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Mean platelet volume and mortality risk in a national incident hemodialysis cohort

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ABSTRACT

Background: Higher mean platelet volume (MPV) is an indicator of larger, reactive platelets, and has been associated with a higher risk of thrombosis and cardiovascular events in the general population. Hemodialysis patients have a higher risk for cardiovascular death and predisposition to platelet dysfunction (thrombosis and bleeding diathesis), but the relationship between MPV and mortality in this population is unknown.
Methods: Among a 5-year cohort (1/2007–12/2011) of 149,118 incident hemodialysis patients from a large national dialysis organization, we examined the association between MPV and all-cause mortality. In primary analyses, we granularly analyzed MPV across five categories: 7.2–7.5, >7.5–9.5, >9.5–11.5, >11.5–13.5, and >13.5–15.0 fL. In secondary analyses, we examined MPV categorized as low, normal, and high based on thresholds in the general population: 7.2–7.5, >7.5–11.5, and >11.5 fL, respectively. Associations between baseline and time-dependent MPV with mortality were estimated using traditional and time-dependent Cox models in order to determine long-term and short-term exposure—mortality associations, respectively, using three adjustment levels: unadjusted, case-mix, and case-mix + laboratory models.
Results: In primary analyses, higher baseline and time-dependent MPV levels were associated with incrementally higher death risk in case-mix + laboratory analyses (reference: >9.5–11.5 fL). In secondary analyses, high baseline and time-dependent MPV levels were associated with higher mortality, whereas low MPV was associated with lower death risk across all multivariable models (reference: normal MPV).
Conclusions: Hemodialysis patients with higher MPV have heightened mortality risk. Further studies are needed to determine the pathophysiologic basis for the higher risk, and if modification of MPV ameliorates mortality in this population.

1. Introduction

Platelets play a pivotal role in the pathogenesis of atherosclerotic lesions, plaque destabilization, and atherothrombosis that underlie coronary heart disease events [1]. While studies examining the association between platelet count and cardiovascular outcomes in the general population have largely been negative, platelet reactivity is an established risk factor and therapeutic target in cardiovascular disease [1,2]. Numerous biomarkers of platelet reactivity have been investigated as predictors of thrombosis and thromboembolism,

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but their use has primarily been restricted to the research setting due to being technically difficult, time consuming, and costly [3,4].

An increasing body of evidence suggests that mean platelet volume (MPV), the most commonly used measure of platelet size, is a potential marker of platelet reactivity and is reported with every complete blood count [5–7]. Larger platelets are more metabolically active, and bear a greater content of prothrombotic material. In the general population, a number of studies show that higher MPV levels are associated with cardiovascular risk factors (e.g., hypertension, diabetes, dyslipidemia [8–10]) as well as higher risk of thrombosis, coronary heart disease events, congestive heart failure, ischemic stroke, and death [1,2,11–19].

End-stage renal disease patients undergoing maintenance hemodialysis have a 7 to 10-fold higher mortality risk compared to the general population, largely due to cardiovascular causes (40% of deaths) [20]. Furthermore, hemodialysis patients commonly demonstrate platelet dysfunction, manifested as amplified platelet reactivity which in part contributes to their heightened risk of thrombosis, and may also function as a novel pathway for cardiovascular death in this population [21,22]. In addition, hemodialysis patients have higher bleeding risk, relating to uremic platelet dysfunction, altered platelet—endothelial wall interactions, abnormal expression of platelet membrane glycoproteins, dialytic heparin use, and anemia. In hemodialysis patients, platelet count indices alone may not adequately discern between thrombotic and bleeding propensity, nor accurately predict cardiovascular risk. Hence, MPV may serve as a simple, inexpensive, and widely accessible method to assess platelet reactivity, stratify cardiovascular mortality risk, and guide clinical decision making in hemodialysis patients [1].

To date, one small cross-sectional analysis has shown a relationship between higher MPV quintile and risk for coronary heart disease in hemodialysis patients, but there have been no studies examining the impact of MPV level upon mortality in this population [23]. Thus, to address this knowledge gap, we sought to examine the association between MPV level and all-cause mortality in a national cohort of incident hemodialysis patients receiving care in facilities operated by a large dialysis organization in the United States.

2. Material and methods

2.1. Study population

We undertook this study of incident hemodialysis patients using data from a large dialysis organization in the United States with comprehensive capture of baseline and longitudinal data on sociodemographics, comorbidities (ascertained from ICD-9 codes), laboratory tests, dialysis treatment characteristics, clinical events, and vital status [24]. Patients were included if at study entry they: (1) were 18 years old or older; (2) had a dialysis vintage of ≤91 days; (3) were undergoing in-center thrice-weekly hemodialysis; (4) had at least one MPV measurement in their baseline quarter (i.e., first 91 days following the initiation of dialysis), and (5) received care between January 1, 2007 to December 30, 2011. Patients were excluded if at study entry they: (1) were receiving peritoneal dialysis or home hemodialysis; (2) had missing censor/death date information; or (3) had an outlier MPV value (<7.2 or >15.0 femtoliter [fL], corresponding to the <0.5th and >99.5th percentiles of observed MPV values, respectively) (Supplementary Fig. 1).

