Age-sex specific ranges of platelet count and all-cause mortality: prospective findings from
the MOLI-SANI study

Marialaura Bonaccio*, Augusto Di Castelnuovo , Simona Costanzo, Amalia De Curtis, Maria
Benedetta Donati, Chiara Cerletti, Giovanni de Gaetano, Licia Iacoviello, on behalf of the Moli-sani
Investigators**.

Authors’ affiliation

Department of Epidemiology and Prevention, IRCCS Istituto Neurologico Mediterraneo
NEUROMED, Pozzilli (IS), Italy

*Fellow of the Fondazione Veronesi, Milan, Italy

**MOLI-SANI study Investigators are listed in Appendix 1

Correspondence:

Marialaura Bonaccio, PhD
Laboratory of Molecular and Nutritional Epidemiology
Department of Epidemiology and Prevention
IRCCS Istituto Neurologico Mediterraneo NEUROMED
Via dell’Elettronica
86077 Pozzilli (IS), Italy
Mail: marialaura.bonaccio@neuromed.it
Phone: +39 0865929665
Fax: +39 0865927575

Platelet count has been reportedly associated in a non-linear way with a number of health outcomes
among older subjects (1, 2) or in high risk groups (3). One early study has investigated the
involvement of platelet count for cardiovascular mortality in a general middle-aged population of
healthy men (4).

Platelet count decreases during aging, is higher in women than in men (5, 6) and varies in
populations of different origin (6, 7). More recently, it has been reported that platelet count is also
influenced by dietary habits (8).
A great majority of epidemiological studies still relies on the use of single reference intervals regardless of age and sex differences in platelet count (9). Similarly, most laboratories in Western Countries still adopt standard ranges for classification of thrombocytopenia or thrombocytosis (generally 150 to 400 or 450x10⁹ platelets/L for all people).

A recent paper (9) has provided new data to overcome the use of single reference interval by identifying age-sex specific cut-offs derived from a large epidemiological setting including three population-based studies on 40,987 inhabitants of seven Italian areas.

We aimed to assess the predictive values of these newly defined ranges of platelet count (Table 1) for all-cause mortality.

According to Biino et al (9), low platelet count was defined as platelet number <156x10⁹/L or <140x10⁹/L for women aged <64 years and for those aged >64 years respectively; for men, low platelet count was defined as platelet number<141x10⁹/L or <122x10⁹/L for men aged <64 years or >64 years, respectively. Cut-offs for high platelet count were set when platelet count > 405x10⁹/L or > 379x10⁹/L for women aged <64 years or >64 years, respectively; high platelet count was defined as platelet count> 362x10⁹/L or >350x10⁹/L for men aged <64 or >64 years, respectively.

We performed analyses on 24,325 adult individuals aged ≥35 randomly recruited from the general population of the MOLI-SANI study between March 2005 and April 2010 (8,10). Subjects not Caucasian, with hepatitis, hematological diseases, missing values for platelet count, unreliable medical questionnaire, lost to follow-up were not included in the analyses. The final sample consisted of 21,635 subjects.

