

## 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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*Citation: Santimone I, Di Castelnuovo A, De Curtis A, Spinelli M, Cugino D, Gianfagna F, Zito F, Donati MB, Cerletti C, de Gaetano G and Iacoviello L on behalf of the Moli-sani Project Investigators. 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. Haematologica 2011;96(8):1180-1188. doi:10.3324/haematol.2011.043042*

### Online supplementary

#### Design and Methods

#### Anthropometric and blood pressure (bp) measurements

Body weight and height were measured on a standard beam balance scale with an attached ruler with subjects wearing no

shoes and only light indoor clothing. Body mass index (BMI) was calculated as kg/m<sup>2</sup>. Waist circumferences were measured according to the NIH, Heart, Lung, and Blood guidelines.<sup>1</sup>

Blood pressure was measured by an automatic device (OMRON-HEM-705CP) 3 times on the non-dominant arm and the average of the last 2 values was taken as the blood pressure.<sup>2</sup> Measurements were made in a quiet room with comfortable temperature with participants lying down for at least 5 min.

### References

1. Expert Panel on Detection Evaluation and Treatment of High Blood Cholesterol In Adults: Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). JAMA 2001;285(19):2486-97.
2. O'Brien E, Weber B, Parati G, Staessen J, Myers MG. Blood pressure measuring devices: recommendations of the European Society of Hypertension. BMJ 2001;322(7255):531-6.

**Online Supplementary Table S1. Characteristics of the study population by sex.**

All	Men	Women	P value
18,097	(No 8,696; 48%)	(No 9,401; 52%)	
Age (years)	55±12	56 ±12	<0.0001
Body mass index (Kg/m <sup>2</sup> )	28.2±4.0	27.8±5.3	<0.0001
Waist to hip-ratio	0.95±0.06	0.90±0.08	<0.0001
Total cholesterol (mg/dL)	214±41.6	221±41.6	<0.0001
HDL cholesterol (mg/dL)	53 ±13	64 ±14.6	<0.0001
LDL cholesterol (mg/dL)	132±35	134±35.3	<0.0001
Triglycerides (mg/dL)	152±101.4	114±64.8	<0.0001
Blood glucose (mg/dL)	107.5±27	98.3±21.2	<0.0001
Systolic blood pressure (mmHg)	145±19.0	139±21.9	<0.0001
Diastolic blood pressure (mmHg)	84.2±9.5	81±9.6	<0.0001
Red blood cells (x10 <sup>12</sup> /L)	5.12±0.45	4.7±0.39	<0.0001
White blood cells (x10 <sup>9</sup> /μL)	6.5±1.65	5.9±1.53	<0.0001
Mean corpuscular volume (fL)	90±5.5	87±5.8	<0.0001
C-reactive protein (mg/L)	1.99±1.81	2.11±1.95	<0.0001
D-dimers (mg/L)	221±310	229±256	0.07
Physical activity (met/day)	43.5±9.8	42.4±7.6	<0.0001
Smokers (%)	2,270 (26.1)	1,882 (20.0)	<0.0001
Ex smokers (%)	3,589 (41.3)	1,263 (13.4)	<0.0001
Factor1 (dietary pattern)	-0.05±0.01	0.06±0.01	<0.0001
Factor2 (dietary pattern)	0.4±1.00	-0.4±0.7	<0.0001
Factor3 (dietary pattern)	0.09±0.9	-0.08±0.8	<0.0001
Total calorie consumption (Kcal/day)	2,307±725	1,913±590	<0.0001
Alcohol consumption (g ethanol/day)	27.4±28	6.2±10.0	<0.0001
Predicted cardiovascular disease risk	7.6±7.3	2.4 ±3.1	<0.0001
Thrombocytopenia (%)	412 (4.7)	211 (2.2)	<0.0001
Thrombocytosis (%)	100 (1.5)	259 (2.8)	<0.0001
Anemia (%)	803 (9.2)	933 (10.0)	0.12
Leukopenia (%)	213 (2.4)	547 (5.9)	<0.0001
Hepatitis B/C (%)	279 (3.2)	227 (2.4)	0.001
Cancer (%)	199 (2.3)	364 (3.9)	<0.0001
Hematologic disease (%)	185 (2.1)	269 (2.9)	0.002
Coronary heart disease (%)	489 (5.7)	182 (2.0)	<0.0001
Cardiovascular disease (%)	674 (7.8)	339 (3.7)	<0.0001
Metabolic syndrome (%)	2,584 (29.9)	2,496 (26.3)	<0.0001
Diabetes (%)	515 (6.0)	324 (3.5)	<0.0001
Dyslipidemia (%)	706 (9.0)	709 (8.0)	0.08
Hypertension (%)	2,463 (29)	2,702 (29)	0.5
Clopidogrel (%)	33 (0.38)	13 (0.14)	0.001
Ticlopidine (%)	113 (1.31)	59 (0.63)	<0.0001
Aspirin (%)	646 (7.5)	371 (4.0)	<0.0001
Non-steroidal anti-inflammatory drugs (%)	54 (0.62)	173 (1.85)	<0.0001
Antiplatelet therapy (%)	891 (10.3)	529 (5.7)	<0.0001
Menopause (%)	-	5,370 (57.2)	-
Contraceptive pill (%)	-	2,527 (27)	-
Hormone replacement therapy (%)	-	556 (6.0)	-