2.2. Exposure ascertainment

The exposure of interest was MPV. In primary analyses, we categorized MPV into five groups using the following gradations: 7.2–7.5, >7.5–9.5, >9.5–11.5, >11.5–13.5, and >13.5–15.0 fL. In secondary analyses, we examined MPV divided into three groups (7.2–7.5, >7.5–11.5, and >11.5–15.0 fL) which were defined as low, normal, and high MPV, respectively, based upon reference range values in the general population [25]. In sensitivity analyses we sought to more granularly examine the association between MPV and mortality by categorizing MPV into ten empirical deciles.

Published data indicate that in order to maintain a constant circulating platelet mass and hemostatic function, MPV and platelet count may be inversely related. As platelet count has been observed to be an independent predictor of all-cause mortality in the dialysis population [6,26], we conducted secondary analyses in which we sought to determine whether platelet count (categorized into seven groups: <150, 150–<200, 200–<250, 250–<300, 300–<350, 350–<400, and ≥400 $\times 10^9$/L) may similarly predict adverse outcomes in our cohort. We also examined the association between MPV and mortality across these seven platelet count categories to determine whether the MPV—mortality association is modified by platelet count.

Blood samples for MPV, platelet count, and other laboratory tests were drawn using standardized techniques in all clinics of the large dialysis organization, transported to a central laboratory in Deland, FL typically within 24 h, and were measured using automated and standardized methods. Most laboratory parameters (i.e., total-iron binding capacity, bicarbonate, phosphorous, calcium) were measured monthly; intact parathyroid hormone was measured at least quarterly. Most blood samples were collected pre-dialysis, except for post-dialysis serum urea nitrogen to calculate urea kinetics.

2.3. Outcome ascertainment

The outcome of interest was all-cause mortality. Patients were considered at risk for mortality from the day after the baseline MPV measurement to the first of occurrence of death or censoring. Patients were censored on the day of kidney transplantation, transfer to a non-affiliated dialysis center, transfer to another type of dialysis modality (e.g., home hemodialysis or peritoneal dialysis), loss to follow-up, or December 31, 2011 (the end of study follow-up).

2.4. Statistical analyses

The primary objective of our analysis was to investigate the association between MPV and all-cause mortality. We examined both baseline MPV and time-dependent MPV in order to determine the short-term and long-term relationship between MPV and death risk, respectively. We estimated the association between MPV and mortality using Cox proportional hazard models with three incremental levels of covariate adjustment:

(1) *Unadjusted*: Adjusted for patient's calendar quarter at study entry;
(2) *Case-mix*: Adjusted for covariates in the unadjusted model, as well as age, sex, race/ethnicity, vascular access (arteriovenous fistula/graft, catheter, other/unknown access), cause of end stage renal disease (diabetes, hypertension, glomerulonephritis, cystic disease, other), diabetes, congestive heart failure, myocardial infarction, other cardiac disease, hypertension, peripheral vascular disease, cerebrovascular disease, and body mass index (BMI);
(3) *Case-mix + laboratory*: Adjusted for covariates in the case-mix model, as well as bicarbonate, iron saturation, total iron binding capacity, calcium, phosphate, parathyroid hormone, dialysis dose (defined by single pool Kt/V [spKt/V]), total cholesterol, hemoglobin, and platelet count. In time-dependent analyses, laboratory covariates were examined as time-dependent variables summarized over 91-day periods (i.e., mean or median values over the quarter for each patient).
We *a priori* defined the case-mix adjusted model as our primary model, which included core sociodemographic measures and other confounders of the association between MPV and outcomes. The case-mix + laboratory model was designated as an exploratory model which included confounders as well as potential causal pathway intermediates of the MPV—mortality association.

As some data suggest that MPV may be associated with inflammatory and nutritional markers, which in and of themselves are associated with mortality risk, we conducted sensitivity analyses that incrementally adjusted for serum albumin, serum creatinine, and normalized protein nitrogen appearance in addition to covariates in the case-mix + laboratory model [5,27].

We then examined the association between MPV (divided into three categories) and mortality across clinically relevant subgroups of age (≤60 and >60 years), sex, race (non-Hispanic white, non-Hispanic black, Hispanic, and other race/ethnicities), BMI (≤25 vs. >25 kg/m^2), and platelet count (≤300 vs. >300 × 10^9/L). As erythropoiesis-stimulating agents (ESAs) may have direct effect on platelet function, owing to increased density of glycoprotein IIb/IIIa on platelet membranes, improved thrombin-induced phosphorylation of platelet proteins, and enhanced platelet calcium signaling, we also examined associations across subgroups of ESA dose (≤50th percentile vs. >50th percentile of observed values in the initial 91 days of dialysis) [28–30]. As noted above, we additionally examined the association between MPV and mortality across seven platelet count categories. The MPV—mortality associations were also examined across subgroups stratified according the presence vs. absence of specific cardiovascular comorbidities, including prior history of congestive heart failure, myocardial infarction, other cardiac disease, cerebrovascular disease, peripheral vascular disease, and any of the aforementioned cardiovascular comorbidities.

Our secondary objective was to investigate the association between baseline and time-dependent platelet count with all-cause mortality. We examined the platelet count—mortality association using three incremental levels of adjustment. Unadjusted and case-mix models incorporated the same covariates as utilized in the MPV—mortality analyses. In case-mix + laboratory adjusted models, we also adjusted for MPV in addition to the covariates accounted for in the MPV—mortality analyses. Similar to the MPV—mortality analyses, we examined for effect modification of the association between platelet count (divided into three categories: <150, 150–300, >300 × 10^9/L) and mortality on the basis of age, sex, race, BMI, platelet count, and ESA dose.

