White blood cell count, sex and age are major determinants of heterogeneity of platelet indices in an adult general population: results from the MOLI-SANI project

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ABSTRACT

Background
The understanding of non-genetic regulation of platelet indices - platelet count, plateletcrit, mean platelet volume, and platelet distribution width - is limited. The association of these platelet indices with a number of biochemical, environmental and clinical variables was studied in a large cohort of the general population.

Design and Methods
Men and women (n=18,097, 52% women, 56±12 years) were randomly recruited from various villages in Molise (Italy) in the framework of the population-based cohort study "Moli-sani". Hemochromocytometric analyses were performed using an automatic analyzer (Beckman Coulter, IL, Milan, Italy). Associations of platelet indices with dependent variables were investigated by multivariable linear regression analysis.

Results
Full models including age, sex, body mass index, blood pressure, smoking, menopause, white and red blood cell counts, mean corpuscular volume, D-dimers, C-reactive protein, high-density lipoproteins, low-density lipoproteins, triglycerides, glucose, and drug use explained 16%, 21%, 1.9% and 4.7% of platelet count, plateletcrit, mean platelet volume and platelet distribution width variability, respectively; variables that appeared to be most strongly associated were white blood cell count, age, and sex. Platelet count, mean platelet volume and plateletcrit were positively associated with white blood cell count, while platelet distribution width was negatively associated with white blood cell count. Platelet count and plateletcrit were also positively associated with C-reactive protein and D-dimers ($P<0.0001$).
Each of the other variables, although associated with platelet indices in a statistically significant manner, only explained less than 0.5% of their variability.
Platelet indices varied across Molise villages, independently of any other platelet count determinant or characteristics of the villages.

Conclusions
The association of platelet indices with white blood cell count, C-reactive protein and D-dimers in a general population underline the relation between platelets and inflammation.

Key words: platelet, inflammation, C-reactive protein, white blood cells, age, gender.

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The online version of this article has a Supplementary Appendix.

Introduction

Platelets are essential for primary hemostasis and endothelial repair, but also play a key role in atherogenesis and thrombus formation.[1] Epidemiological studies suggest that platelet indices are related to the risk of cardiovascular disease. Platelet count has been associated with vascular and non-vascular death[2,3] and a recent meta-analysis showed that mean platelet volume is a predictor of cardiovascular risk.[4] However, our understanding of the regulation of platelet indices at a population level is limited.

Genetics may contribute to a certain variation in platelet count and mean platelet volume. Various studies found that inherited components explain a large part of the variability of such indices in normal people.[5-8] A recent meta-analysis identified 15 loci associated with variation in platelet count and mean platelet volume, but these explained only a minor part of trait variance.[9]

The understanding of the non-genetic regulation of platelet indices is even more limited. A few studies have identified only age, sex and ethnicity as variables influencing platelet count.[10-13] Bain first showed that platelet count varied according to age, gender and ethnicity,[11] findings which were subsequently confirmed by Segal.[12] More recently, Biino *et al.*[13] showed, in a Sardinia geographic isolate population including subjects with a large age range, that platelet count progressively decreased during aging, with a consequent increase of cases with thrombocytopenia and a decrease of cases with thrombocytosis in the elderly.

We took advantage of a large adult population-based survey we performed in the Molise region, Italy[14,15] to study the association of each of the four major platelet indices – namely, platelet count, plateletcrit, mean platelet volume, and platelet distribution width – with the other three indices and with a number of biochemical, environmental and clinical variables. Moreover, since Biino *et al.*[13] found large differences in platelet count levels across Sardinia villages, we also studied the levels of platelet indices across Molise villages.

Design and Methods

Population

The cohort of the Moli-Sani Project was recruited in the Molise region from city hall registries by a multistage sampling. First, townships were sampled in major areas by cluster sampling; then, within each township, participants aged 35 years or over were selected by simple random sampling. Exclusion criteria were pregnancy at the time of recruitment, disturbances in understanding or willingness, current multiple trauma or coma, or refusal to sign the informed consent. Thirty percent of subjects refused to participate; based on a short questionnaire on risk factors and major diseases administered by telephone, those who refused were older and had a higher prevalence of cardiovascular disease and cancer.

Subjects (n=24,318) from 30 Molise cities and villages of different sizes attended either of the two recruiting centers: the Catholic University in Campobasso (n=19,211; 79%) and San Timoteo Hospital in Termoli (n=5,107; 21%). The recruitment strategies were carefully defined and standardized across the two centers. The Moli-sani study was approved by the Catholic University ethical committee. All participants enrolled provided written informed consent.