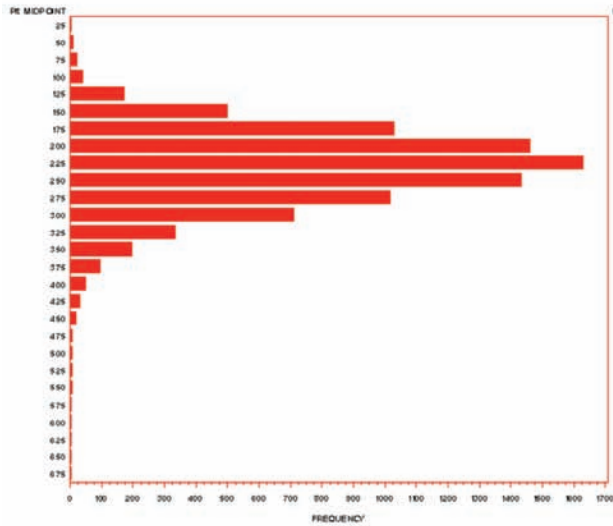
Quantitative variables are presented as mean±SD, categorical variables are presented as n° (%)

**Online Supplementary Table S2. Variables associated with plateletcrit in the Moli-sani population.**

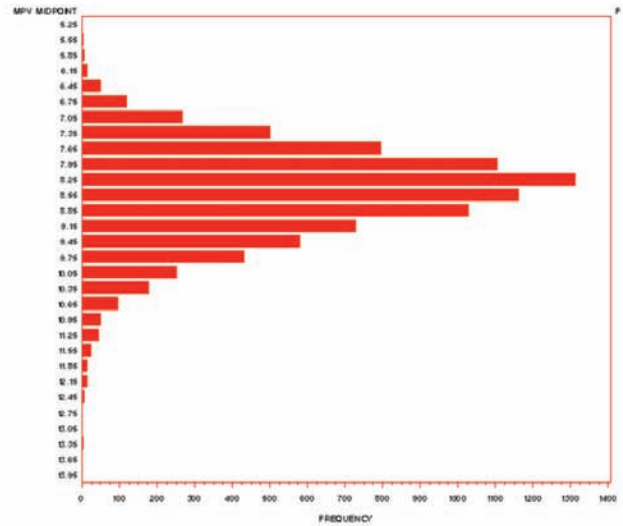
Plateletcrit (%) Variables	Quintiles	N.	Mean	SEM	Age-adjusted P value	Multivariable P value	Partial R <sup>2</sup> (%)
White blood cells (×10 <sup>9</sup> /L)	(1.4-4.9)	3807	0.194	0.00077	<0.0001	<0.0001	9.1
	(5.0-5.6)	3552	0.205	0.00080			
	(5.7-6.3)	3499	0.212	0.00081			
	(6.4-7.3)	3596	0.218	0.00079			
	(7.4-18)	3632	0.230	0.00079			
Sex	Women	9401	0.225	0.00049	<0.0001	<0.0001	3.2
	Men	8696	0.198	0.00051			
Age (years)	(35-44)	3774	0.220	0.00080	<0.0001	<0.0001	2.02
	(44-51)	3710	0.220	0.00080			
	(51-58)	3608	0.212	0.00082			
	(58-67)	3401	0.206	0.00084			
	(67-99)	3604	0.201	0.00082			
Mean corpuscular volume (fL)	(57-85)	3377	0.226	0.00084	<0.0001	<0.0001	1.2
	(85-88)	3623	0.212	0.00081			
	(88-90)	3614	0.211	0.00081			
	(90-92)	3722	0.210	0.00080			
	(92-124)	3760	0.203	0.00080			
D-dimer (ng/dL)	(18-129)	2395	0.214	0.00100	<0.0001	<0.0001	0.76
	(130-165)	3363	0.208	0.00084			
	(166-194)	3658	0.213	0.00081			
	(195-239)	3855	0.219	0.00079			
	(240-9588)	3377	0.219	0.00086			
HDL-cholesterol (mg/dL)	(14-44)	3039	0.201	0.00088	<0.0001	<0.0001	0.52
	(45-52)	3753	0.207	0.00080			
	(53-59)	3504	0.212	0.00082			
	(60-69)	3891	0.216	0.00078			
	(70-135)	3836	0.220	0.00079			
Red blood cells (×10 <sup>12</sup> /L)	(2.67-4.51)	3759	0.216	0.00080	<0.0001	<0.0001	0.20
