Vascular Pharmacology 120 (2019) 106564

Contents lists available at ScienceDirect

Vascular Pharmacology

journal homepage: www.elsevier.com/locate/vph

Vitamin D levels and platelet reactivity in diabetic patients receiving dual antiplatelet therapy

Monica Verdoia[a,b], Patrizia Pergolini[d], Matteo Nardin[c], Roberta Rolla[d], Federica Negro[a], Elvin Kedhi[e], Harry Suryapranata[f], Marco Marcolongo[b,c], Alessandro Carriero[g], Giuseppe De Luca[a,*], on behalf of the Novara Atherosclerosis Study Group (NAS)

[a] Division of Cardiology, Azienda Ospedaliera-Universitaria "Maggiore della Carità", EasternPiedmontUniversity, Novara, Italy
[b] Cardiology, Ospedale degli Infermi, ASL Biella, Italy
[c] Internal Medicine, ASST Spedali Civili, University of Brescia, Brescia, Italy
[d] ClinicalChemistry, Azienda Ospedaliera-Universitaria "Maggiore della Carità", EasternPiedmontUniversity, Novara, Italy
[e] Department of Cardiology, ISALA Hospital, Zwolle, the Netherlands
[f] Department of Cardiology, UMC St Radboud, Nijmegen, the Netherlands
[g] Radiology, Azienda Ospedaliera-Universitaria "Maggiore della Carità", EasternPiedmontUniversity, Novara, Italy

ARTICLE INFO

Keywords:
Diabetes mellitus
Vitamin D
Platelet aggregation
Dual antiplatelet therapy

ABSTRACT

Background: Hypovitaminosis D represents an emerging cardiovascular risk factor, and especially among higher-risk subsets of patients, such as in those with diabetes mellitus. The anti-inflammatory and anti-thrombotic properties of vitamin D, in fact, could be even more beneficial among diabetics, where platelet hyperreactivity and suboptimal response to antiplatelet drugs has been associated with poorer outcomes. However, no study has so far evaluated the impact of vitamin D levels on platelet reactivity and high-on treatment platelet reactivity (HRPR) among diabetic patients receiving dial antiplatelet therapy (DAPT).

Methods: Our population is represented by a consecutive cohort ofdiabetic patients treated with DAPT (ASA + clopidogrel or ticagrelor or dose-adjusted prasugrel) for an acute coronary syndrome or elective PCI, undergoing platelet reactivity assessment at 30–90 days post-discharge. Aggregation was assessed by multiple-electrode aggregometry. HRPR was defined for values above the lower limit of normality (in non-treated patients).

Results: We included 440 patients, that were divided according to quartiles values of vitamin D (< 9.4; 9.4–15.59; 15.6–21.64; ≥ 21.65 ng/ml). Among them, 31 were excluded as chronically treated with vitamin D supplementation. Lower vitamin D quartiles were associated with more advanced age ($p = 0.01$), female gender ($p = 0.04$), renal failure ($p = 0.005$), history of previous MI (p = 0.01), CABG and use of diuretics ($p = 0.003$), severe coronary disease ($p = 0.002$), but lower ejection fraction ($p = 0.001$), treatment with statins (p = 0.04) and new ADP-antagonists (p = 0.002). Vitamin D levels related with higher HbA1c (p = 0.001), cholesterol ($p = 0.02$) and creatinine ($p = 0.004$) and lower hemoglobin (p = 0.004).

The prevalence of HRPR with ASA was low and not related to vitamin D quartiles (3.4% vs 2.7% vs 1.8% vs 2.1%, $p = 0.44$; adjusted OR[95%CI] = 1.16[0.60–2.26], $p = 0.67$).

The prevalence of HRPR for ADP antagonists was associated to hypovitaminosis D (40.2% vs 29.1% vs 29.4% vs 25.5%, $p = 0.03$; (adjusted OR[95%CI] = 1.76[1.04–2.98], $p = 0.036$for I vs II-IV quartile). The impact of vitamin D quartiles, was significant only in patients on new ADP antagonists ($n = 225$, of whom 81 on prasugrel 5 mg; p = 0.03; adjusted OR[95%CI] = 3.12[1.34–7.49], $p = 0.009$) but not with clopidogrel ($p = 0.85$, adjusted OR[95%CI] = 1.05[0.49–2.24], $p = 0.89$).

Conclusions: Among diabetic patients receiving dual antiplatelet therapy for an acute coronary syndrome or elective percutaneous coronary intervention, severe vitamin D deficiency is associated with a higher ADP-mediated platelet reactivity and rate of HRPR, and especially for new ADP-antagonists over clopidogrel.

* Corresponding author at: Ospedale "Maggiore della Carità", EasternPiedmontUniversity, C.so Mazzini, 18, 28100 Novara, Italy.
E-mail address: giuseppe.deluca@maggioreosp.novara.it (G. De Luca).

https://doi.org/10.1016/j.vph.2019.106564
Received 20 January 2019; Received in revised form 8 April 2019; Accepted 27 May 2019
Available online 07 June 2019

1. Background

Dual antiplatelet therapy (DAPT) represents a pillar treatment for patients with coronary artery disease, after an acute cardiovascular event or percutaneous coronary intervention (PCI), preventing peri-procedural thrombotic complications and recurrent ischemic events [1–3].

Pharmacological innovations [4], especially in the field of oral antiplatelet agents, have progressively improved the effectivenessof the drugs partnering ASA in the DAPT treatment, with Ticagrelor and Prasugrel providing a faster and more predictable platelet inhibition that the traditional clopidogrel, translating into a positive prognostic impact [5,6].

However, the persistence of an elevated platelet reactivity on DAPT (high-on treatment platelet reactivity, HRPR) has been described in 10 to 30% of patients, being associated with a more than doubled risk of major cardiovascular events [7,8].

Despite the determinants of HRPR have not been, so far, completely defined, several factors, as advanced age, smoking, diabetes mellitus, genetic asset and pro-inflammatory conditions have emerged as potential determinants of a suboptimal response to DAPT [9].

Vitamin D deficiency has been recently claimed as a principal cardiovascular risk factor, affecting a broad part of the population worldwide [10]. In fact, the loss of the pleiotropic antioxidant, antithrombotic and cardioprotective effects of vitamin D has been linked to the prevalence and extent of CAD and to an increase of platelet reactivity and cardiovascular mortality, even among DAPT treated patients [11–13].

Moreover, the consequences of hypovitaminosis D could be even more relevant in the settings of diabetes, where hyperglycemia and the pro-inflammatory milieu induce a marked platelet dysfunction and hyper-reactivity [14].

Nevertheless, the impact of vitamin D on platelet function has never been addressed in diabetic patients and especially among those receiving antiplatelet agents. Therefore, the aim of the present study was to evaluate the relationship between vitamin D levels and platelet reactivity or the rate of HRPR among diabetic patients treated with DAPT.