Structured questionnaires were administered by research staff, carefully trained to collect personal and clinical information, including socio-economic status, physical activity, medical history, drug use and dietary habits, risk factors for cardiovascular disease and other diseases, including cancer, liver disorders, hematologic and thrombotic events and family/personal history of cardiovascular disease and/or cancer.

The Italian European Prospective Investigation into Cancer and Nutrition (EPIC) Food Frequency questionnaire was used to determine daily nutritional intakes consumed in the past year.[16]

After the exclusion of subjects with incomplete questionnaires (n=188, 1%), or missing platelet count, plateletcrit, mean platelet volume and platelet distribution width values (n=926, 5%), and all subjects recruited in the Termoli center, because a different cell counter was used (n=5,107), 18,097 subjects were finally analyzed.

Anthropometric and blood pressure measurements

The anthropometric and blood pressure measurements are described in the *Online Supplementary Design and Methods*.

Definition of risk factors

Hypertension, diabetes and dyslipidemia were defined as present when an individual self-reported a health professional's diagnosis of one of these conditions and was using anti-hypertensive, anti-diabetics or lipid-lowering medication. Subjects were classified as non-smokers if they had smoked less than 100 cigarettes in their lifetime or they had never smoked cigarettes, ex-smokers if they had smoked cigarettes in the past and had stopped smoking for at least 1 year, and current smokers if they reported having smoked at least 100 cigarettes in their lifetime and still smoked or had quit smoking within the preceding year.[17] Socio-economic status was defined by a score based on eight variables [(income, education, employment, housing, ratio between the number of live-in partners and the number of rooms (both current and in childhood) and availability of hot water at home during childhood)]; the higher the score, the higher the socio-economic level.[15] Physical activity was assessed by a structured questionnaire and expressed as daily energy expenditure in metabolic equivalent task-hours (MET-h).[18] Metabolic syndrome was defined using the ATP III criteria.[19]

Biochemical measurements

Blood samples were obtained between 07:00 and 09:00 from participants who had fasted overnight and had refrained from smoking for at least 6 h. Biochemical analyses were performed in the centralized Moli-sani laboratory. All hemochromocytometric analyses were performed using the same cell counter (Coulter HMX, Beckman Coulter, IL, Milan, Italy) within 1 hour of venipuncture. Coefficients of variation (CV) were 4.0 %, for platelet count, 5.0% for plateletcrit (calculated by multiplying platelet count by mean platelet volume), 1.4% for mean platelet volume and 2.0% for platelet distribution width. Thrombocytopenia was defined as a platelet count less than 150×10^9/L; thrombocytosis as a platelet count higher than 400×10^9/L; anemia as hemoglobin levels lower than 14.0 g/dL in men and 12.3 g/dL in women; and leukopenia as a white blood cell count lower than 4×10^9/L.

Serum lipids and glucose were assayed by enzymatic reaction methods using an automatic analyzer (ILab 350, Instrumentation Laboratory, Milan, Italy). The concentration of low-density lipoprotein (LDL) cholesterol was calculated using Friedewald's formula. High sensitivity C-reactive protein was measured in fresh serum, by a latex particle-enhanced immunoturbidimetric assay (IL Coagulation Systems on ACL9000). Inter- and intra-day CV were 5.5% and 4.2%, respectively. D-dimer concentrations were measured in fresh citrate plasma, utilizing HemosIL, an automated latex immunoassay on an IL coagulation System ACL9000. Inter and intra-day CV were 5.4% and 7.6%, respectively.

Statistical analysis

All continuous variables were tested for normality using Shapiro's test and are reported as means± standard deviation (SD) or standard error of the mean (SEM). Categorical variables are reported as frequencies and percentages. Correlations among platelet parameters were calculated using Pearson's approach. Differences in platelet parameter distribution among potential determinants were investigated by a series of linear regression analyses. Factors considered for association with platelet indices were age, sex, smoking, physical activity, total cholesterol, high-density lipoprotein (HDL) cholesterol, LDL cholesterol, triglycerides, glucose, systolic and diastolic blood pressure, red blood cells, white blood cells, mean corpuscular volume, C-reactive protein, D-dimers, cancer, hepatitis B/C, hematologic diseases, coronary heart disease (angina, myocardial infarction, revascularisation procedures), cardiovascular disease (coronary heart disease, stroke, transient ischemic attacks, peripheral arterial disease), diabetes, dyslipidemia, hypertension, metabolic syndrome, use of oral contraception, hormone replacement therapy, non-steroidal anti-inflammatory drugs, anti-platelets drugs, ticlopidine, clopidogrel or aspirin, predicted risk of cardiovascular disease, body mass index, menopause, alcohol consumption, total calorie consumption and dietary pattern. Continuous determinants were categorized in quintiles, separately for men and women. For each platelet parameter, a specific multivariable model was built, including in the full model the parameters associated with the dependent variable with a *P* value less than 0.10 in a first step analysis adjusted for age. Principal component analysis, conducted on the correlation matrix of 45 food groups derived from the EPIC questionnaire, was used to identify dietary patterns and to further reduce food groups. Two-sided 95% confidence intervals (95% CI) and *P* values were calculated. *P* values less than 0.05 were considered statistically significant. The data were analyzed using SAS/STAT software, version 9.1.3 of the SAS System for Windows©2009 (SAS Institute Inc., Cary, NC, USA).