To address missing covariate data, we implemented multiple imputation which generates complete datasets (i.e., replaces missing values in the dataset) by borrowing information from other covariates and accounts for uncertainty associated with the estimation of missing values when estimating the regression parameters using the MICE package in R [31,32]. All covariates had <1% missing values except for vascular access (17.8%), iron saturation (1.2%), total iron binding capacity (1.2%), parathyroid hormone (1.1%), spKt/V (1.5%), and total cholesterol (49.5%). Proportional hazards assumptions were checked by graphical and formal testing including Schoenfeld residuals. Residual diagnostic did not indicate strong departures from the proportional hazards assumption Analyses and figures were conducted using R version 3.1.1 (R Foundation for Statistical Computing, Vienna, Austria) and SigmaPlot Version 12.5 (Systat Software, San Jose, CA).

3. Results

3.1. Study cohort description

After applying the eligibility criteria, there were 149,118 incident hemodialysis patients in the final study cohort (Supplementary Fig. 1). The mean (SD) and median (IQR) of observed MPV values in the overall cohort were 10.2 (1.3) fL and 10.1 (9.2, 11.0) fL, respectively (Supplementary Fig. 2). Comparison of included (n = 149,118) vs. excluded patients (n = 59,702) showed that included patients tended to be older; were more likely to have an arteriovenous fistula/graft or central venous catheter as their initial access; were more likely to have a history of diabetes, congestive heart failure, myocardial infarction, other cardiovascular disease, hypertension, and peripheral vascular disease; and were more likely to have higher parathyroid hormone levels and lower cholesterol levels (Supplementary Table 1).

Table 1 presents the baseline characteristics of the cohort according to baseline MPV across five categories. Compared to the lowest MPV category, patients in the highest MPV category tended to be older and female; were more likely to be non-Hispanic black; were less likely to have an arteriovenous fistula/graft as their vascular access; were more likely to have underlying congestive heart failure and less likely to have hypertension; and had lower parathyroid hormone, cholesterol, and platelet count values.

3.2. Mean platelet volume and mortality

We observed 40,595 deaths during 192,482 patient-years of follow-up. The median (IQR) follow-up period was 0.8 (0.2, 2.0) years. In primary analyses of baseline MPV divided into five groups, we observed a graded association between higher MPV category and higher mortality risk in unadjusted, case-mix, and case-mix + laboratory adjusted models; these associations were even stronger in analyses of time-dependent MPV and mortality indicating a stronger local effect of MPV on the risk of death (Fig. 1 and Supplementary Table 2). These associations remained robust in sensitivity analyses incrementally adjusted for markers of protein-energy wasting and inflammation (Supplementary Table 3).

In secondary analyses of baseline MPV divided into three categories based on general population thresholds, we found that low MPV was associated with lower mortality risk and high MPV with higher mortality risk across all three multivariable models (reference group: normal MPV). In parallel analyses of time-dependent MPV, we observed an even stronger association between higher MPV category and higher mortality risk across all three multivariable models (Supplementary Fig. 3 and Supplementary Table 4). These associations persisted upon incremental adjustment for markers of protein-energy wasting and inflammation (Supplementary Table 3). When examined as deciles, we observed a monotonic relationship between higher MPV level and mortality in both baseline and time-dependent analyses adjusted for case-mix covariates (Fig. 2 and Supplemental Table 5).

When we examined baseline MPV and all-cause mortality across clinically relevant subgroups, we found that high MPV was consistently associated with higher death risk across all patient subcategories in case-mix adjusted analyses. Point estimates for low MPV and mortality suggested lower death risk across all subgroups, but did not reach statistical significance for those who were female, non-Hispanic black, Hispanic, or of other race/ethnicity; had a BMI >25 kg/m^2 or platelet count <300 × 10^9/L; received an ESA dose <50th percentile of observed values; or had a history of congestive heart failure, myocardial infarction, cerebrovascular disease, peripheral vascular disease, or any cardiovascular comorbidity. In subgroup analyses of time-dependent MPV and all-cause mortality, we found an even stronger association between high MPV and higher death risk consistent across all subgroups. Similarly, point estimates for low MPV and mortality suggested lower death risk across all subgroups, but were not statistically significant among those who were female, non-Hispanic black, Hispanic, received an ESA dose <50th percentile of observed values, or had prior history of congestive heart failure, myocardial infarction, other cardiac disease, cerebrovascular disease, or peripheral vascular disease (Fig. 3 and Supplementary Table 6).

3.3. Platelet count and mortality

In unadjusted analyses, we observed a U-shaped relationship between baseline platelet count and mortality in which there was a strong association between lower platelet counts <200 × 10^9/L and death risk, and a comparatively weaker association between higher platelet counts ≥400 × 10^9/L and death risk in unadjusted analyses (reference group: platelet count 200–<250 × 10^9/L). These associations were mildly

Table 1
Baseline characteristics according to mean platelet volume (MPV) category.