2. Methods

We included patients admitted at the Division of Cardiology, “Maggiore della Carità” Hospital, Eastern Piedmont University in Novara, Italy, from September 2011 to June 2017 requiring DAPT for an acute coronary syndrome (ACS) or after PCI for stable CAD. All patients receiving at discharge dual antiplatelet therapy with ASA (100 to 160 mg daily) and an ADP-antagonist (clopidogrel 75 mg daily or ticagrelor 90 mg b.i.d or prasugrel 10 mg daily - or 5 mg daily in case of age ≥ 75 years or body weight < 60 Kg) were scheduled for chemistry and platelet function tests evaluation at 30–90 days from discharge. Among them, only the diabetic subgroup of patients was analyzed. Diabetes mellitus was defined as previous diagnosis, specific treatment administration (oral drug or insulin), random glycaemia > 200 mg/dL, fasting glycaemia > 126 mg/dL or HbA1c > 6.5% [15]. Chronic renal failure was considered for history of renal failure or an admission glomerular filtrate (GFR) < 60 mol/min/1.73 m2 by MDRD (Modification of Diet in renal Disease) formula. Hypertension was defined as systolic pressure > 140 mmHg and/or diastolic pressure > 90 mmHg or if the individual was taking antihypertensive medications.

The study was approved by our local Ethical Committee and informed consent was obtained by all patients. Main demographic, clinical and angiographic data, together with the indication to DAPT were recorded at discharge and included in a dedicated database, protected by password.

3. Biochemical measurements

Blood samples were drawn in the early morning, following a fasting period of 12 h. Glucose, creatinine, glycosylated hemoglobin and lipid profile were determined as previously described [18]. Blood cells count was performed in a blood sample collected in tripotassium EDTA (7.2 mg) tubes. These blood samples were analyzed within 2 h of venepuncture by automatic blood cells counter (A Sysmex XE-2100).

Vitamin D measurement was performed by chemiluminescence method through LIAISON® Vitamin D assay (Diasorin Inc). The normal range for 25-OH D3 levels in our laboratory is from 30 to 100 ng/ml, according to literature reference (16). Hypovitaminosis D was considered for severe deficiency (< 10 ng/ml).

4. Platelet function assessment on DAPT

Platelet aggregation was measured at 30–90 days from discharge by impedance aggregometry (Multiplate®- multiple platelet function analyser; Roche Diagnostics AG). For Multiplate a whole blood sample was stored in Vacutainer standard lithium heparin tubes and analyzed within 1–2 h from collection [17]. Tests with different agonists were performed: arachidonic acid (AA), collagen, ADP and prostaglandin E1 and thrombin receptor activating peptide (TRAP-6). Results were expressed as arbitrary Aggregation Units (AU) and plotted against time, defining platelet function as the area under curve (AUC or AU*min). HRPR was considered for AU*min values above lower limit normal for ASA (HAPR), [range: 862–1344] or after ADP stimulation [range: 417–1030], respectively [18,19]. The test was repeated in patients with HRPR to confirm the findings.

5. Statistical analysis

Statistical analysis was performed using SPSS 22.0 statistical package. Continuous data were expressed as mean ± SD and categorical data as percentage. Analysis of variance and the chi-square test were used for continuous and categorical variables, respectively. Patients were grouped according to vitamin D quartiles. A p value < 0.05 was considered statistically significant. Multivariate logistic regression analysis was performed to evaluate the relationship between vitamin D and HRPR, after correction for baseline differences that were entered in the model in block. A linear regression analysis was performed to evaluate the relationship between vitamin D levels and absolute levels of platelet reactivity with different activating stimuli.

6. Results

We included in our study 440 diabetic patients, that were divided according to vitamin D quartiles values (< 9.4 ng/ml, 9.4–15.59 ng/ml; 15.6–21.64 ng/ml; ≥21.65 ng/ml). Among them, 31 patients (7%) were excluded as chronically treated with vitamin D supplementation.

Main clinical and demographic features are reported in Table 1. Lower vitamin D quartiles were associated with more advanced age ($p = 0.01$), female gender ($p = 0.04$), renal failure ($p = 0.005$), history of previous MI (p = 0.01), CABG and use of diuretics ($p = 0.003$), severe coronary disease ($p = 0.002$), while inversely to ejection fraction ($p = 0.001$), treatment with statins (p = 0.04) and new ADP-antagonists (p = 0.002).

Vitamin D levels related with higher HbA1c (p = 0.001), cholesterol ($p = 0.02$) and creatinine ($p = 0.004$) and lower hemoglobin (p = 0.004). Mean platelet reactivity was non-significantly higher for lower vitamin D levels (Table 1).

At linear regression analysis, we observed a trend for higher ADP-mediated platelet reactivity with lower levels of vitamin D ($r = -0.09$, $p = 0.07$), but not for other activating stimuli, as shown in Fig. 1A (ASPI test: $r = -0.03$, $p = 0.54$), 1B (COL test: $r = -0.06$; $p = 0.22$) and 1C (TRAP: $r = 0.05$, $p = 0.31$).

Table 1
Main clinical and demographic features in study population.