Blood samples were obtained from participants who had fasted overnight and had refrained from smoking for at least 6 h. All subjects underwent 1 platelet count measurement. All hemocromocytometric analyses were performed by the same instrument (Coulter HMX, Backman Coulter, IL Milan, Italy) within 3 h from blood collection.
The cohort was followed up until May 2015 with the main outcome of interest being all-cause mortality. The latter was assessed by linkage with Offices of vital statistics of the Molise region. Cox proportional hazard ratios (HRs) with 95% confidence intervals (95%CI) were used to assess the association between all-cause mortality and different categories of platelet count with age-sex specific normal values as the reference category. Multivariable model included age, sex, education, cardiovascular disease, history of cancer, diabetes, hypertension, hypercholesterolemia, leisure-time physical activity, body mass index, smoking, leukocyte count, mean platelet volume, C-reactive protein, haematocrit, use of antiplatelet drugs and site of recruitment. During a median follow-up of 7.6 years (interquartile range: 6.7 to 8.6 years; 163,659 person/years), 1,001 deaths were recorded. The incidence rates and the risk of all-cause mortality among the participants in the different platelet count groups are shown in Table 1. As compared to the normal range, lower platelet number was significantly related to increased risk of mortality in the multivariable model (HR=2.17; 95%CI 1.55-3.05). Conversely higher platelet count was not associated with higher risk of death (Table 1). Comparison with usual ranges used for classifying thrombocytopenia and thrombocytosis (platelet count <150x10^9/L or >400x10^9/L, respectively, 5) is also reported in Table 1. According to the standard classification, which does not account for age and sex differences in platelet count, about 2.9% of the study population would be classified as having thrombocytopenia. At variance, the newly defined ranges in platelet count only identified 1.8% of the population as being at higher risk of death because of low platelet count. We also found that subjects classified as thrombocytopenic by traditional range intervals (150-400x10^9/L) had a significantly higher risk of all-cause mortality than subjects with normal platelet count or thrombocytosis (HR 1.62; 95%CI 1.28-2.05). As compared to subjects included in the normal platelet count category for both criteria, those who shifted from thrombocytopenia - as defined by usual ranges (<150 x10^9/L) - to normal platelet count according to new ranges (n= 278; n of deaths=50) had a risk of death of 1.37 (95%CI 1.02-1.85). For comparison, subjects who were
considered as having thrombocytopenia according to both classifications, (n=358, n of deaths=36) had a relative risk of death of 2.22 (95%CI 1.57-3.13). The number of individuals who shifted from normal values (by traditional ranges) to thrombocytopenia (according to new ranges) was too small (n=36, n of deaths=1; HR=4.01; 95%CI 0.56-28.8) to allow any meaningful risk interpretation. A cubic spline was also generated and showed a U-shaped relationship between platelet number and overall mortality. Findings revealed a non-linear relationship (p value =0.0015) with lower platelet count at higher risk for total mortality, in comparison with normal or higher values (Figure 1). To the best of our knowledge, this is the first study to test the association of age and sex specific ranges of platelet count with risk of all-cause mortality in a large population-based epidemiological cohort. Using personalized (sex and age specific) rather than traditional reference intervals of platelet count reduces the number /proportion of subjects with thrombocytopenia, as lately observed in a sample of Italian adult patients to whom the newly identified cut-offs were applied (11). The group of possibly true thrombocytopenic subjects (identified by personalized range intervals) had a higher risk of total mortality as compared to subjects classified as thrombocytopenic by traditional range intervals. Strengths of the present study are represented by the large number of subjects and its prospective design. In addition, a large number of possible confounding factors have been considered, including C-reactive protein, mean platelet volume and use of antiplatelet drugs. A major limitation is the unavailability of cause-specific deaths. In conclusion, it is suggested that introducing the new platelet count ranges into clinical practice would reduce the number/proportion of normal individuals unduly considered at high risk of all-cause mortality and would be useful to better identify those subjects at higher mortality risk, possibly related to a low platelet count.
ACKNOWLEDGMENTS
The MOLI-SANI research group thanks the Associazione Cuore Sano Onlus (Campobasso, Italy) for its financial support and the Azienda Sanitaria Regionale del Molise (ASReM, Campobasso, Italy), the Offices of vital statistics of the Molise region and the Molise Dati Spa (Campobasso, Italy) for their collaboration and support provided during the follow-up activities.

Funding: The enrolment phase of the MOLI-SANI Study was conducted at the Research Laboratories of the Catholic University in Campobasso (Italy) and supported by research grants from Pfizer Foundation (Rome, Italy), the Italian Ministry of University and Research (MIUR, Rome, Italy)—Programma Triennale di Ricerca, Decreto no.1588 and Instrumentation Laboratory, Milan, Italy. The follow up of the MOLI-SANI cohort is being conducted at the IRCCS Neuromed, Pozzilli, Italy. Funders had no role in study design, collection, analysis, and interpretation of data; in the writing of the manuscript and in the decision to submit the article for publication. All Authors were and are independent from funders. MB was supported by a Fondazione Umberto Veronesi Fellowship. The present analyses were partially supported by the Italian Ministry of Health 2013 (Young investigator grant to MB, number: GR-2013-02356060).