	MPV category (fL)					p-Value[a]
	7.2 to 7.5	>7.5 to 9.5	>9.5 to 11.5	>11.5 to 13.5	>13.5 to 15	
N	995	45,398	78,266	22,173	2286	N/A
Age, in years (Mean [SD])	59.7 (16.1)	61.1 (15.5)	63.1 (15.2)	65.3 (14.7)	66.3 (14.9)	<0.001
Body Mass Index (kg/m^2) (Mean [SD])	27.8 (7.4)	28.4 (7.5)	28.2 (7.5)	27.8 (7.4)	27.2 (6.7)	<0.001
Female (%)	40	41	44	47	47	<0.001
Race/ethnicity (%)						
Non-Hispanic White	51	50	51	51	52	<0.001
Non-Hispanic Black	27	28	29	29	30	
Hispanics	14	13	14	14	13	
Asians/other	9	8	6	5	4	
Initial vascular access (%)						
AVF/AVG	20	18	15	12	12	<0.001
Central venous catheter	62	61	62	62	56	
Other/Unknown	18	21	23	26	32	
Cause of ESRD (%)						
Diabetes	40	43	43	42	39	<0.001
Hypertension	30	28	28	29	29	
Glomerulonephritis	10	11	10	10	11	
Cystic Disease	2	2	1	1	1	
Other	17	17	16	17	21	
Comorbidities (%)						
Diabetes	55	54	56	57	53	<0.001
Congestive heart failure	12	12	14	14	15	<0.001
Myocardial infarction	12	11	11	11	11	0.2
Other cardiac	8	7	8	8	8	<0.001
Hypertension	33	28	26	24	22	<0.001
Peripheral vascular disease	9	7	8	8	9	<0.001
Cerebrovascular disease	2	2	2	2	1	0.7
Laboratory variables Median (IQR)						
Bicarbonate (mEq/L)	23.40 (21.50, 25.25)	23.67 (22.00, 25.50)	23.67 (22.00, 25.50)	23.75 (22.00, 25.67)	24.00 (22.00, 26.00)	<0.001
Iron saturation (%)	21.00 (17.25, 26.50)	21.00 (17.00, 26.50)	21.25 (17.00, 26.75)	21.60 (17.00, 27.67)	22.50 (17.00, 29.81)	<0.001
Total iron binding capacity (mcg/dl)	223.25 (194.00, 253.80)	224.33 (193.50, 255.50)	222.33 (190.00, 254.67)	218.33 (183.75, 252.00)	215.25 (176.00, 251.75)	<0.001
Calcium (mg/dl)	9.13 (8.75, 9.45)	9.10 (8.77, 9.43)	9.10 (8.78, 9.43)	9.13 (8.80, 9.47)	9.13 (8.80, 9.47)	<0.001
Phosphate (mg/dl)	4.86 (4.14, 5.72)	4.80 (4.10, 5.60)	4.73 (4.03, 5.50)	4.63 (3.92, 5.41)	4.55 (3.80, 5.33)	<0.001
Parathyroid hormone (pg/ml)	305.53 (187.88, 473.85)	306.00 (186.00, 482.00)	302.71 (185.00, 478.00)	294.50 (179.27, 467.31)	281.23 (170.38, 441.13)	<0.001
spKt/V	1.43 (1.24, 1.69)	1.42 (1.24, 1.64)	1.42 (1.24, 1.64)	1.42 (1.23, 1.64)	1.40 (1.21, 1.61)	<0.001
Total cholesterol (mg/dl)	150.00 (125.00, 184.25)	148.00 (122.00, 178.50)	145.00 (119.00, 176.00)	141.00 (113.70, 171.50)	133.00 (107.00, 168.00)	<0.001
Hemoglobin (g/dl)	11.04 (10.14, 11.96)	11.00 (10.13, 11.80)	11.02 (10.15, 11.84)	11.05 (10.16, 11.88)	10.93 (10.05, 11.75)	<0.001
Platelet count ($\times 10^9$/L)	283.00 (209.70, 338.50)	265.00 (214.00, 327.00)	235.00 (185.00, 290.00)	192.00 (142.00, 246.00)	140.17 (97.38, 199.19)	<0.001

Abbreviations: AVF, arteriovenous fistula; AVG, arteriovenous graft; ESRD, end-stage renal disease.
[a] P-values estimated with ANOVA, chi-square, or Kruskal Wallis tests according to data type.

attenuated in case-mix and case-mix + laboratory adjusted analyses (Supplementary Fig. 4 and Supplementary Table 7).

In time-dependent analyses, we observed a comparatively stronger U-shaped association between platelet count and mortality in which both lower platelet counts $<200 \times 10^9$/L and higher platelet counts $>300 \times 10^9$/L were associated with higher death risk in unadjusted analyses (reference group: platelet count 200–$<250 \times 10^9$/L). While associations were only mildly attenuated in case-mix adjusted analyses, in case-mix + laboratory adjusted analyses the high platelet count—mortality association was markedly attenuated whereas the low platelet count—mortality association remained robust (Supplementary Fig. 4 and Supplementary Table 7).

When we examined baseline platelet count and all-cause mortality across clinically relevant subgroups, we observed that low platelet count was associated with higher death risk across all patient subcategories, whereas an association between high platelet count and mortality was not consistently observed in case-mix adjusted analyses. In subgroup analyses of time-dependent platelet count and all-cause mortality, both low and high platelet count were associated with higher death risk across all patient subcategories (Supplementary Fig. 5 and Supplementary Table 8).