Results

The major characteristics of the study population are shown in *Online Supplementary Table S1*. Among 18,097 subjects (aged more than 35 years), 52% were women. The crude prevalences of anemia (P=0.12), cancer (P<0.0001), leukopenia (P<0.0001) and hematologic diseases (P=0.002) were higher in women than in men, while hepatitis (B or C) (P=0.001), coronary heart disease (P<0.0001) and cardiovascular disease (P<0.0001) were more frequent in men. The prevalence of thrombocytopenia (P<0.0001) was 4.7% in men and 2.2% in women, whereas the prevalence of thrombocytosis (P<0.0001) was 1.5% in men and 2.8% in women (*Online Supplementary Table S1*).

Distribution of platelet indices and mutual correlations

Online Supplementary Figure S1 shows the distribution of platelet parameters in men and women. All four parameters considered were normally distributed and followed a Gaussian trend. The distribution in men and women was comparable.

Platelet count and plateletcrit were strongly positively correlated (r=0.90, P<0.0001), platelet count instead was inversely correlated with both mean platelet volume (r=-0.36, P<0.0001) and platelet distribution width (r=-0.24, P<0.0001), while plateletcrit was inversely correlated with platelet distribution width (r=-0.22, P<0.0001). No correlation was apparent between mean platelet volume and either platelet distribution width (r=0.095) or plateletcrit (r=0.048).

Platelet indices and environmental, biochemical or clinical variables

Numerous variables were associated with platelet indices in the simple linear regression analysis (*data not shown*). We only report here the variables that remained associated with platelet indices in the analyses adjusted for age (Tables 1-3, *Online Supplementary Table S2*). The full model explained 16%, 21%, 1.9% and 4.7% of the variability of platelet count, plateletcrit, mean platelet volume and platelet distribution width, in the whole sample. The determinants that explained most of the variability of all four platelet indices were white blood cell count, age and sex (except for platelet distribution width).

Platelet indices and white blood cell count

Platelet count, plateletcrit and platelet distribution width were associated with white blood cell count, which explained 6.1%, 9.1% and 0.78% of their variability, respectively. Platelet count, mean platelet volume and plateletcrit were positively, and platelet distribution width was negatively associated with white blood cell count (Figure 1A-D, Tables 1-3, *Online Supplementary Table S2*). Mean platelet volume was also directly associated with white blood cell count, which explained 0.26% of the 1.9% of its variability (Table 2). Platelet count and plateletcrit were significantly associated with C-reactive protein and D-dimer levels in a way similar to that with the white blood cell count (Figure 1A-C, Table 1, *Online Supplementary Table S2*).

Platelet indices: association with gender and age

Tables 1-3 and *Online Supplementary Table S2* report the four platelet indices by gender: women had significantly higher platelet counts, plateletcrit and mean platelet volume than men, 261±64 *versus* 235±59×10^9/L (P≤0.0001), 0.22±0.05 *versus* 0.20±0.04 % (P≤0.0001) and 8.67±0.94 *versus* 8.50 ±0.92 fL (P≤0.0001), respectively, while platelet distribution width was slightly higher in men (16.3±0.57 *versus* 16.4±0.58 fL (P≤0.0001), respectively.

The sex difference was present in all age ranges (Figure 2A-D). Figure 2A shows a progressive decline of platelet number during aging in both men and women and *Online Supplementary Figure S2* illustrates the relationship of thrombocytopenia and thrombocytosis frequency with age: the former increased while the latter decreased with age. On average, a 10-year increase in age corresponds to a sex-adjusted decrease of 10×10^9/L in the platelet count. Like the platelet count, plateletcrit decreased with age in both men and women (Figure 2C). While it was not possible to identify a clear relation between mean platelet volume and age in women, in men it increased with age until 79 years and then decreased (Figure 2B). Finally platelet distribution width increased with age in both men and women (Figure 2D).

Platelet indices and other variables

Variables such as body mass index, HDL cholesterol, LDL cholesterol, glucose, triglycerides, smoking habit, systolic or diastolic blood pressure and antiplatelet drug use, although associated in a statistically significant manner, each explained less than 0.5% of the variability in platelet indices (Tables 1-3, *Online Supplementary Table S2*).