	(4.52-4.77)	3738	0.217	0.00080			
	(4.78-5.00)	3529	0.212	0.00082			
	(5.01-5.28)	3598	0.209	0.00082			
	(5.29-7.75)	3473	0.205	0.00083			
C-reactive protein (mg/L)	(0.01-0.62)	3401	0.205	0.00084	<0.0001	<0.0001	0.17
	(0.63-1.11)	3548	0.207	0.00081			
	(1.12-1.81)	3517	0.211	0.00082			
	(1.82-3.15)	3436	0.214	0.00083			
	(3.16-10.0)	3445	0.221	0.00083			
Body mass index (Kg/m <sup>2</sup> )	(16-24)	3662	0.214	0.00082	<0.0001	<0.0001	0.13
	(24-26)	3610	0.211	0.00082			
	(26-29)	3606	0.210	0.00082			
	(29-31)	3628	0.210	0.00082			
	(31-59)	3579	0.215	0.00083			
Blood glucose (mg/dL)	(12-86)	2723	0.215	0.00094	<0.0001	<0.0001	0.10
	(87-93)	3638	0.216	0.00082			
	(94-100)	3966	0.212	0.00078			
	(101-110)	3880	0.211	0.00079			
	(111-443)	3818	0.207	0.00081			
LDL-cholesterol (mg/dL)	(29-101)	3140	0.203	0.00087	<0.0001	0.0005	0.08
	(101-120)	3381	0.209	0.00084			
	(120-137)	3571	0.213	0.00082			
	(137-158)	3691	0.215	0.00080			
	(158-321)	3970	0.219	0.00078			
Menopause (%)	No	12719	0.206	0.00044	<0.0001	0.0006	0.07
	Yes	5370	0.225	0.00070			
Smokers (%)	No smoke	9073	0.215	0.00051	<0.0001	0.0079	0.04
	Smoke	4152	0.212	0.00077			
	Ex smoke	4852	0.206	0.00071			

Online Supplementary Figure S1 A-H. Distribution of platelet parameters in men and in women. (A) Platelet count in men. (B) Mean platelet volume in men. (C) Plateletcrit in men. (D) Platelet distribution width in men. (E) Platelet count in women. (F) Mean platelet volume in women. (G) Plateletcrit in women. (H) Platelet distribution width in women. All four parameters were normally distributed in both men and women.

