	0.4 (0.2–1.3)	0.4 (0.1–1.3)	0.9 (0.3–2.0)	0.7 (0.2–2.0)	<0.001	<0.001
Systolic BP (mmHg)	136 ± 18	134 ± 17*	138 ± 19	136 ± 17	140 ± 22	138 ± 20	0.041	0.010
Diastolic BP (mmHg)	81 ± 10	79 ± 9*	79 ± 11	78 ± 9	80 ± 12	79 ± 11	0.191	0.146
Antihypertensive drugs (n)	2.0 ± 1.2	2.2 ± 1.2*	2.2 ± 1.2	2.4 ± 1.3*	2.1 ± 1.3	2.3 ± 1.3*	0.143	0.203
Use of RAS (%)	69.4	74.4*	68.8	73.7*	63.4	65.6	0.396	0.115
Iron supplementation (%)	5.0	6.5	4.9	14.3*	9.9	19.8*	0.073	<0.001

Data are mean ± SD unless stated otherwise. Iron deficiency defined as TSAT <20% and/or ferritin<100 ng/mL. *$P < 0.05$ versus baseline. Conversion factors: cholesterol in mg/dL to mmol/L, × 0.02586; phosphorus in mg/dL to mmol/L, × 0.3229.

[36 ± 13 mL/min/1.73 m^2 and 0.4 g/day (IQR 0.1–1.5)] and without diabetes [37 ± 13 mL/min/1.73 m^2 and 0.4 g/day (IQR 0.1–1.1)]. We also found that higher serum phosphate predicted anemia onset. Similarly, males were at higher risk of mild anemia but not of severe anemia, likely because of the different upper limit of Hb for defining mild anemia in men and women (Table 3). No significant interaction was found between either CKD stage, diabetes or proteinuria and all variables included in the model.

An algorithm for detecting severe anemia within 3 years of follow-up is generated by using decision classification tree analysis (Supplementary data, Figure S3). We confirmed that CKD stage was the most important factor predicting severe anemia, with proteinuria category A3 and serum phosphate level >3.8 mg/dL discriminating a higher incidence in patients with stage 3 and stage 4–5, respectively. The third node was the sex-specific median of Hb (in proteinuria category A3) and older age (in proteinuria categories A1–A2).

Table 3. Multinomial regression analysis to estimate baseline characteristics associated with development of mild and severe anemia

	Mild anemia			Severe anemia		
Variable	OR	95% CI	P-value	OR	95% CI	P-value
Age (years)	1.01	0.99–1.02	0.318	1.01	0.99–1.02	0.541
Men versus women	3.79	2.49–5.76	<0.001	1.32	0.78–2.23	0.298
BMI (kg/m^2)	0.99	0.95–1.02	0.424	0.91	0.87–0.95	<0.001
Diabetes mellitus (yes versus no)	1.72	1.16–2.56	0.008	1.87	1.11–3.16	0.019
History of CVD (yes versus no)	1.14	0.79–1.63	0.488	0.83	0.50–1.36	0.452
Hb (g/dL)	0.40	0.33–0.49	<0.001	0.53	0.41–0.67	<0.001
Serum phosphate (mg/dL)	1.41	1.11–1.79	0.004	1.56	1.16–2.11	0.004
TSAT (%)	1.01	0.99–1.03	0.189	1.00	0.98–1.02	0.946
Ferritin (ng/mL)	1.00	0.998–1.002	0.967	1.00	0.998–1.002	0.891
eGFR categories						
G3a (45–60 mL/min/1.73m^2)		Reference			Reference	
G3b (30–44 mL/min/1.73 m^2)	1.67	1.07–2.60	0.025	1.19	0.63–2.26	0.589
G4 (15–29 mL/min/1.73 m^2)	2.83	1.73–4.62	<0.001	3.32	1.75–6.31	<0.001
G5 (<15 mL/min/1.73 m^2)	3.64	1.48–9.00	0.005	9.05	3.50–23.4	<0.001
Proteinuria categories						
A1 (<0.15 g/day)		Reference			Reference	
A2 (0.15–0.50 g/day)	1.67	1.09–2.57	0.019	1.70	0.89–3.25	0.106
A3 (>0.50 g/day)	1.70	1.12–2.58	0.013	3.19	1.78–5.72	<0.001
Use of RAS inhibitors	0.93	0.65–1.32	0.679	1.01	0.64–1.61	0.952

OR, odds ratio.

Table 4. Adjusted risks of ESKD and all-cause death in patients who develop either mild or severe anemia

	ESKD					All-cause death				
Variable	*n/N*	Incidence rate (% patient-years)	HR	95% CI	P-value	*n/N*	Incidence rate (% patient-years)	HR	95% CI	P-value
Not developing anemia	85/634	3.78	Ref.			64/634	2.84	Ref.		
Mild anemia	78/266	11.27	1.42	1.02–1.98	0.038	48/266	6.94	1.55	1.04–2.32	0.032
Severe anemia	69/131	21.51	1.73	1.20–2.51	0.003	23/131	7.17	1.83	1.05–3.19	0.034

Adjusted for age, sex, diabetes mellitus, prior cardiovascular disease, BMI, serum phosphate, total cholesterol, eGFR, log-transformed proteinuria, systolic blood pressure and use of RAS inhibitors.