Table 1. Variables associated with platelet count in the Moli-sani population.

Platelet count (x10^9/L) Variables	Quintiles	N.	Mean	SEM	Age-adjusted *P* value	Multivariable *P* value	Partial R^2 (%)
White blood cells (×10^9/L)	(1.4-4.9)	3807	229	1.00	<0.0001	<0.0001	6.1
	(5.0-5.6)	3552	242	1.00			
	(5.7-6.3)	3499	249	1.00			
	(6.4-7.3)	3596	256	1.00			
	(7.4-18)	3632	269	1.00			
Age (years)	(35-44)	3774	258	1.00	<0.0001	<0.0001	1.5
	(44-51)	3710	259	1.00			
	(51-58)	3608	249	1.00			
	(58-67)	3401	242	1.00			
	(67-99)	3604	234	1.00			
Sex	Women	9401	261	1.00	<0.0001	<0.0001	1.0
	Men	8696	235	1.00			
Mean corpuscular volume (fL)	(57-85)	3377	262	2.00	<0.0001	<0.0001	0.88
	(85-88)	3623	249	1.00			
	(88-90)	3614	247	1.00			
	(90-92)	3722	246	1.00			
	(92-124)	3760	241	1.00			
D-dimer (ng/dL)	(18-129)	2395	236	1.00	<0.0001	<0.0001	0.56
	(130-165)	3363	245	1.00			
	(166-194)	3658	249	1.00			
	(195-239)	3855	256	1.00			
	(240-9588)	3377	257	1.00			
HDL-cholesterol (mg/dL)	(14-44)	3039	237	1.00	<0.0001	<0.0001	0.46
	(45-52)	3753	243	1.00			
	(53-59)	3504	249	1.00			
	(60-69)	3891	253	1.00			
	(70-135)	3836	258	1.00			
Red blood cells (×10^{12}/L)	(2.67-4.51)	3759	255	1.00	<0.0001	<0.0001	0.41
	(4.52-4.77)	3738	254	1.00			
	(4.78-5.00)	3529	249	1.00			
	(5.01-5.28)	3598	246	1.00			
	(5.29-7.75)	3473	239	1.00			
C-reactive protein (mg/L)	(0.01-0.62)	3401	240	1.00	<0.0001	<0.0001	0.24
	(0.63-1.11)	3548	242	1.00			
	(1.12-1.81)	3517	247	1.00			
	(1.82-3.15)	3436	251	1.00			
	(3.16-10.0)	3445	260	1.00			
Body mass index (Kg/m^2)	(16-24)	3662	251	1.00	0.0609	<0.0001	0.17
	(24-26)	3610	248	1.00			
	(26-29)	3606	247	1.00			
	(29-31)	3628	247	1.00			
	(31-59)	3579	250	1.00			
Blood glucose (mg/dL)	(12-86)	2723	252	1.20	<0.0001	<0.0001	0.14
	(87-93)	3638	253	1.00			
	(94-100)	3966	249	1.00			
	(101-110)	3880	247	1.00			
	(111-443)	3818	243	1.00			
LDL-cholesterol (mg/dL)	(29-101)	3140	237	1.00	<0.0001	<0.0001	0.10
	(101-120)	3381	245	1.00			
	(120-137)	3571	250	1.00			
	(137-158)	3691	253	1.00			
	(158-321)	3970	257	1.00			
Aspirin (%)	No	17965	249	0.46	0.0107	0.0007	0.07
	Yes	19	285	14.3			
Triglycerides (mg/dL)	(21-72)	3435	246	1.00	0.0019	0.0020	0.06
	(73-95)	3492	249	1.00			
	(96-123)	3641	250	1.00			
	(124-169)	3734	251	1.00			
	(170-950)	3722	247	1.00			

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Ticlopidine (%)	No	17812	249	0.46	0.0233	0.016	0.04
	Yes	172	260	4.80			
Smokers (%)	No smoke	9073	252	1.00	<0.0001	0.0547	0.02
	Smoke	4152	247	1.00			
	Ex smoke	4852	244	1.00			
Menopause (%)	No	12719	243	1.00	<0.0001	0.0665	0.02
	Yes	5370	262	1.00			

Table 2. Variables associated with mean platelet volume in the Moli-sani population.