Clinical features	Overall population	I quart < 9.4 (*n* = 116)	II quart 9.4–15.59 (*n* = 112)	III quart 15.6–21.64 (*n* = 113)	IV quart ≥21.65 (*n* = 99)	*P* value
Age (mean ± SD)	69.2 ± 9.7	70.2 ± 9.8	68.9 ± 9.6	66.1 ± 10.4	67.8 ± 9.3	0.01
Male sex (%)	75.5	68.1	74.1	85	76.5	0.04
BMI (mean ± SD)	28.8 ± 5.8	28.9 ± 4.9	29.1 ± 4.6	29.6 ± 8.3	27.7 ± 4.4	0.13
Hypertension (%)	80.3	84.3	74.1	78.8	79.6	0.54
Active smokers (%)	22.9	27.8	20.5	24.8	21.4	0.08
Hypercholesterolemia (%)	68.9	73.1	70.5	67.3	67.3	0.44
Previous MI (%)	25.5	33.9	25	23.9	18.6	0.01
Previous PCI (%)	44.5	46.1	39.3	47.8	46.9	0.62
Previous CABG (%)	14	22.6	14.3	14.3	7.1	0.003
Renalfailure (%)	23.6	32.8	20	15	18.4	0.005
Ejectionfraction (mean ± SD)	51.5 ± 10.1	47.9 ± 11.6	51.9 ± 10.7	53.4 ± 9.3	51.8 ± 10.1	0.001
Indication to DAPT (%)						0.04
Stable angina /silent ischemia	36.2	34.5	32.1	41.6	38.8	
ACS	55.4	50	62.5	54	55.1	
Dilated CMP/arrhythmias	8.5	15.5	5.4	4.4	6.1	
Therapy atadmission						
ACE inhibitors (%)	48.2	43.1	56.3	39.8	55.1	0.39
ARBs (%)	29	33.6	19.6	37.2	23.5	0.54
Statins (%)	85	78.4	90.2	85.8	89.8	0.04
Beta blockers (%)	81.2	81	83.9	76.1	82.7	0.82
Nitrates (%)	49	58.6	44.6	49.6	42.9	0.05
Ca-antagonists (%)	30	30.2	27.7	35.4	26.5	0.92
Diuretics (%)	44.4	56.9	38.4	39.8	35.7	0.003
P2Y12 inhibitor (%)						0.18
Clopidogrel	42	50.9	46	48.2	50.5	
New P2Y12 inhibitor	58	49.1	60.8	51.8	49.5	
Antidiabetic therapy (%)						0.06
Insulin	32.2	47.1	34.1	24.4	21.3	
Oraldrugs	45.5	28.7	53.4	46.3	54.7	
Multivessel CAD	73.3	77.7	73.4	73.6	68.5	0.15
Left main/trivessel CAD	45.5	56.3	47.7	42.7	34.8	0.002
Main chemistryparameters						
Glycaemia(mean ± SD)	142.7 ± 42.5	160 ± 70.3	142 ± 55.9	155.2 ± 50.2	144.3 ± 41.8	0.76
HbA1c (mean ± SD)	7.2 ± 1	7.7 ± 1.5	7.1 ± 1.2	7.3 ± 1.6	6.9 ± 1	0.001
Creatinine (mean ± SD)	1.1 ± 0.7	1.2 ± 0.85	0.99 ± 0.4	0.98 ± 0.4	0.98 ± 0.4	0.004
Cholesterol total (mean ± SD)	140.3 ± 35.9	148.6 ± 42.7	138 ± 33.8	138.7 ± 34.8	133.8 ± 29.6	0.02
CholesterolHDL(mean ± SD)	40 ± 13.7	41.2 ± 14.1	41.8 ± 17	38.1 ± 12.5	38 ± 10.2	0.08
C reactive protein (mg/dl, ± SD)	0.794 ± 1.7	1 ± 1.5	0.6 ± 0.9	1.1 ± 2.3	0.4 ± 1	0.008
Platelets (10^5/ml; mean ± SD)	241.4 ± 72	242.7 ± 69.4	241 ± 75.1	226.7 ± 58.6	251.5 ± 77.1	0.008
Haemogobin (mean ± SD)	13.2 ± 1.8	13 ± 1.9	13.2 ± 1.7	13.7 ± 1.5	12.9 ± 1.8	0.08
WBC (10^3/ml; mean ± SD)	8.3 ± 2.3	8.5 ± 2.4	8.1 ± 2	8.8 ± 2.7	8.2 ± 2.1	0.12
COL test (AUC; mean ± SD)	450.8 ± 163	481.9 ± 168.2	461.4 ± 193	459.1 ± 156	450.6 ± 177	0.61
ASPI test (AUC; mean ± SD)	391. 4 ± 207	401 ± 246.3	387.6 ± 198	390.7 ± 196.2	380.4 ± 183.8	0.64
TRAP test (AUC; mean ± SD)	1116 ± 329	1096.7 ± 334	1097.6 ± 304	1090.3 ± 306	1142 ± 335	0.76
ADP test (AUC, mean ± SD)	370.3 ± 204	420.1 ± 228	397.5 ± 228	384.3 ± 205	361.3 ± 195	0.27

The prevalence of HRPR with ASA was low and not significantly related to vitamin D quartiles (3.4% vs 2.7% vs 1.8% vs 2.1%, $p = 0.44$), as shown in Fig. 2. Results were confirmed after correction for baseline differences at multivariate analysis (adjusted OR [95%CI] = 1.16[0.60–2.26], $p = 0.67$).

The prevalence of HRPR for ADP antagonists was associated to hypovitaminosis D (40.2% vs 29.1% vs 29.4% vs 25.5%, $p = 0.03$, Fig. 3A).

In a multivariate regression model, the lowest vitamin D quartile (< 9.4 ng/ml) was significantly associated to the occurrence of HRPR (adjusted OR[95%CI] = 1.76[1.04–2.98], $p = 0.036$).

However, the impact of vitamin D quartiles, was significant only in patients on new ADP antagonists ($n = 225$), including 81 patients on prasugrel, of whom 16 receiving the low-dose of 5 mg (33.3% vs 10.5% vs 17.2% vs 12.9%, $p = 0.03$, Fig. 3B). The results were confirmed at multivariate analysis (adjusted OR[95%CI] = 3.12[1.34–7.49], $p = 0.009$). On the contrary, vitamin D did not affect the effectiveness of clopidogrel (45.8% vs 52.2% vs 45.5% vs 50%, $p = 0.85$, adjusted OR[95%CI] = 1.05[0.49–2.24], $p = 0.89$), as shown in Fig. 3C. Results did not change when excluding the subgroup of elderly patients treated with low-dose prasugrel (33.3% vs 8.3% vs 16.7% vs 5%, p = 0.03). (adjusted OR[95%CI] = 1.88[1.01–3.24], $p = 0.02$ for I vs II-IV quartile) or according to the intensity of statin therapy (high-intensity statins, $n = 307$: 43.8% vs 26.1% vs 30.4% vs 27.6%, $p = 0.11$; adjusted OR[95%CI] = 1.84[0.97–3.49], $p = 0.08$).

7. Discussion

The present study represents the largest cohorts of diabetic patients where we assessed the impact of vitamin D levels on platelet reactivity and the rate of high-residual platelet reactivity (HRPR) on DAPT.

We found a significant impact of severe vitamin D deficiency on ADP-mediated platelet reactivity, and the rate of HRPR, especially with the newer antiplatelet drugs Ticagrelor and Prasugrel than with clopidogrel.

Diabetics represent the most challenging subpopulation of CAD patients [20-24], displaying a reduced effect of antiplatelet drugs in diabetic patients, with suboptimal platelet inhibition with the different available drugs [25–32].