Prognostic role of new-onset anemia

After excluding 20 subjects lost to follow-up (Supplementary data, Figure S1), the effect of anemia development on the subsequent risk of adverse outcomes was ascertained in 1011 patients. During a median follow-up of 3.1 years (IQR 2.0–5.2), 232 patients reached ESKD and 135 died. We detected an increase in the incidence of ESKD and all-cause death across subgroups (Table 4). Compared with non-anemic patients, adjusted risks of ESKD increased by 42% and 73% for mild and severe anemia, respectively, while mortality risk significantly increased by 55% and 83% in patients with mild and severe anemia, respectively (Table 4). In a sensitivity analysis, we added to the models the rate of eGFR decline during the period of anemia assessment (i.e. before starting outcome assessment). In this model, the adjusted risk of ESKD associated with mild [hazard ratio (HR) 1.44 (95% CI 1.03–2.00)] and severe anemia [HR 1.82 (95% CI 1.24–2.65)] remained significantly increased; the same occurred for mortality risk [HR 1.56 (95% CI 1.05–2.34) and HR 1.78 (95% CI 1.02–3.11)] for mild and severe anemia, respectively.

DISCUSSION

This study adds novel and comprehensive information on CKD-related anemia by evidencing that more than one-third of the non-anemic CKD population seen in renal clinics develop anemia during a 3-year observation period with an incidence rate of mild and severe anemia of ∼14 cases and 6 cases/100 patient-years, respectively. Measures of kidney damage (eGFR and proteinuria) are the main factors predicting the occurrence of anemia, as well as diabetes, hyperphosphatemia and Hb levels. These findings are relevant because development of mild and severe anemia has a negative impact on patient and renal survival.

Few studies have evaluated the incidence of anemia in non-dialysis CKD patients by reporting an incidence rate of new-onset anemia ranging from 10.8 to ∼14/100 person-years [8–10]. All these estimates are lower when compared with our cohort (20.4/100 patient-years) likely because of a substantial difference in the two main predictors of anemia (either eGFR or proteinuria level) (Figure 1 and Table 3). Indeed, Portolés *et al*. [8] enrolled only CKD patients in stage 3, while we included patients with more advanced CKD (32% of our population had stage 4–5). Furthermore, in comparison with the Chronic Renal Insufficiency Cohort study, we enrolled patients with similar eGFRs but higher proteinuria (44% had severe proteinuria) [9], while in the KNOW-CKD cohort, 24 h proteinuria was similar but patients had a higher mean eGFR (63.3 mL/min/1.73 m^2) with a greater prevalence of initial stages of CKD (53% in stage 1–2) [10].

An original finding of our work, not analyzed by previous studies, was the distinction between mild and severe anemia. This information can be useful for correctly planning the allocation of resources, because different therapeutic approaches are indicated for different degrees of anemia. Mild anemia, in fact, can be exclusively and efficaciously treated by iron supplementation, while severe anemia also benefits from ESA therapy. The knowledge that the rate of this complication is consistent (~20% of patients every year) may prompt nephrologists to a more proactive approach to limit the currently high rates of therapeutic inertia that lead to anemia worsening [6, 18, 19]. In this regard, recognizing the factors predicting anemia onset may represent a useful tool to prevent anemia. Lower eGFR acts as a predictor of anemia as an expression of inadequate erythropoietin (EPO) production, while proteinuria, particularly in category A3, is associated with anemia due to either urinary loss of transferrin [20] or proteinuria-induced tubulointerstitial damage, leading to impairment of EPO production and release [21, 22]. It has been reported that CKD patients with diabetes are more frequently affected by anemia than their counterparts without diabetes [1, 23, 24]; we now extend this association to the incidence of anemic status by showing that patients with diabetes are exposed to an increased risk of new-onset mild or severe anemia (Table 3). The finding that eGFR and proteinuria did not differ according to the presence of diabetes supports the hypothesis that other factors, besides renal function (eGFR) and glomerular damage (proteinuria), may play a role in increasing the susceptibility of diabetic patients in developing anemia. In this regard, peritubular and interstitial damage, which is common in diabetic kidney disease, may blunt EPO release in response to a hypoxic stimulus, thus resulting in anemia onset [21, 22]. Additional proposed mechanisms of impaired erythropoiesis in diabetic CKD are chronic hyperglycemia, increased production of advanced glycation end products, elevated levels of free radicals, increased oxidative stress, reduced nitric oxide production, decreased stabilization of hypoxia-inducible factors (HIFs), enhanced endothelial dysfunction and chronic inflammation (with ensuing disturbance of iron metabolism) [25, 26].