Mean platelet count (x10^9/L) Variables	Quintiles	N.	Mean	SEM	Age-adjusted *P* value	Multivariable *P* value	Partial R^2 (%)
Sex	Women	9401	8.67	0.0096	<0.0001	<0.0001	0.8
	Men	8696	8.50	0.0100			
Red blood cells (x10^{13}/µL)	(2.67-4.51)	3759	8.58	0.0152	0.0008	<0.0001	0.28
	(4.52-4.77)	3738	8.59	0.0153			
	(4.78-5.00)	3529	8.59	0.0157			
	(5.01-5.28)	3598	8.56	0.0155			
	(5.29-7.75)	3473	8.65	0.0159			
White blood cells (×10^9/L)	(1.4-4.9)	3807	8.55	0.0151	<0.0001	<0.0001	0.26
	(5.0-5.6)	3552	8.57	0.0157			
	(5.7-6.3)	3499	8.58	0.0158			
	(6.4-7.3)	3596	8.60	0.0156			
	(7.4-18)	3632	8.65	0.0155			
Age (years)	(35-44)	3774	8.58	0.0152	0.0234	<0.0001	0.23
	(44-51)	3710	8.56	0.0153			
	(51-58)	3608	8.59	0.0155			
	(58-67)	3401	8.60	0.0160			
	(67-99)	3604	8.63	0.0155			
Menopause (%)	No	12719	8.56	0.0085	<0.0001	<0.0001	0.09
	Yes	5370	8.66	0.0136			
Triglicerydes (mg/dL)	(21-72)	3435	8.64	0.0161	<0.0001	0.0002	0.08
	(73-95)	3492	8.61	0.0158			
	(96-123)	3641	8.60	0.0155			
	(124-169)	3734	8.60	0.0153			
	(170-950)	3722	8.55	0.0153			
Body mass index (Kg/m^2)	(16-24)	3662	8.62	0.0156	<0.0001	0.0020	0.06
	(24-26)	3610	8.56	0.0155			
	(26-29)	3606	8.56	0.0155			
	(29-31)	3628	8.56	0.0155			
	(31-59)	3579	8.66	0.0157			
Diastolic blood pressure (mm/Hg)	(46-74)	3627	8.65	0.0155	0.0004	0.0016	0.05
	(75-79)	3419	8.60	0.0160			
	(80-84)	3700	8.58	0.0153			
	(85-90)	3744	8.56	0.0152			
	(91-130)	3598	8.57	0.0156			
Ticlopidine (%)	No	17812	8.59	0.0070	0.0032	0.0200	0.03
	Yes	172	8.38	0.0718			

Platelet indices across Molise villages

Average platelet indices were significantly different across Molise villages (*Online Supplementary Figure S3*). Platelet count ranged from 228×10^9/L to 269×10^9/L, plateletcrit from 0.19 fL to 0.23 fL, mean platelet volume from 8.0 fL to 8.9 fL and platelet distribution width from 16.3% to 16.5%. In particular, average platelet count differed by more than 50×10^9/L between Gildone and Sant'Angelo Limosano. Correspondingly, the prevalence of thrombocytopenia differed from 0.7% to 6.8% between these two villages. This variation could not be explained by differences in either other platelet count determinants, or liver disorders or cancer, the altitude of the villages above sea level or their distance from the recruitment center (*data not shown*).

Within villages, increasing average platelet volume was associated with a decrease in platelet count, whereas an increase in platelet count was associated with an increase in plateletcrit. Villages whose inhabitants had the highest platelet counts also showed the highest levels of plateletcrit but the lowest levels of mean platelet volume.

Adjustment for age, sex, other platelet count determinants, liver disorders, cancer, altitude of the villages above sea level or their distance from the recruitment center did not modify what whas observed across Molise villages (*data not shown*).

Discussion

Platelet indices are receiving increasing attention as potential markers of platelet activation, since they are easily measurable in the context of epidemiological studies given the widespread availability of reliable automated blood cell counters. These counters provide platelet count as part of the full blood count, in addition to derived indices related to platelet size, such as plateletcrit (total volume of platelets in a given volume of blood), mean platelet volume (plateletcrit divided by total platelet number) and platelet distribution width, which directly measures the variability in platelet size.

In a large population sample recruited at random from a general adult population we studied the relation of platelet indices with each other and with a number of non-genetic variables. We found a significant inverse correlation between platelet count and mean platelet volume, in agree-

Table 3. Variables associated with platelet distribution width in the MOLI-SANI population.