Therefore, several efforts have been addressed to the identification of those factors modulating platelet reactivity among diabetic patients, aiming at the identification of those patients where a persistent HRPR

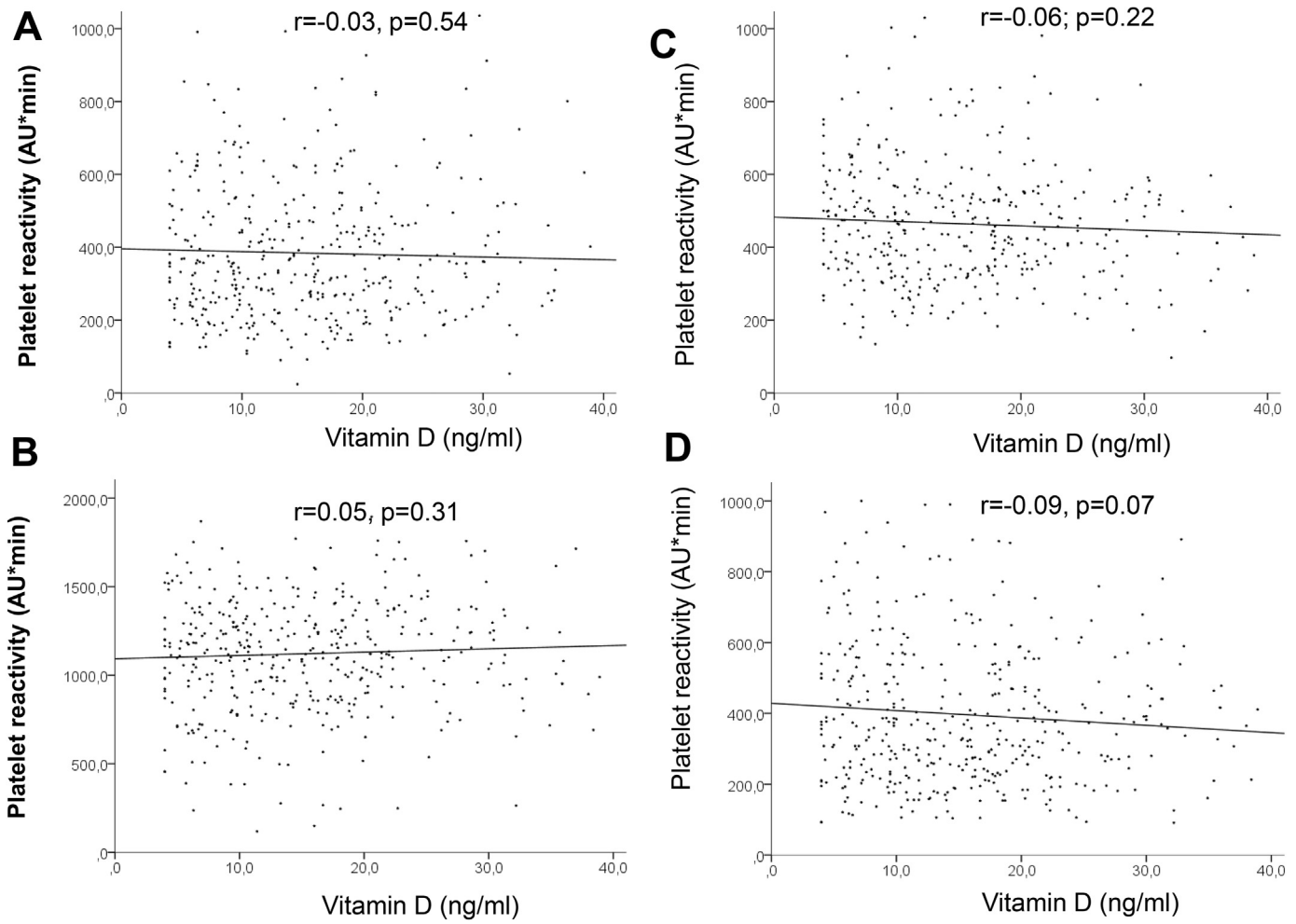

Fig. 1. Linear relationship between vitamin D levels and platelet reactivity at ASPI test (1A, upper left), COL test (1B, upper right), TRAP test (1C, lower left) and ADP (1D, lower right).

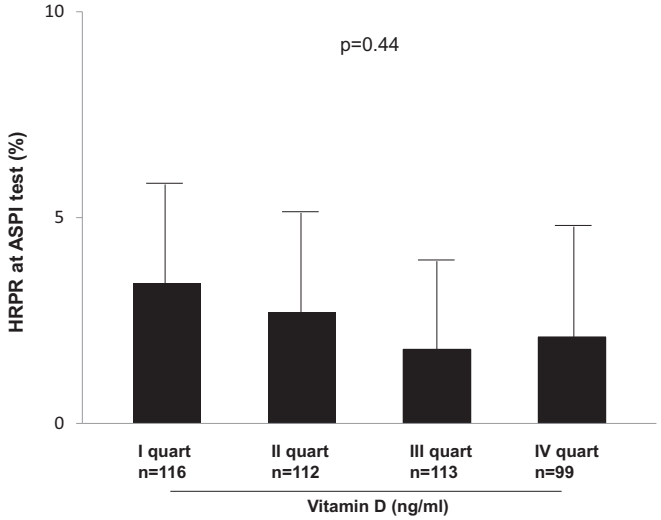

Fig. 2. Bar graph displaying the prevalence of residual high-on treatment platelet reactivity (HAPR) for Acetylsalicylic acid (ASA) in diabetic patients according to vitamin D quartiles.

could condition the risk of major thrombotic events [14]. However, inconclusive results have been achieved when focusing on platelet morphology and turnover [33,34]. Moreover, the evaluation of hypoglycemic therapy and aggressive glucose control [35] has not provided so far the expected benefits, therefore shifting the attention to other modulators of platelet function in diabetic patients.

Vitamin D deficiency represents a rising pandemic social problem, affecting up to 50% of the healthy population worldwide [36].

Moreover, an emerging link has been established between hypovitaminosis D and cardiovascular risk, since the pleiotropic, non-calcium related, effects of vitamin D on the cardiovascular system could modulate those inflammatory, oxidant and immunitary processes leading to the progression of atherosclerosis and its acute thrombotic complications [37,38].

In fact, vitamin D could exert a regulatory effect also on platelets, since its deficiency has been associated to endothelial dysfunction and the levels of nitric oxide and moreover, vitamin D receptor (VDR) has been recently identified also in platelets [39], where studies in vitro and in vivo have demonstrated that the vitamin D-VDR system plays a pivotal role in antithrombogenicity [40]. In a previous study, Lopez-Farrè et al. [41] reported higher vitamin D binding protein (DBP) levels in ASA resistant subjects. Moreover, Verdoia et al. [42] showed a significantly higher ADP-mediated platelet aggregation in patients with lower vitamin D levels, despite the use of DAPT.

In addition, diabetic patients could display even more reduced levels of vitamin D. In fact, its deficiency has been related to the pathogenesis of metabolic disorders and especially to those inflammatory or immuno-mediated processes leading to the development of diabetes mellitus [43,44].