3.4. Effect modification by platelet count

We then examined the association between MPV and mortality across platelet count categories adjusted for case-mix covariates (Fig. 4). In baseline analyses, we observed that the high MPV—mortality association was consistently observed across all platelet count strata. While point estimates for low MPV and mortality suggested lower death risk across all platelet count strata, these associations did not

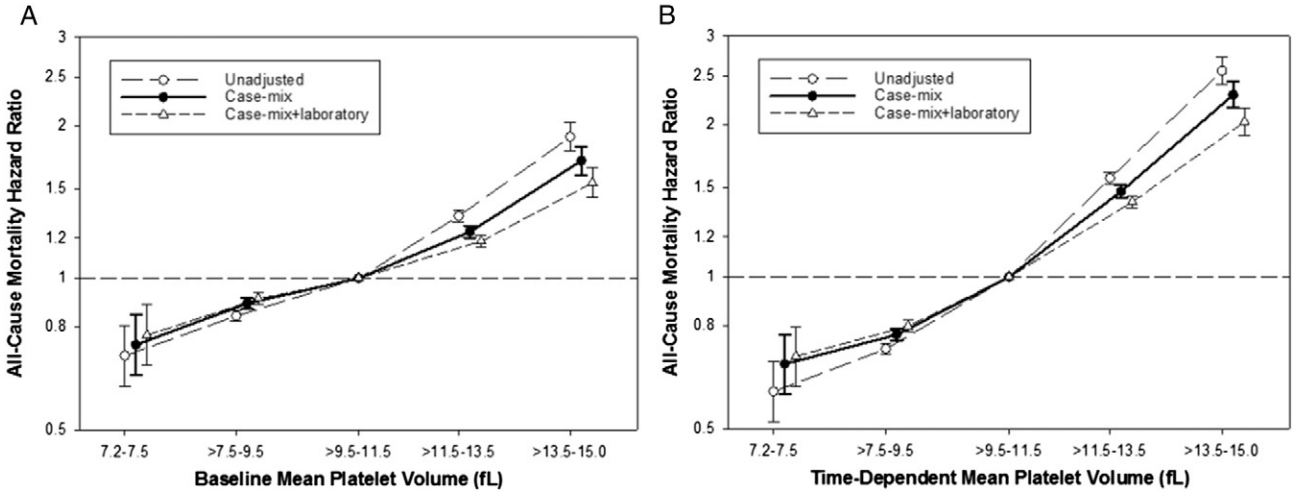

Fig. 1. Association of baseline (Panel A) and time-dependent (Panel B) mean platelet volume in 5 categories with all-cause mortality. Unadjusted model is adjusted for entry calendar quarter. Case-mix adjusted model is adjusted for covariates in the unadjusted model, plus age, sex, race/ethnicity, access, cause of end stage renal disease, diabetes, congestive heart failure, myocardial infarction, other cardiac disease, hypertension, peripheral vascular disease, cerebrovascular disease, and body mass index. Case-mix + laboratory adjusted model is adjusted for covariates in the case-mix adjusted model, plus bicarbonate, iron saturation, total iron binding capacity, calcium, phosphate, parathyroid hormone, spKt/V, total cholesterol, hemoglobin, and platelet count.

achieve statistical significance. In time-dependent analyses, the high MPV—mortality association was observed across all strata except for platelet counts $<150 \times 10^9$/L, whereas a consistent association between low MPV—mortality was not observed.

4. Discussion

In a nationally representative cohort of incident hemodialysis patients from a large dialysis organization, we observed that higher MPV levels were associated with higher death risk. The higher MPV—mortality relationship was robust across a number of secondary and sensitivity analyses, including those that accounted for confounders of inflammation and protein-energy wasting, and examined potential effect modification by platelet count strata.

In the general population, MPV has gained recognition as a predictor of thrombosis, thromboembolism, and adverse primary and secondary cardiovascular outcomes [1,2,11,12,14,16–19]. A Norwegian population-based study of 25,923 participants observed that higher MPV levels had a strong and independent association with venous thromboembolism in the absence of surgery, trauma, immobilization, or malignancy [12]. Higher MPV levels have also been associated with higher risk of ischemic stroke, as well as larger volumes of cerebral damage and early death in the post-stroke period [33,34]. In a subsequent meta-analysis of 3184 patients across three cohort studies who underwent percutaneous coronary intervention, higher MPV was associated with higher death risk [1]. Associations between higher MPV and coronary heart disease outcomes were corroborated by a study of 39,531 participants from the Copenhagen General Population Study, in which higher MPV quintiles (i.e., second quintile and higher, as compared with the first quintile) were associated with higher risk of myocardial infarction; in contrast, this study did not observe an association between platelet count and myocardial infarction [16].