Platelet distribution width (fL) Variables	Quintiles	N.	Mean	SEM	Age-adjusted *P* value	Multivariable *P* value	Partial R^2 (%)
White blood cells ($x10^9$/L)	(1.4-4.9)	3807	16.41	0.0094	<0.0001	<0.0001	0.78
	(5.0-5.6)	3552	16.38	0.0097			
	(5.7-6.3)	3499	16.38	0.0098			
	(6.4-7.3)	3596	16.37	0.0097			
	(7.4-18)	3632	16.33	0.0096			
Age (years)	(35-44)	3774	16.34	0.0094	<0.0001	<0.0001	0.53
	(44-51)	3710	16.33	0.0095			
	(51-58)	3608	16.37	0.0097			
	(58-67)	3401	16.38	0.0099			
	(67-99)	3604	16.44	0.0097			
Mean corpuscular volume (fL)	(57-85)	3377	16.47	0.0100	<0.0001	<0.0001	0.41
	(85-88)	3623	16.38	0.0096			
	(88-90)	3614	16.36	0.0096			
	(90-92)	3722	16.33	0.0094			
	(92-124)	3760	16.34	0.0095			
LDL-cholesterol (mg/dL)	(29-101)	3140	16.43	0.0103	<0.0001	<0.0001	0.3
	(101-120)	3381	16.40	0.0099			
	(120-137)	3571	16.36	0.0097			
	(137-158)	3691	16.35	0.0095			
	(158-321)	3970	16.33	0.0092			
Red blood cells ($x10^{12}$/L)	(2.67-4.51)	3759	16.29	0.0094	<0.0001	<0.0001	0.24
	(4.52-4.77)	3738	16.32	0.0094			
	(4.78-5.00)	3529	16.35	0.0097			
	(5.01-5.28)	3598	16.42	0.0096			
	(5.29-7.75)	3473	16.51	0.0098			
HDL-cholesterol (mg/dL)	(14-44)	3039	16.49	0.0104	<0.0001	<0.0001	0.20
	(45-52)	3753	16.41	0.0094			
	(53-59)	3504	16.38	0.0097			
	(60-69)	3891	16.35	0.0092			
	(70-135)	3836	16.27	0.0093			
Sex	Women	9401	16.30	0.0059	<0.0001	<0.0001	0.17
	Men	8696	16.46	0.0062			
Triglycerides (mg/dL)	(21-72)	3435	16.33	0.0099	<0.0001	<0.0001	0.1
	(73-95)	3492	16.34	0.0098			
	(96-123)	3641	16.36	0.0096			
	(124-169)	3734	16.38	0.0095			
	(170-950)	3722	16.46	0.0095			
Blood glucose (mg/dL)	(12-86)	2723	16.33	0.0111	<0.0001	<0.0001	0.09
	(87-93)	3638	16.33	0.0096			
	(94-100)	3966	16.37	0.0092			
	(101-110)	3880	16.37	0.0093			
	(111-443)	3818	16.45	0.0095			
Menopause (%)	No	12719	16.42	0.0053	<0.0001	<0.0001	0.08
	Yes	5370	16.27	0.0084			
Body mass index (Kg/m^2)	(16-24)	3662	16.35	0.0097	0.0066	0.0070	0.04
	(24-26)	3610	16.37	0.0097			
	(26-29)	3606	16.38	0.0097			
	(29-31)	3628	16.40	0.0096			
	(31-59)	3579	16.37	0.0097			

ment with other studies,[20-22] or platelet distribution width. This finding could be explained by the possibility that, in order to maintain constant platelet functional mass,[23] platelet count would be decreased in the presence of bigger platelets. This could be the case of increased production by the bone marrow of young (reticulated) platelets that are larger than older (exhausted) platelets.[24-25]

Platelets, hematologic cells and inflammation

By analyzing a large number of variables, we observed, apparently for the first time, that both platelet count and plateletcrit were strongly associated with white blood cell count, explaining 6.2 out of 16% and 9.0 out of 21% of their total variability, respectively. Although our model explained a smaller part of mean platelet volume and platelet distribution width heterogeneity, white blood cell count was again a major associated variable.

These findings support the hypothesis of a common regulation of blood cells, as recently suggested by the presence of a common genetic component.[9] The link between platelets and inflammation, suggested by previous studies[26-28] is also supported by our present findings. Indeed platelet indices are not only associated with white blood cell count, but are also significantly and independently related to a soluble inflammatory marker such as C-reactive protein.