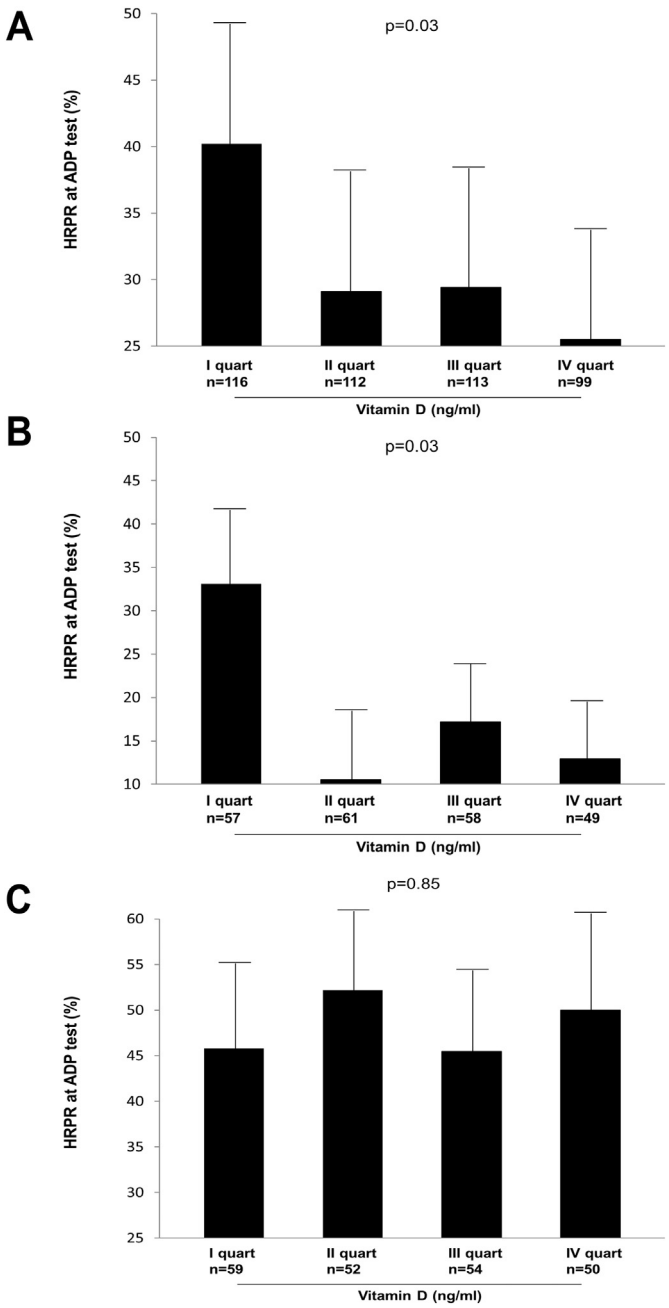

Fig. 3. Bar graph displaying the prevalence of residual high-on treatment platelet reactivity (HRPR) to ADP antagonists in diabetic patients according to vitamin D quartiles (overall population- A, upper graph; new ADP-antagonists- B, mid graph and clopidogrel- C, lower graph).

Therefore, an even more relevant role of vitamin D in conditioning platelet reactivity and the thrombotic risk could be expected among diabetic patients. However, this issue has not been addressed so far, especially among patients treated with antiplatelet agents.

In the present study we documented in a large cohort of diabetic patients a high rate of poor responders to DAPT, despite a large use of the new ADP-antagonists.

Vitamin D levels among our diabetic patients did not affect ASA response, but were independently associated, when severely reduced, with an increased ADP-mediated platelet reactivity and rate of HRPR with new ADP-antagonists but not Clopidogrel. Indeed, it could be argued that Clopidogrel response is much depending on metabolic and genetic factors conditioning its hepatic transformation, while the action of new agents, that are not pro-drugs, could be more dependent from baseline platelet reactivity. In addition, patients with more severe vitamin D deficiency displayed a higher inflammatory status and a poorer control of glucose homeostasis. Indeed, the relationship between the levels of glycosylated hemoglobin and platelet function has been previously reported in several studies, as in Singla et al. [45] for levels of HbA1c ≥ 7 g/dl. Moreover, Vivas et al., [46] showed a significant reduction of platelet ADP-mediated aggregation in post-ACS patients receiving intensive glucose control treatment with insulin. However, in our population, patients with lower vitamin D were more often receiving insulin than on oral drugs, suggesting either a more complex and inveterate diabetic disease or a potential interaction of vitamin D levels in modulating insulin levels and the sensitivity to insulin.

In fact, vitamin D has been shown to condition directly and indirectly insulin secretion and effectiveness in diabetic patients. In Kumar et al., the administration of supplemental vitamin D to subjects with elevated blood glucose levels has resulted in an improvement in insulin secretion [47] and similarly, von Hurst et al. reported in a randomized trial that improving vitamin D status in insulin resistant women resulted in improved insulin resistance [48].In fact, insulin sensitivity is often impaired among diabetic patients, enhancing their thrombotic risk [49].

Therefore, it could be hypothesized that vitamin D might counteract insulin resistance and increase the response to insulin in diabetic platelets', reducing then their activated status and preventing HRPR during DAPT treatment.

However, future dedicated studies are certainly needed to define the interactions between vitamin D and diabetes on platelet function and the potential benefits of its supplementation, especially in higher cardiovascular risk subsets of patients as among diabetics.

8. Limitations

A first limitation could be considered the absence of a clinical follow-up. Therefore, we could not evaluate the impact of diabetes or vitamin D levels on the occurrence of thrombotic or bleeding events. In addition, interindividual clinical variability in our study population could certainly have conditioned our study results. In particular, we did not consider the duration of diabetes or the impact of antidiabetic therapies in our patients, and in particular of more recent oral drugs, whose anti-inflammatory and anti-thrombotic effects could have modulated platelet reactivity [50,51] However, insulin was used in the majority of our vitamin D deficient patients, and data were confirmed interindividual Moreover, despite the positive association of hypovitaminosis D with C reactive protein, we did not consider other parameters of inflammation and oxidative stress, that are known to condition platelet reactivity, as isoprostanes of soluble NOX2-dp [52].

In addition, we could not provide data on the role of vitamin D supplementation, since treated patients represented only a minor part of our population, and extremely heterogeneous for the dosing and the adequacy of the levels reached with the supplementation. Neither, as now stated in our limitations, we could provide data on the genetic variants associated to vitamin D polymorphisms, since genotyping was not performed in the overall cohort of the patients but only in part of them. Therefore, we preferred not to perform such analysis to avoid the risk of underpowered analysis and false negative results. However, such evaluation certainly represents a future implementation of our studies.

Despite the intensity of statin treatment did not affect platelet reactivity in our study population, the majority of our diabetic patients were receiving high-intensity statins, and whether the differential use of statins could affect the levels of vitamin D, as suggested in our previous studies [53], could not be assessed.

Finally, being our study one of the first exploratory study on this issue, we did not perform a sample size calculation. However, based on an expected prevalence of severe hypovitaminosis D of 30%, with an anticipated two-sided test for differences in independent binomial

proportions at the 5% significance level, with a power of 80%, a total of 353 patients would have been needed to show a significant increase in HRPR from 25% to 40%. The inclusion of final population of > 400 patients may have limited the risk of underpower, based on this clinically reasonable assumption.

9. Conclusions

Among diabetic patients receiving dual antiplatelet therapy for an acute coronary syndrome or elective percutaneous coronary intervention, lower vitamin D levels are associated with a higher ADP-mediated platelet reactivity and rate of HRPR, and especially for new ADP-antagonists over clopidogrel.