Despite the hemodialysis population's predisposition towards thrombosis and exceedingly high risk of cardiovascular disease and death, there are a limited number of studies that have examined the association between MPV and outcomes in this high risk group. In a

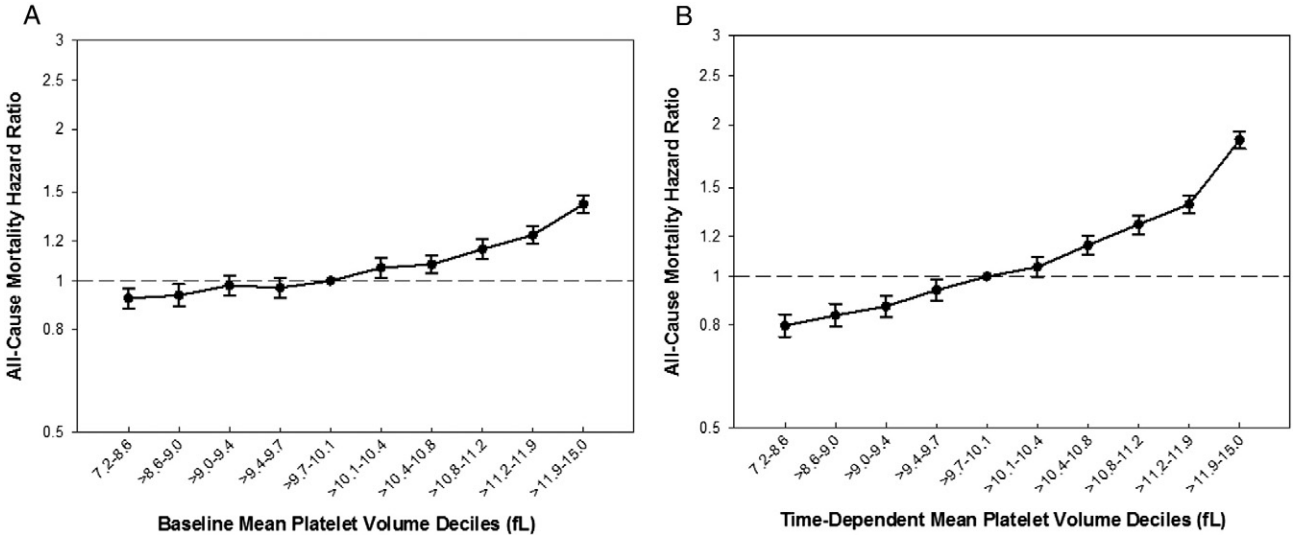

Fig. 2. Association of baseline (Panel A) and time-dependent (Panel B) mean platelet volume in deciles with all-cause mortality. Analyses are adjusted for entry calendar quarter, age, sex, race/ethnicity, access, cause of end stage renal disease, diabetes, congestive heart failure, myocardial infarction, other cardiac disease, hypertension, peripheral vascular disease, cerebrovascular disease, and body mass index.