Contribution: LI, GdG, MB, CC, MBD, ADiC contributed to the conception and design of the work, and interpretation of data; SC, ADC managed data collection; MB, ADiC analysed the data; MB wrote the paper; MBD, GdG, CC and LI originally inspired the research and critically reviewed the manuscript.

Disclosure of Conflicts of Interest
None of the Authors had a personal or financial conflict of interest.
REFERENCES


intervals for platelet count reduce the number of subjects with unexplained thrombocytopenia.
*Haematologica.* 2015;100(9):e338-40.
Table 1 Association of platelet count with overall mortality according to new or traditional cut-offs for thrombocytosis and thrombocytopenia

<table>
<thead>
<tr>
<th>Age/sex-specific platelet count ranges*</th>
<th>N of deaths/n of subjects</th>
<th>Incidence rate (%)</th>
<th>Model 1 HR (95% CI)</th>
<th>Model 2 HR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lower</td>
<td>37/394</td>
<td>9.4</td>
<td>1.92 (1.38-2.66)</td>
<td>2.17 (1.55-3.05)</td>
</tr>
<tr>
<td>Normal</td>
<td>929/20686</td>
<td>4.5</td>
<td>-1-</td>
<td>-1-</td>
</tr>
<tr>
<td>Higher</td>
<td>35/555</td>
<td>6.3</td>
<td>1.39 (0.99-1.95)</td>
<td>1.09 (0.77-1.55)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Usual platelet count ranges</th>
<th>N of deaths/n of subjects</th>
<th>Incidence rate (%)</th>
<th>Model 1 HR (95% CI)</th>
<th>Model 2 HR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lower (&lt;150 ×10^9/L)</td>
<td>86/636</td>
<td>13.5</td>
<td>1.51 (1.21-1.89)</td>
<td>1.62 (1.28-2.05)</td>
</tr>
<tr>
<td>Normal (150-400 ×10^9/L)</td>
<td>897/20606</td>
<td>4.4</td>
<td>-1-</td>
<td>-1-</td>
</tr>
<tr>
<td>Higher (&gt;400 ×10^9/L)</td>
<td>18/393</td>
<td>4.6</td>
<td>1.48 (0.93-2.37)</td>
<td>1.21 (0.75-1.95)</td>
</tr>
</tbody>
</table>

*Lower platelet count was defined as platelet number <156 ×10^9/L or <140×10^9/L for women aged <64 years and for those aged >64 years respectively; platelet number<141×10^9/L or <122×10^9/L for men aged <64 years or >64 years, respectively. Higher platelet count was set when platelet count > 405×10^9/L or > 379×10^9/L for women aged <64 years or >64 years, respectively; as platelet count> 362×10^9/L or >350×10^9/L for men aged <64 or >64 years, respectively (data from reference 9).

Model 1 adjusted for age, sex, hematocrit, and site of recruitment.

Model 2 as Model 1 further adjusted for education, cardiovascular disease, cancer, diabetes, hypertension, hypercholesterolemia, leisure-time physical activity, body mass index, smoking, leukocyte count, mean platelet volume, C-reactive protein and use of antiplatelet drugs.
**Figure 1** Adjusted HR for all-cause mortality according to platelet count in the MOLI-SANI study population.
Figure 1 Adjusted HR for all-cause mortality according to platelet count in the MOLI-SANI study population.
Age-sex specific ranges of platelet count and all-cause mortality: prospective findings from the MOLI-SANI study

Marialaura Bonaccio, Augusto Di Castelnuovo, Simona Costanzo, Amalia De Curtis, Maria Benedetta Donati, Chiara Cerletti, Giovanni de Gaetano and Licia Iacoviello