Disclosures

The Authors declare no conflict of interest nor funding source to disclose.

Acknowledgments

This research was conducted with the unconditioned research funding of the Università del Piemonte Orientale.

References

[1] M. Valgimigli, H. Bueno, R.A. Byrne, J.P. Collet, F. Costa, A. Jeppsson, P. Jüni, A. Kastrati, P. Kolh, L. Mauri, G. Montalescot, F.J. Neumann, M. Petricevic, M. Roffi, P.G. Steg, S. Windecker, J.L. Zamorano, G.N. Levine, ESC Scientific Document Group, ESC Committee for Practice Guidelines (CPG), ESC National Cardiac Societies, 2017 ESC focused update on dual antiplatelet therapy in coronary artery disease developed in collaboration with EACTS: the task force for dual antiplatelet therapy in coronary artery disease of the European Society of Cardiology (ESC) and of the European Association for Cardio-Thoracic Surgery (EACTS), Eur. Heart J. 39 (3) (2018) 213–260.

[2] G. De Luca, H. Suryapranata, P. Marino, Reperfusion strategies in acute ST-elevation myocardial infarction: an overview of current status, Prog. Cardiovasc. Dis. 50 (5) (2008) 352–382.

[3] M. Verdoia, A. Schaffer, L. Barbieri, E. Cassetti, R. Piccolo, G. Galasso, P. Marino, F. Sinigaglia, G. De Luca, Benefits from new ADP antagonists as compared with clopidogrel in patients with stable angina or acute coronary syndrome undergoing invasive management: a meta-analysis of randomized trials, J. Cardiovasc. Pharmacol. 63 (4) (2014) 339–350.

[4] G. De Luca, H. Suryapranata, Recent advances in optimal adjunctive antithrombotic therapy in STEMI patients undergoing primary angioplasty: an overview, Curr. Vasc. Pharmacol. 13 (5) (2015) 594–615.

[5] S.D. Wiviott, E. Braunwald, D.J. Angiolillo, S. Meisel, A.J. Dalby, F.W. Verheugt, S.G. Goodman, R. Corbalan, D.A. Purdy, S.A. Murphy, C.H. McCabe, Antman EM; TRITON-TIMI 38 investigators. Greater clinical benefit of more intensive oral antiplatelet therapy with prasugrel in patients with diabetes mellitus in the trial to assess improvement in therapeutic outcomes by optimizing platelet inhibition with prasugrel-thrombolysis in myocardial infarction 38, Circulation. 118 (16) (2008) 1626–1636.

[6] L. Wallentin, R.C. Becker, A. Budaj, Cannon CP, Emanuelsson H, held C, Horrow J, Husted S, James S, Katus H, Mahaffey KW, Scirica BM, Skene a, Steg PG, storey RF, Harrington RA; PLATO investigators, Freij a, Thorsén M. Ticagrelor versus clopidogrel in patients with acute coronary syndromes, N. Engl. J. Med. 361 (2009) 1045–1057.

[7] U.S. Tantry, L. Bonello, D. Aradi, M.J. Price, Y.H. Jeong, D.J. Angiolillo, G.W. Stone, N. Curzen, T. Geisler, J. TenBerg, A. Kirtane, J. Siller-Matula, E. Mahla, R.C. Becker, D.L. Bhatt, R. Waksman, S.V. Rao, D. Alexopoulos, R. Marcucci, J.L. Reny, D. Trenk, D. Sibbing, Gurbel PA; working group on on-treatment PlateletReactivity. Consensus and update on the definition of on-treatment platelet reactivity to adenosine diphosphate associated with ischemia and bleeding, J. Am. Coll. Cardiol. 62 (24) (2013) 2261–2273.

[8] G.W. Stone, B. Witzenbichler, G. Weisz, M.J. Rinaldi, F.-J. Neumann, D.C. Metzger, et al., Platelet reactivity and clinical outcomes after coronary artery implantation of drug-eluting stents (ADAPT-DES): a prospective multicentre registry study, Lancet. 382 (9892) (2013 Aug 17) 614–623.

[9] M. Amsallem, S. Manzo-Silberman, J.G. Dillinger, G. Sideris, S. Voicu, Bal ditSollier C, Drouet L, Henry P. predictors of high on-aspirin platelet reactivity in high-risk vascular patients treated with single or dual antiplatelet therapy, Am. J. Cardiol. 115 (9) (2015) 1305–1310.

[10] S.H. Pearce, T.D. Cheetham, Diagnosis and management of vitamin D deficiency, BMJ 340 (2010) b566.

[11] M. Verdoia, A. Schaffer, C. Sartori, L. Barbieri, E. Cassetti, P. Marino, G. Galasso, G. De Luca, Vitamin D deficiency is independently associated with the extent of coronary artery disease, Eur. J. Clin. Invest. 44 (7) (2014 Jul) 634–642.

[12] E. Giovannucci, Y. Liu, B.W. Hollis, E.B. Rimm, 25-hydroxyvitamin D and risk of myocardial infarction in men: a prospective study, Arch. Intern. Med. 168 (2008) 1174–1180.

[13] M. De Metrio, V. Milazzo, M. Rubino, A. Cabiati, M. Moltrasio, I. Marana, J. Campodonico, N. Cosentino, F. Veglia, A. Bonomi, M. Camera, E. Tremoli, G. Marenzi, Vitamin D plasma levels and in-hospital and 1-year outcomes in acute coronarysyndromes: a prospectivestudy, Medicine (Baltimore) 94 (19) (2015) e857.

[14] J.L. Ferreiro, J.A. Gómez-Hospital, D.J. Angiolillo, Platelet abnormalities in diabetes mellitus, Diab. Vasc. Dis. Res. 7 (4) (2010) 251–259.

[15] G. De Luca, G.G. Secco, M. Santagostino, L. Venegoni, S. Iorio, E. Cassetti, et al., Uric acid does not affect the prevalence and extent of coronary artery disease. results from a prospective study, Nutr. Metab. Cardiovasc. Dis. 22 (5) (2012 May) 426–433.

[16] M.F. Holick, Vitamin D deficiency, N. Engl. J. Med. 357 (2007) 266–281.

[17] M. Verdoia, P. Pergolini, C. Camaro, M. Restifo, R. Rolla, A. Schaffer, G. Di Giovine, P. Marino, G. Bellomo, H. Suryapranata, G. De Luca, Novara AtherosclerosisStudy Group (NAS), PlA(1)/PlA(2) polymorphism does not influence response to Gp IIb-IIIa inhibitors in patients undergoing coronary angioplasty, Blood Coagul. Fibrinolysis 24 (2013) 411–418.

[18] M. Vidali, R. Rolla, M. Parrella, C. Cassani, M. Manzini, M.R. Portalupi, R. Serino, M.D. Prando, G. Bellomo, P. Pergolini, Role of the laboratory in monitoring patientsreceiving dual antiplatelet therapy, Int. J. Lab. Hematol. 34 (2012) 484–494.