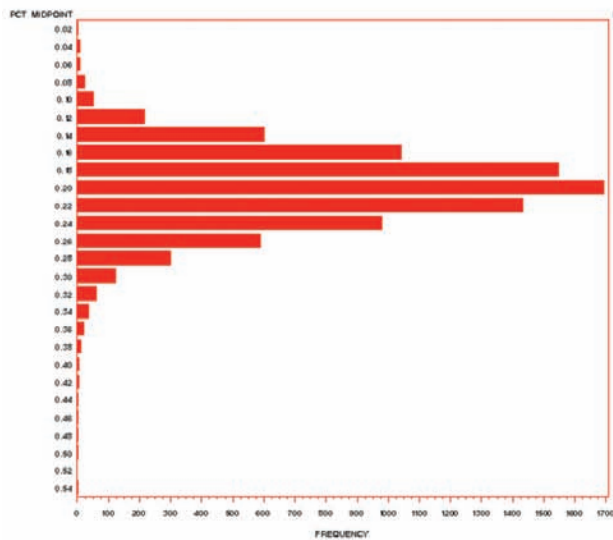
### A. Platelet count



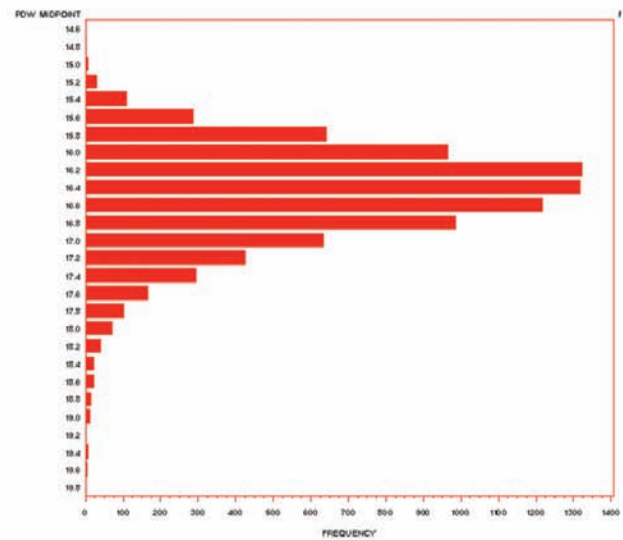
### B. Mean platelet volume



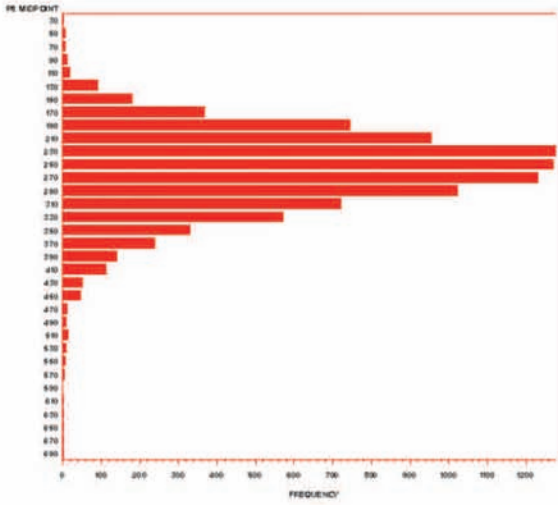
### C. Plateletcrit



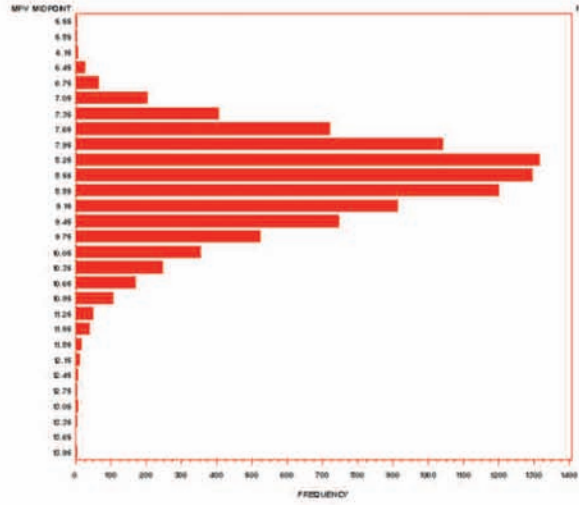
### D. Platelet distribution width



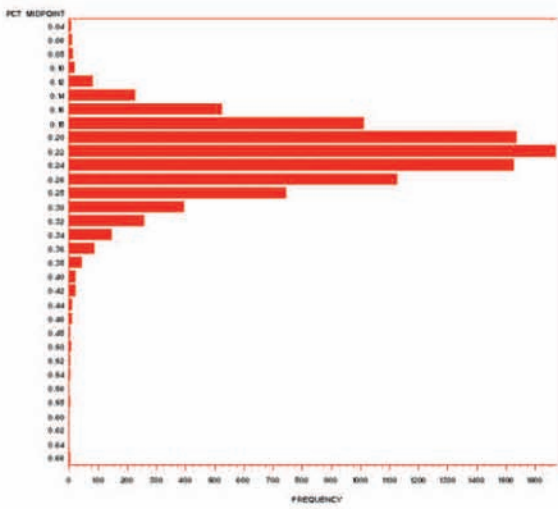