Platelets are considered as inflammatory cells since their

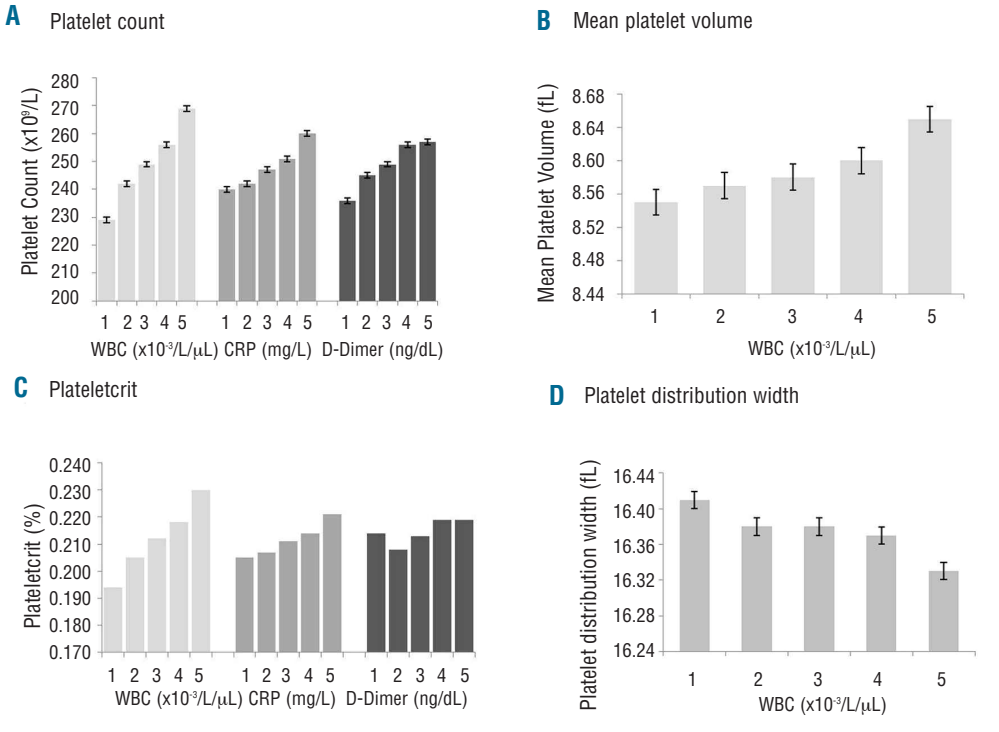

Figure 1 A-D. Platelet parameters by white blood cell count, C-reactive protein and D-dimer levels. (A) Platelet count. (B) Mean platelet volume. (C) Plateletcrit. (D) Platelet distribution width.

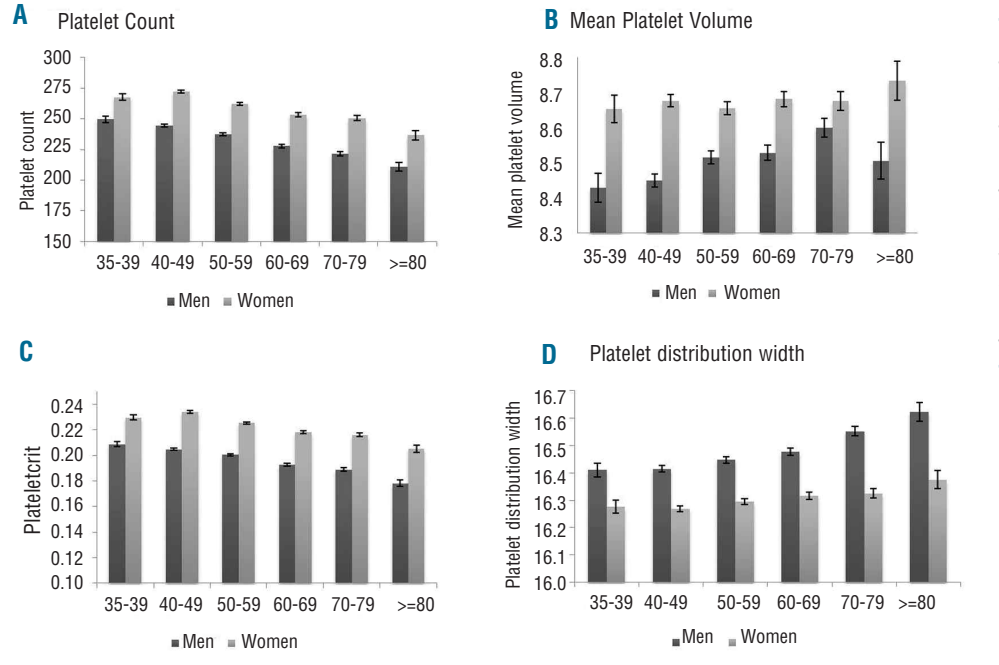

Figure 2 A-D. Platelet parameters by age and sex. (A) Platelet count. (B) Mean platelet volume. (C) Plateletcrit. (D) Platelet distribution width. The difference between men and women holds in any age range. The *P* values for the differences according to age were *P*<0.0001 for all platelet parameters in men, whereas in women they were *P*<0.0001 for platelet count and plateletcrit, *P*=0.77 for mean platelet volume and *P*=0.0024 for platelet distribution width.