[19] L. Bonello, U.S. Tantry, R. Marcucci, R. Blindt, D.J. Angiolillo, R. Becker, D.L. Bhatt, M. Cattaneo, J.P. Collet, T. Cuisset, C. Gachet, G. Montalescot, L.K. Jennings, D. Kereiakes, D. Sibbing, D. Trenk, J.W. Van Werkum, F. Paganelli, M.J. Price, R. Waksman, P.A. Gurbel, Working group on high on-treatment platelet reactivity. consensus and future directions on the definition of high on-treatment platelet reactivity to adenosine diphosphate, J. Am. Coll. Cardiol. 56 (2010) 919–933.

[20] G. De Luca, C.M. Gibson, F. Bellandi, M. Noc, D. Dudek, U. Zeymer, et al., Diabetes mellitus is associated with distal embolization, impaired myocardial perfusion, and higher mortality in patients with ST-segment elevation myocardial infarction treated with primary angioplasty and glycoprotein IIb-IIIa inhibitors, Atherosclerosis 207 (2009) 181–185.

[21] G. De Luca, A. Schaffer, M. Verdoia, H. Suryapranata, Meta-analysis of 14 trials comparing bypass grafting vs drug-eluting stents in diabetic patients with multivessel coronary artery disease, Nutr. Metab. Cardiovasc. Dis. 24 (2014) 344–354.

[22] J.R. Timmer, I.C. van der Horst, G. de Luca, J.P. Ottervanger, J.C. Hoorntje, M.J. de Boer, H. Suryapranata, J.H. Dambrink, M. Gosselink, F. Zijlstra, Van T. Hof AW, Zwolle myocardial infarction study group. Comparison of myocardial perfusion after successful primary percutaneous coronary intervention in patients with ST-elevation myocardial infarction with versus without diabetes mellitus, Am. J. Cardiol. 95 (11) (2005) 1375–1377.

[23] G. De Luca, L.A. Małek, P. Maciejewski, W. Wasek, M. Niewada, B. Kamiński, J. Drzewiecki, M. Kośmider, J. Kubica, W. Ruzyłło, J.Z. Peruga, D. Dudek, G. Opolski, S. Dobrzycki, R.J. Gil, A. Witkowski, STEMI 2003 registry collaborators. impact of diabetes on survival in patients with ST-segment elevation myocardial infarction treated by primary angioplasty: insights from the POLISH STEMI registry, Atherosclerosis 210 (2) (2010) 516–520.

[24] M. Ferlini, G. Musumeci, N. Grieco, R. Rossini, S. De Servi, A. Mafrici, C. Sponzilli, A. Demarchi, E.M. Assanelli, P. Camisasca, G. Chizzola, E. Corrada, A. Farina, C. Pedrinazzi, C. Lettieri, Visconti L. Oltrona, The paradox of clopidogrel use in patients with acute coronary syndromes and diabetes: insight from the Diabetes and Acute Coronary Syndrome Registry, Coron. Artery Dis. 29 (4) (2018 Jan 5) 309–315.

[25] G. De Luca, M.T. Dirksen, C. Spaulding, H. Kelbæk, M. Schalij, L. Thuesen, B. van der Hoeven, M.A. Vink, C. Kaiser, C. Musto, T. Chechi, G. Spaziani, Diaz de la Llera LS, Pasceri V, Di Lorenzo E, Violini R, Suryapranata H, Stone GW; DESERT cooperation.Impact of diabetes on long-term outcome after primary angioplasty: insights from the DESERT cooperation, Diabetes Care 36 (4) (2013 Apr) 1020–1025.

[26] S.R. Mehta, S. Yusuf, R.J. Peters, M.E. Bertrand, B.S. Lewis, M.K. Natarajan, et al., Effects of pretreatment with clopidogrel and aspirin followed by long-term therapy in patients undergoing percutaneous coronary intervention: the PCI-CURE study, Lancet 358 (9281) (2001 Aug 18) 527–533.

[27] J.P. Bae, S.D. Candrilli, J. Fortenberry, J.L. Meyers, J.A. Jakubowski, D. Drenning, Point-of-care platelet reactivity determination with VerifyNow-P2Y12 following administration of clopidogrel or prasugrel: data from a real-world, clinical care inpatient setting, Hosp. Pract. 42 (4) (2014 Oct) 7–15 1995.

[28] D.J. Angiolillo, A. Fernandez-Ortiz, E. Bernardo, C. Ramírez, M. Sabaté, P. Jimenez-Quevedo, et al., Platelet function profiles in patients with type 2 diabetes and coronary artery disease on combined aspirin and clopidogrel treatment, Diabetes. 54 (8) (2005 Aug) 2430–2435.

[29] D.J. Angiolillo, J.J. Badimon, J.F. Saucedo, A.L. Frelinger, A.D. Michelson, J.A. Jakubowski, B. Zhu, C.K. Ojeh, B.A. Baker, M.B. Effron, A pharmacodynamic comparison of prasugrel vs. high-dose clopidogrel in patients with type 2 diabetes mellitus and coronary artery disease: results of the optimizing anti-platelet therapy IndiabetesMellitUS (OPTIMUS)-3 trial, Eur. Heart J. 32 (7) (2011 Apr) 838–846.

[30] F. Franchi, F. Rollini, N. Aggarwal, J. Hu, M. Kureti, A. Durairaj, V.E. Duarte, J.R. Cho, L. Been, M.M. Zenni, T.A. Bass, D.J. Angiolillo, Pharmacodynamic comparison of prasugrel versus Ticagrelor in patients with type 2 diabetes mellitus and coronary artery disease: the OPTIMUS (optimizing antiplatelet therapy in diabetes mellitus)-4 study, Circulation 134 (11) (2016) 780–792.

[31] M. Nardin, M. Verdoia, C. Sartori, P. Pergolini, R. Rolla, L. Barbieri, A. Schaffer, G. Bellomo, H. Suryapranata, G. De Luca, Novara Atherosclerosis Study Group (NAS), Diabetes mellitus, glucose control parameters and platelet reactivity in ticagrelor treated patients, Thromb. Res. 143 (2016) 45–49.