### E. Platelet count



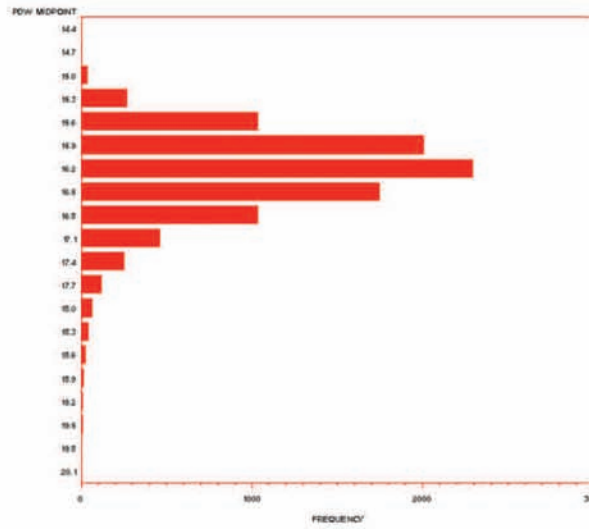
### F. Mean platelet volume



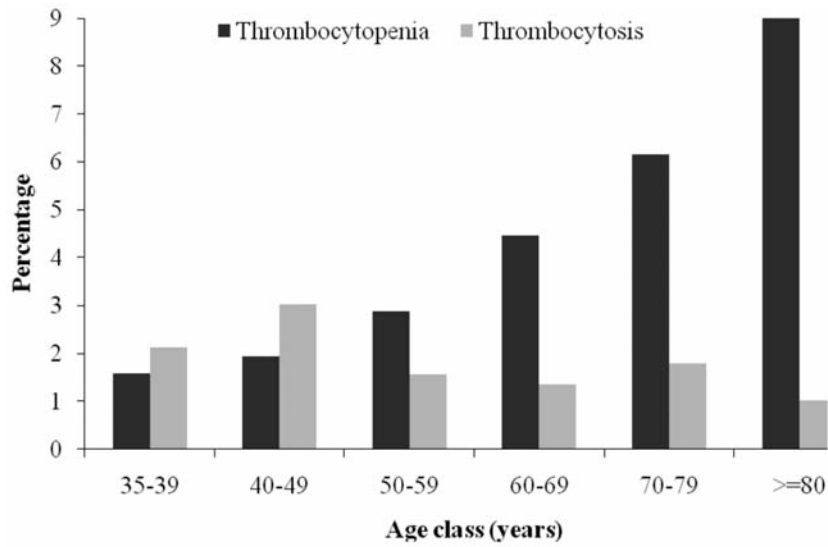
### G. Plateletcrit



### H. Platelet distribution width



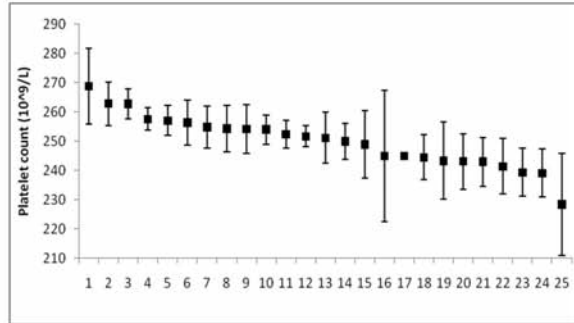
**Online Supplementary Figure S2.** Prevalence of thrombocytopenia and thrombocytosis in different age classes.