Serum phosphate level also acted as a predictor of mild and severe anemia; this is in agreement with clinical and experimental studies supporting a role of mineral bone disorders in the pathogenesis of CKD-related anemia. Indeed, high parathyroid hormone levels are associated with bone marrow fibrosis and EPO resistance [27]; furthermore, vitamin D increases EPO-receptor expression and synergistically stimulates the proliferation of erythroid precursors along with EPO and also has anti-inflammatory effects that may improve EPO responsiveness, namely a reduction of interleukin-6 and hepcidin levels [28–30]. The lack of significant interaction of serum phosphate with eGFR categories in our study reasonably excludes the possibility that hyperphosphatemia merely reflects the effect of reduced eGFR on anemia onset. When focusing on severe anemia (Hb <11 g/dL or ESA use), CKD stage represents the main factor discriminating patients who will or will not develop this complication. In patients with more advanced CKD (stages 4–5), serum phosphate can further improve discrimination, while in those with stage 3, the proteinuria category may help identify subjects who are more likely to develop severe anemia. At this level, older age and Hb levels should be considered further for better discrimination (Supplementary data, Figure S3).

Interestingly, in our study, the use of RAS inhibitors did not modify the probability of becoming anemic (Table 3); this is in contrast with the evidence that these drugs may promote anemia by blocking angiotensin IIa-mediated EPO transcription [31]; a potential explanation for this discrepancy is that RAS inhibitors were prescribed to the vast majority of the cohort (68%).

It should be noted that compared with non-anemic patients, those developing anemia displayed a higher risk of adverse outcomes (Table 4). Indeed, new onset of either mild or severe anemia heralded a higher risk of ESKD (+42% and +73% for mild and severe anemia, respectively) and all-cause death (+55% and +83% for mild and severe anemia, respectively). The significant association of anemia with adverse outcome represents a convincing reason for promoting a more extensive measurement of anemia parameters (Hb, TSAT and ferritin) that is now sub optimal, even in a tertiary care setting where these indices are not measured in >40% of anemic patients [7].

The importance of our findings is further highlighted when considering that the negative role of anemia on ESKD and mortality is independent from risk factors of CKD progression that *per se* increase the risk of developing anemia. As an example, eGFR decline is associated with a higher risk of anemia occurrence, thus suggesting that anemia may be a marker of faster CKD progression; however, the observation that the anemia-associated risk of ESKD and death was not modified by adding the change of eGFR in the previous 3 years to the survival models may suggest that anemia acts in the natural history of CKD as an independent contributor to adverse outcomes in these patients along with the presence of faster eGFR decline. This finding is consistent with the 'chronic hypoxia hypothesis', that is, intrarenal hypoxia due to anemia, even of mild degree, triggers a fibrotic response in tubulointerstitial cells and stimulates the infiltration and maturation of immune cells that, in turn, impact on remnant nephrons, thus expanding the damage and worsening renal injury [32, 33].

Limitations of the present study relate to the presence of a residual confounding due to unmeasured variables, such as C-reactive protein as a marker of inflammation. In addition, we included only white patients, thus precluding generalizability to other racial groups. Finally, the observational nature of this study does not allow assessing any cause–effect relationship and therefore we cannot affirm that interventions aimed at delaying anemia onset are effective in retarding CKD progression. In this regard, large randomized trials in a CKD setting failed in demonstrating that the risk of ESKD can be reduced by anemia correction [34–36]. However, these disappointing results were markedly influenced by the use of a very high ESA dose that eventually had a negative impact on prognosis and ended up masking the favorable effect of anemia correction [37–39]. Whether the new anemia drugs (HIF stabilizers), by inducing a lower increase of circulating EPO levels [40] and/or ameliorating erythropoietic response due to anti-inflammatory effects and improved availability of circulating iron [41, 42], may potentially reveal a nephroprotective effect remains to be demonstrated.

In conclusion, we provide evidence that new-onset anemia is frequent, particularly in patients with more severe renal damage (low eGFR and higher proteinuria), as in those with diabetes mellitus. The occurrence of anemia, even if of a mild degree, is associated with faster progression to ESKD and significantly affects mortality risk.

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AUTHORS' CONTRIBUTIONS

R.M. and L.D.N. conceptualized the research idea and study design. S.B., C.G., M.E.L. and V.B. were responsible for data acquisition. R.M., S.B., P.C., M.P. and V.B. were responsible for data analysis/interpretation. R.M., L.D.N., G.C. and M.A. drafted and/or revised the article. Each author contributed important intellectual content during manuscript drafting or revision and approved the final version of the manuscript.

CONFLICT OF INTEREST STATEMENT

R.M. has been a member of advisory boards for Amgen and Astellas and an invited speaker at meetings supported by Amgen, Astellas and Vifor Pharma. L.D.N. has received fees for scientific consultation and/or lectures from Amgen, Astellas, AstraZeneca, Mundibiopharma and Vifor Pharma. The remaining authors have declared no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data underlying this article will be shared on reasonable request to the corresponding author.

APPENDIX

Complete list of investigators in each cohort study:

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