[32] D. Alexopoulos, C. Vogiatzi, K. Stavrou, N. Vlassopoulou, A. Perperis, I. Pentara, I. Xanthopoulou, Diabetes mellitus and platelet reactivity in patients under prasugrel or ticagrelor treatment: an observational study, Cardiovasc. Diabetol. 14 (2015) 68.
[33] M. Verdoia, P. Pergolini, M. Nardin, R. Rolla, L. Barbieri, A. Schaffer, P. Marino, G. Bellomo, H. Suryapranata, G. De Luca, Impact of diabetes on immature platelets fraction and its relationship with platelet reactivity in patients receiving dual antiplatelet therapy, J. Thromb. Thrombolysis 42 (2) (2016) 245–253.
[34] G. De Luca, M. Verdoia, E. Cassetti, A. Schaffer, G. Di Giovine, A. Bertoni, C. Di Vito, S. Sampietro, G. Aimaretti, G. Bellomo, P. Marino, Sinigaglia F; Novara AtherosclerosisStudy (NAS) group. Mean platelet volume is not associated with platelet reactivity and the extent of coronary artery disease in diabetic patients, Blood Coagul. Fibrinolysis 24 (6) (2013) 619–624.
[35] F. Mangiacapra, A.J. Peace, W. Wijns, E. Barbato, Lack of correlation between platelet reactivity and glycaemic control in type 2 diabetes mellitus patients treated with aspirin and clopidogrel, J. Thromb. Thrombolysis 32 (1) (2011) 54–58.
[36] M.F. Holick, The vitamin D deficiency pandemic: approaches for diagnosis,treatment and prevention, Rev. Endocr. Metab. Disord. 18 (2) (2017) 153–165.
[37] M. Verdoia, A. Schaffer, C. Sartori, L. Barbieri, E. Cassetti, P. Marino, G. Galasso, G. De Luca, Vitamin D deficiency is independently associated with the extent of coronary artery disease, Eur. J. Clin. Investig. 44 (7) (2014) 634–642.
[38] H.A. Sheerah, E.S. Eshak, R. Cui, H. Imano, H. Iso, Tamakoshi a; Japan collaborative cohort study group. relationship between dietary vitamin D and deaths from stroke and coronary heart disease: the Japan collaborative cohort study, Stroke 49 (2) (2018) 454–457.
[39] F. Silvagno, E. De Vivo, A. Attanasio, V. Gallo, G. Mazzucco, G. Pescarmona, Mitochondrial localization of vitamin D receptor in human platelets and differentiated megakaryocytes, PLoS ONE 5 (2010) e8670.
[40] K. Aihara, H. Azuma, M. Akaike, Y. Ikeda, M. Yamashita, T. Sudo, H. Hayashi, Y. Yamada, F. Endoh, M. Fujimura, T. Yoshida, H. Yamaguchi, S. Hashizume, M. Kato, K. Yoshimura, Y. Yamamoto, S. Kato, T. Matsumoto, Disruption of nuclear vitamin D receptor gene causes enhanced thrombogenicity in mice, J. Biol. Chem. 279 (2004) 35798–35802.
[41] A.J. López-Farré, P.J. Mateos-Cáceres, D. Sacristán, L. Azcona, E. Bernardo, T.P. de Prada, S. Alonso-Orgaz, M. Fernández-Arquero, A. Fernández-Ortiz, C. Macaya, Relationshipbetweenvitamin D bindingprotein and aspirinresistance in coronaryischemicpatients: a proteomicstudy, J. Proteome Res. 6 (2007) 2481–2487.
[42] M. Verdoia, P. Pergolini, R. Rolla, C. Sartori, M. Nardin, A. Schaffer, L. Barbieri, V. Daffara, P. Marino, G. Bellomo, H. Suryapranata, G.D. Luca, Novara atherosclerosis study group (NAS). vitamin D levels and high-residual platelet reactivity in patients receiving dual antiplatelet therapy with clopidogrel or ticagrelor, Platelets 27 (6) (2016) 576–582.
[43] A.M. Pello, C. Cristóbal, N. Tarín, A. Huelmos, Á. Aceña, R. Carda, M.L. González-Casaus, J. Alonso, Ó. Lorenzo, L. Blanco-Colio, J.L. Martín-Ventura, J.A. Franco Peláez, I. Mahíllo-Fernández, J. Farré, L. López-Bescós, J. Egido, J. Tuñón, Differential profile in inflammatory and mineral metabolism biomarkers in patients with ischemic heart disease without classical coronary risk factors, J. Cardiol. 66 (2015) 22–27.
[44] V. Hirani, R.G. Cumming, D.G. Le Couteur, V. Naganathan, F. Blyth, D.J. Handelsman, L.M. Waite, M.J. Seibel, Low levels of 25-hydroxy vitamin d and active 1,25-dihydroxyvitamin d independently associated with type 2 diabetes mellitus in older Australian men: the concord health and ageing in men project, J. Am. Geriatr. Soc. 62 (2014) 1741–1747.
[45] A. Singla, M.J. Antonino, K.P. Bliden, U.S. Tantry, P.A. Gurbel, The relation between platelet reactivity and glycemic control in diabetic patients with cardiovascular disease on maintenance aspirin and clopidogrel therapy, Am. Heart J. 158 (5) (2009 Nov) (784.e1–6).
[46] D. Vivas, J.C. García-Rubira, E. Bernardo, D.J. Angiolillo, P. Martín, A. Calle-Pascual, et al., Effects of intensive glucose control on platelet reactivity in patients with acute coronary syndromes. results of the CHIPS study ("control de Hiperglucemia y ActividadPlaquetariaenPacientes con SindromeCoronarioAgudo"), Heart 97 (10) (2011 May) 803–809.
[47] S. Kumar, M. Davies, Y. Zakaria, Improvement in glucose tolerance and beta-cell function in a patient with vitamin D deficiency during treatment with vitamin D, Postgrad. Med. J. 70 (1994) 440–443.
[48] P.R. von Hurst, W. Stonehouse, J. Coad, Vitamin D supplementation reduces insulin resistance in south Asian women living in New Zealand who are insulin resistant and vitamin D deficient - a randomised, placebo-controlled trial, Br. J. Nutr. 103 (4) (2010) 549–555.
[49] R. Paniccia, E. Antonucci, N. Maggini, E. Romano, A.M. Gori, R. Marcucci, et al., Assessment of platelet function on whole blood by multiple electrode aggregometry in high-risk patients with coronary artery disease receiving antiplatelet therapy, Am. J. Clin. Pathol. 131 (2009) 834–842.
[50] G. Anfossi, I. Russo, M. Trovati, Platelet resistance to the anti-aggregating agents in the insulin resistant states, Curr. Diabetes Rev. 2 (4) (2006 Nov) 409–430.
[51] M. Verdoia, P. Pergolini, R. Rolla, C. Ceccon, M. Caputo, G. Aimaretti, H. Suryapranata, G. De Luca, Use of metformin and platelet reactivity in diabetic patients treated with dual antiplatelet therapy, Exp. Clin. Endocrinol. Diabetes (2018) (Epub ahead of print).
[52] F. Violi, P. Pignatelli, Platelet oxidative stress and thrombosis, Thromb. Res. 129 (3) (2012 Mar) 378–381.
[53] M. Verdoia, P. Pergolini, R. Rolla, M. Nardin, A. Schaffer, L. Barbieri, V. Daffara, P. Marino, G. Bellomo, H. Suryapranata, De Luca G; Novara AtherosclerosisStudy group (NAS). Impact of high-dose statins on vitamin D levels and platelet function in patients with coronary artery disease, Thromb. Res. 150 (2017) 90–95.