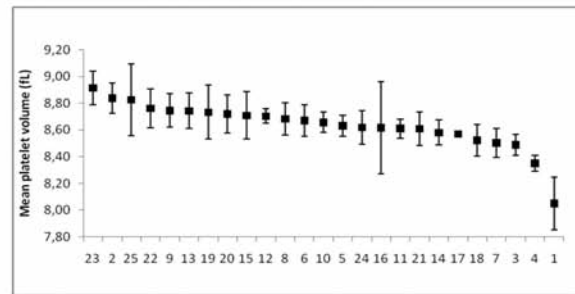
Values correspond to the percentage of cases of thrombocytopenia or thrombocytosis. For further details, see the Design and Methods section.

**Online Supplementary Figure S3 A-D.** Platelet parameters in Molise villages. (A) Platelet count. (B) Mean platelet volume. (C) Plateletcrit. (D) Platelet distribution width.

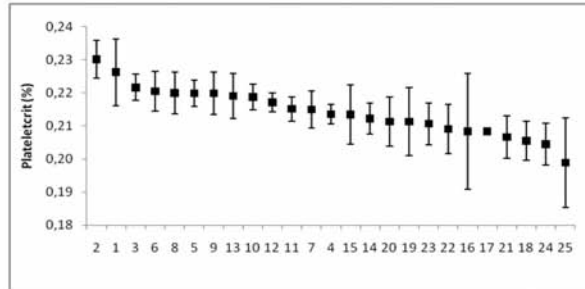
**A Platelet count**



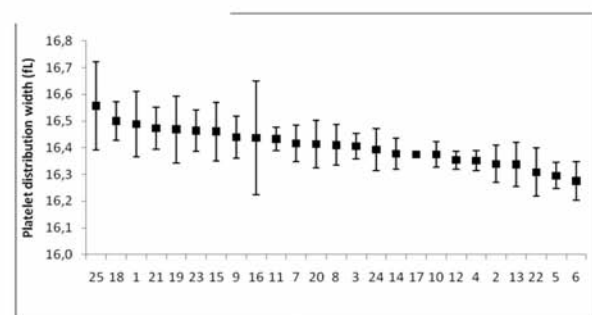
**B Mean platelet volume**



**C Plateletcrit**



**D Platelet distribution width**



Mean values are age and sex adjusted. Vertical bars represent 95% confidence intervals. The P values for difference among villages are  $P < 0.0001$  for all platelet parameters.

Code for the Molise villages: Gildone =1; Mirabello Sannitico =2; Baranello =3; Frosolone =4; Ripalimosani =5; Campodipietra =6; Busso =7; Toro =8; Gambatesa =9; Vinchiatiuro =10; Ferrazzano =11; Bojano =12; Petrella Tifernina =13; Fossalto =14; Riccia =15; Molise =16; Campobasso =17; Matrice =18; San Giovanni in Galdo =19; Oratino =20; Sepino =21; Montagano =22; Spinete =23; Cercemaggiore =24; Sant'Angelo Limosano =25.

(Number of subjects from each Molise village: Gildone=84; Mirabello Sannitico = 258; Baranello= 551; Frosolone = 968; Ripalimosani = 536; Campodipietra = 241; Busso = 278; Toro = 219; Gambatesa = 207; Vinchiatiuro = 565; Ferrazzano = 641; Bojano =1086; Petrella Tifernina = 186; Fossalto = 378; Riccia = 105; Molise = 28; Campobasso = 10439; Matrice = 239; San Giovanni in Galdo = 81; Oratino = 158; Sepino = 205; Montagano = 156; Spinete = 213; Cercemaggiore = 210; Sant'Angelo Limosano = 46).

## Online Supplementary Appendix

### *Moli-sani project Investigators*

**Chairperson:** Licia Iacoviello

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**Call Center:** Giovanna Galuppo (till June 2009), Maura Di Lillo (till March 2009), Concetta Castaldi (till September 2008), Dolores D'Angelo (till May 2008), Rosanna Ramacciato (till May 2008).