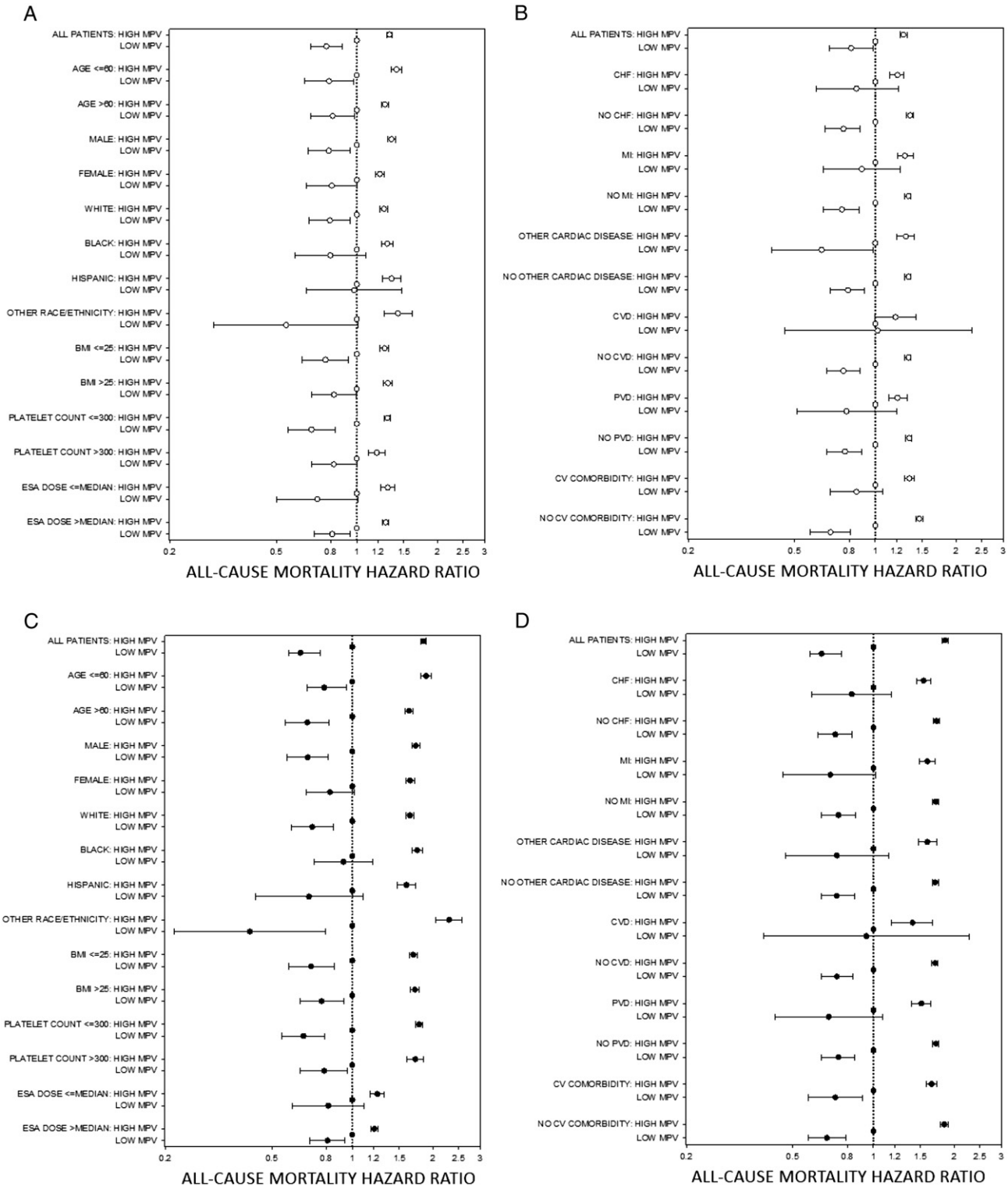

Fig. 3. Association of baseline (Panels A and B) and time-dependent (Panels C and D) mean platelet volume with all-cause mortality across clinically relevant subgroups. Analyses are adjusted for entry calendar quarter, age, sex, race/ethnicity, access, cause of end stage renal disease, diabetes, congestive heart failure, myocardial infarction, other cardiac disease, hypertension, peripheral vascular disease, cerebrovascular disease, and body mass index.

multi-center cross-sectional analysis of 518 hemodialysis patients, those with MPV levels in the highest quintile (>9.2 fL) had a higher risk of prevalent coronary heart disease compared to patients in the lowest quintile (<7.2 fL), independent of age, smoking, blood pressure, and laboratory measures [23]. While it has been suggested that MPV may be influenced by changes in extracellular fluid volume and composition, a sub-study of 30 patients showed that pre-dialysis and post-dialysis MPV values were not significantly different.

To our knowledge, ours is the first study to demonstrate a strikingly consistent association between higher MPV level and death risk in incident hemodialysis patients, independent of case-mix and laboratory covariates. While high MPV was associated with higher mortality in all

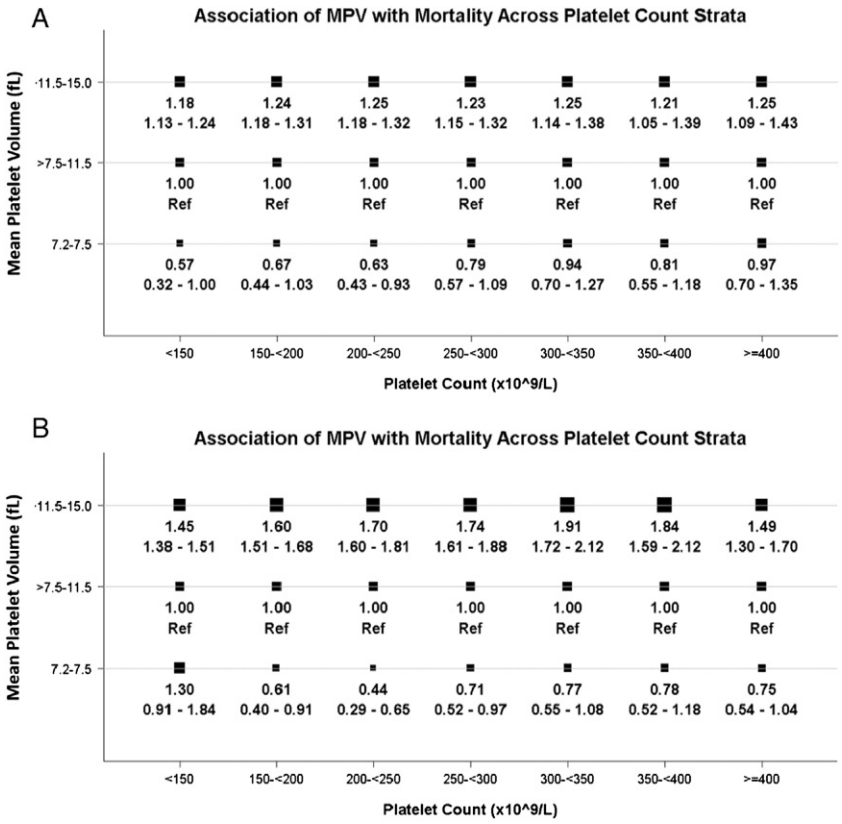

Fig. 4. Association of baseline (Panel A) and time-dependent (Panel B) mean platelet volume with all-cause mortality across platelet count strata. Analyses are adjusted for entry calendar quarter, age, sex, race/ethnicity, access, cause of end stage renal disease, diabetes, congestive heart failure, myocardial infarction, other cardiac disease, hypertension, peripheral vascular disease, cerebrovascular disease, and body mass index.

subgroup analyses, low MPV was not consistently associated with survival benefit, likely owing to the relatively small sample size in the low MPV group (n = 995). We also showed that the association between these platelet parameters and death risk were independent of potent markers of inflammation and nutrition, including serum albumin, serum creatinine, and normalized protein nitrogen appearance. For example, in addition to thrombopoietin, several inflammatory cytokines (e.g., IL-1, IL-6, tumor necrosis factor-alpha) are regulators of thrombopoiesis [35]. It has been hypothesized that intensity of systemic inflammation has differential impact upon platelet size, such that higher-grade inflammation may be associated with circulation of smaller-sized platelets, while lesser degrees of inflammation or inflammatory disease in remission are associated with larger-sized platelets [5].