count increases in many inflammatory states and platelet count has been associated with the level of other acute-phase proteins.[27] During inflammation higher thrombopoietin levels have also been observed,[28] which could, at least in part, represent the link to the increase in platelet production. In turn, platelets are a source of inflammatory mediators and can be activated by several inflammatory triggers during the process of atherothrombosis.[26,29] The positive significant association between platelet count and plateletcrit with D-dimer levels further underlies the link between inflammation, platelets and activation of blood coagulation.[30]

Sex and age

Women had significantly higher platelet indices than men (except for platelet distribution width, which was slightly higher in men), suggesting a hormonal influence in their regulation. The process by which megakaryocytes proceed to proplatelet formation and platelet production is reportedly under the influence of autocrine estrogen.[31] Additionally, estrogen-receptor antagonists inhibit platelet production *in vivo*, supporting a role of estrogens in platelet production.[12] In agreement with a previous study on platelet count,[11] we found that the differences between men and women persisted for all platelet indices at any age range, as well as after the menopause. Contraceptives and hormone therapy, used by only a relatively small proportion of women in our sample, did not significantly influence any platelet parameter.

Although the higher platelet count in women might be the result of greater inflammation, even in the setting of a lower white cell count, differences between men and women were not attenuated after adjustment for inflammatory variables.

All platelet indices varied with age. In particular platelet count and plateletcrit decreased with age in both men and women. A similar trend was observed for the prevalence of thrombocytosis, which progressively decreased with age, and the prevalence of thrombocytopenia, which, in contrast, increased, up to the level of 9% in subjects over 80 years old. In a recent publication, Biino *et al.*[13] provided for the first time an estimate of the prevalences of thrombocytosis and thrombocytopenia in the general population of the Ogliastra region, in Sardinia, clearly showing that the prevalence of mild thrombocytopenia was higher in older people. Although differences in platelet count with age and sex have been previously reported,[10-13] normal laboratory ranges of platelet count are not usually distinguished for sex and age. This might possibly lead to some overestimation of platelet count defects in the elderly. The decline in platelet counts we observed in older age is difficult to interpret because of the cross-sectional nature of our data: it may reflect a reduction in hematopoietic stem-cell reserve during aging or a survival advantage in those subjects with lower platelet counts.

Platelet distribution width increased with age in both men and women, while the relation between mean platelet volume and age differed by sex: it increased with age until 79 years and then decreased in women over 80 years, while it remained constant over age in men. Previous studies yielded contrasting results on the association between mean platelet volume and age, but a sex-specific analysis was not performed in any of them.[32-34]

Other variables

Many other biological and clinical variables were associated with platelet indices, such as body mass index, HDL cholesterol, LDL cholesterol, triglycerides, glucose, diastolic blood pressure and antiplatelet drug use; however, although statistically significant, each explained less than 0.5% of the variability of platelet indices. As the association of these variables was mainly directed towards a risk profile for cardiovascular disease, such an association might become stronger in pathological conditions, such as diabetes, obesity and hypertension.[35-37]

Environmental variables, including dietary habits, did not show any relevant association with platelet indices.

Molise villages

Since a recent Italian study[13] found significant variations in both platelet number and prevalence of thrombocytopenia among some Sardinian villages, we investigated the distribution of platelet indices across our villages, finding that they were significantly different across the villages. This variation could not be explained by differences in other platelet count determinants, in liver disorders or cancer distribution or in the altitude of the villages above the sea or in their distance from the recruitment center.

That platelet count might differ by ethnicity has already been reported;[10-12,38] our findings, together with Biino's data,[13] from a large Caucasian population, indicate that there is a micro-heterogeneity in platelet parameters even among apparently ethnically homogeneous subjects living in the same country and even in the same region.

Conclusions

As our data show that a large number of non-genetic variables explain a small proportion of the heterogeneity in platelet indices, it appears that genetic factors might play the major role. That both platelet count and platelet volume are highly inherited traits is already known.[5-8] We have also recently found in large pedigrees derived from the same population studied here that the genetic component explained 69.0%, 52.1% and 34.0% of mean platelet volume, platelet count and platelet distribution width, respectively, with a small contribution being made by shared household (<10%), while the environmental component was minimal (*data not shown*).[8]

A recent meta-analysis[9] identified 12 loci linked to mean platelet volume or platelet count. These single nucleotide polymorphisms did, however, only explain 8.6% of mean platelet volume variance upon 75% of heritability, suggesting that other genetic or epigenetic factors could be involved. The availability of DNA samples stored in the large Moli-sani biological research bank will help better understanding of the genetic regulation of platelets in the near future.

Authorship and Disclosures

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