In the present work, we also examined alternative platelet indices, and found that lower baseline and time-dependent platelet counts were associated with higher mortality risk across all multivariable models, whereas the association between higher baseline and time-dependent platelet counts and death were attenuated following adjustment for laboratory results. These findings are consistent with a prior study of baseline platelet count and mortality in 40,797 hemodialysis patients, which showed that the association between higher platelet count and mortality was attenuated to the null following comprehensive adjustment for malnutrition-inflammation-cachexia covariates [26]. These data collectively suggest that MPV may be a more prognostically important platelet parameter with potential therapeutic implications in hemodialysis patients.

Our findings warrant further studies comparing MPV with functional platelet reactivity biomarkers, which may shed light into the underlying mechanisms of the high MPV—mortality association in hemodialysis patients. Although the precise biological pathways by which elevated MPV influences the development and progression of cardiovascular disease and death are incompletely understood, several mechanisms may be contributory. First, larger platelets contain more prothrombotic material per unit volume, which promotes the release of substances that amplify platelet activation, platelet adhesion, and vascular neointimal proliferation (e.g., thromboxane A2, alpha-granules) [36,37]. In addition, larger platelets have a greater density of glycoprotein Ib and IIb/IIIa adhesion receptors, and are more reticulated thereby leading to poor antiplatelet therapy response [36,38]. Platelet activation and thrombosis are central to the pathogenesis of occlusive arterial disease, and antiplatelet agents' ability to reduce cardiovascular morbidity and mortality has reinforced the principal role of platelets in thromboembolic disease. It should be noted that not all antiplatelet agents modify MPV levels or associated function. Aspirin, which has specific action on the arachidonic acid cascade, exerts little to no effect on MPV [39,40]. However, purinergic receptor blockers (e.g., clopidogrel) may be effective in controlling platelet size and volume [41,42]. In addition, in a study of 398 patients who sustained an ST-elevation myocardial infarction, among 202 patients who received treatment with the glycoprotein IIb/IIIa inhibitor abciximab, treatment was shown to reduce risk of short-term death only among patients who had high MPV levels [14]. Given that hemodialysis patients experience two opposing complications of platelet dysfunction, namely thrombosis and bleeding diathesis, MPV may potentially serve as a readily available platelet reactivity marker and a practical tool for selecting patients who will benefit from certain antiplatelet agents.

The strengths of this study include its derivation from a large, nationally representative cohort of hemodialysis patients among whom the vast majority underwent MPV measurements; inclusion of an incident hemodialysis cohort whose characteristics are not impacted by survivor bias and whose mortality risk differs from that of prevalent patients [43]; and comprehensive availability of detailed baseline and longitudinal patient-level comorbidity, laboratory, and dialysis-treatment

data. However, several limitations bear mention. First, all patients were required to have one or more MPV measurements, which may have limited the study's generalizability. However, while we could not determine the indications for MPV measurement, this requirement applied equally to all patients irrespective of MPV level, and therefore should not have created for differential bias nor impacted the study's internal validity. Second, we were not able to account for potential sources of imprecision and/or measurement bias relating to biologic variations of platelet measurements, nor inconsistencies in methodologic factors that may have influenced MPV results, including the type of anticoagulation used in blood-drawing tubes, time interval between blood draw and MPV measurement, MPV testing device, or temperature at which MPV was analyzed [6]. However, all laboratory data were collected in the outpatient setting and were uniformly measured at a single laboratory processing all specimens from the large dialysis organization, assuring minimal variability. In addition, it is likely that misclassification of MPV status on this basis would non-differential, leading to an attenuation of the MPV—mortality associations, and rendering our estimates conservative. Third, due to data limitations we were unable to determine which patients were receiving antiplatelet therapy, and if treatment impacted outcomes. Fourth, we were unable to examine cause-specific mortality (e.g., cardiovascular death) or mechanisms underlying the high MPV-mortality association. However, designation of all-cause mortality vs. cardiovascular mortality as the outcome of interest may be less susceptible to outcome misclassification, particularly in large retrospective datasets of dialysis patients in which cardiovascular mortality may be over-reported. It should be noted that at baseline, the prevalence of cardiovascular comorbidities was similar across all MPV categories. One potential explanation may be that these baseline prevalences were examined among an incident cohort of hemodialysis patients, and that with increasing dialysis vintage, differences in the proportion of cardiovascular disease may have developed over time. However, due to data limitations, we were unable to examine the association between MPV and trends in the incidence and prevalence of cardiovascular disease. Fifth, as with all observational studies, we cannot exclude the possibility of residual confounding. However, we did observe consistent results across a range of adjusted analyses including many of the covariates previously considered to be strongly associated with mortality in the hemodialysis population.

In conclusion, our study shows that higher MPV levels are independently associated with higher all-cause mortality risk in a nationally representative cohort of incident hemodialysis patients. At this time, future studies are needed to determine underlying mechanisms, and whether the MPV characteristics of hemodialysis patients can be used in the cardiovascular risk assessment of this population.

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Conflicts of interest and financial disclosures

None of the authors declare any relevant conflicts of interest.

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Portions of these data have been presented in an abstract at the National Kidney Foundation 2015 Spring Clinical Meeting, March 25–29, 2015, Dallas, TX.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ijcard.2016.06.074.

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