Personalized reference intervals for platelet count reduce the number of subjects with unexplained thrombocytopenia

The reference range for any blood test is defined as the set of values which 95% of the normal population falls within. When the value of a parameter varies according to specific physiological characteristics of investigated individuals, a single reference range is not sufficient, and personalized ranges are required. This is the case, for instance, for the normal values for hemoglobin concentration, which vary according to age and sex. The demonstration that platelet count progressively decreases with ageing, and that women have more platelets than men after puberty,[1-3] stimulated the search for personalized ranges for this parameter too, and customized reference intervals for the Italian population have been recently proposed based on the analysis of over 40,000 healthy subjects.[4] According to this study, normal ranges for platelet count are: $165-473 \times 10^9$/L, regardless of gender, under 15 years of age; 136-436 and $120-369 \times 10^9$/L in women and men, respectively, between 15 and 64 years; and 119-396 and $112-361 \times 10^9$/L in women and men, respectively, over 64 years. Thus, both the upper and lower limits of normal platelet count for children are higher than those within the single range currently in use, while those for adults are lower.

To verify the impact of the personalized ranges in clinical practice, we used them retrospectively, together with that presently in use of $150-450 \times 10^9$/L, in a consecutive series of 917 Italian adult patients admitted to the Department of Internal Medicine of the Policlinico San Matteo Foundation, a research and university hospital located in Pavia, in the north of Italy. Platelet counts at admission were used for this study. All patients hospitalized in the period from March 2012 to February 2014 were enrolled in the study.

Clinical records were used to identify the potential causes of thrombocytopenia and thrombocytosis in each patient. The following conditions have been considered as potentially responsible for thrombocytopenia: chronic liver disorders, acute viral hepatitis, HIV infection, helicobacter pylori infection, sepsis, systemic lupus erythematosus, antiphospholipid syndrome, hematological and non-hematological malignancies with at least another cytopenia in addition to thrombocytopenia, bone marrow aplasia/hypoplasia, thrombotic microangiopathies, and immune thrombocytopenia. The following conditions have been regarded as potential causes of thrombocytosis: myeloproliferative disorders, myelodysplasia with del(5q), infections, extensive tissue damage or invasive surgery, splenectomy, hemorrhages, iron deficiency, cancer, hemolysis, and rebound following myelosuppressive chemotherapy. Moreover, we considered any other condition associated with a serum value of C-reactive protein (CRP) at least three times higher than the upper limit of reference interval, as being potentially responsible for thrombocytosis. In fact, it has been shown that the serum level of thrombopoietin, the hormone that stimulates megakary-

Table 1. Main characteristics and prevalence of thrombocytopenia and thrombocytosis in a series of consecutive patients admitted to the department of internal medicine.

Main diagnostic category at discharge	No. of patients (%)	Mean age (SD), years	Male/ Female	No. of thrombocytopenic patients (% of all patients/95% CI)		No. of thrombocytosic patients (% of all patients/95% CI)	
				Traditional reference range	Personalized reference range	Traditional reference range	Personalized reference range
Disorders of the cardiovascular system	190 (20.7)	80.1 (9.2)	94/96	29 (3.2)	18 (2)	14 (1.5)	19 (2.1)
Non-neoplastic disorders of the respiratory system	124 (13.5)	76.2 (12.7)	77/47	17 (1.9)	8 (0.9)	14 (1.5)	16 (1.7)
Hematological malignancies	94 (10.3)	71.8 (13.5)	55/39	48 (5.2)	46 (5)	10 (1.1)	11 (1.2)
Non-neoplastic disorders of the gastrointestinal system	88 (9.6)	70.9 (14.6)	42/46	22 (2.4)	19 (2.1)	10 (1.1)	13 (1.4)
Non-neoplastic disorders of the nervous system	76 (8.3)	74.3 (14.9)	42/34	11 (1.2)	7 (0.8)	4 (0.4)	5 (0.5)
Non-neoplastic hematological disorders	74 (8.1)	67.4 (17.5)	36/38	23 (2.5)	22 (2.4)	7 (0.8)	8 (0.9)
Neoplastic disorders of the gastrointestinal system	44 (4.8)	76.6 (12.3)	26/18	9 (1)	7 (0.8)	6 (0.7)	9 (1)
Non-neoplastic disorders of the kidney and the urinary system	36 (3.9)	77.1 (11.7)	17/19	5 (0.5)	2 (0.2)	3 (0.3)	4 (0.4)
Thromboembolic disorders	34 (3.7)	73 (15.1)	12/22	1 (0.1)	0 (0)	1 (0.1)	3 (0.3)
Neoplastic disorders of the respiratory system	25 (2.7)	71.2 (12.2)	19/6	6 (0.7)	6 (0.7)	2 (0.2)	3 (0.3)
Rare neoplastic disorders	25 (2.7)	67.3 (19.7)	17/8	5 (0.5)	4 (0.4)	1 (0.1)	3 (0.3)
Non-neoplastic disorders of metabolism and the endocrine system	23 (2.5)	68 (18)	11/12	4 (0.4)	3 (0.3)	1 (0.1)	1 (0.1)
Non-neoplastic disorders of soft tissues	17 (1.9)	70.4 (18.4)	7/10	4 (0.4)	3 (0.3)	2 (0.2)	4 (0.4)
Intoxication and poisoning	17 (1.9)	65.5 (22.1)	5/12	1 (0.1)	1 (0.1)	1 (0.1)	2 (0.2)
Systemic infections	15 (1.6)	81.2 (8.4)	7/8	10 (1.1)	9 (1)	1 (0.1)	1 (0.1)
Disorders of the immune system	13 (1.4)	70.5 (13.4)	7/6	0 (0)	0 (0)	3 (0.3)	3 (0.3)
Neoplastic disorders of the female reproductive system	11 (1.2)	74.5 (11.5)	0/11	2 (0.2)	1 (0.1)	2 (0.2)	3 (0.3)
Non-neoplastic disorders of the skeletal system	11 (1.2)	76.1 (15.1)	2/9	0 (0)	0 (0)	1 (0.1)	2 (0.2)
Total	**917 (100)**	**74.2 (14.3)**	**476/441**	**197 (21.5/18.8-24.1)**	**156 (17/14.6/19.5)**	**83 (9/7.2-10.9)**	**110 (12/9.9-14.1)**

Table 2. Prevalence of thrombocytopenia in investigated patients.

	No. of patients (%)	Mean platelet count (SD/25th-75th)	No. of patients with thrombocytopenia (% of all patients/95% C.I.)	
			Traditional reference range	Personalized reference range
With a possible cause of thrombocytopenia*	258 (28.1)	159.4 (115/64-232)	130 (14.2/12-16.6)	119 (13/10.9-15.3)
Chronic liver disorders	152 (16.6)	215.2 (104/152-273)	36 (3.9)	28 (3.1)
Hematological malignancies°	55 (6)	61.3 (43.6/25-88)	54 (5.9)	52 (5.7)
Immune thrombocytopenia	14 (1.5)	22.2 (20.9/5-41)	14 (1.5)	14 (1.5)
Cancer°	9 (1)	73.7 (39.1/53-100)	9 (1)	8 (0.9)
Helicobacter pylori infection	9 (1)	241.2 (83.1/189-276)	1 (0.1)	1 (0.1)
Disseminated intravascular coagulation	8 (0.9)	97.9 (59.2/64-115)	7 (0.8)	7 (0.8)
Bone marrow aplasia/hypoplasia	4 (0.4)	40.3 (29/20-61)	4 (0.4)	4 (0.4)
Sepsis	2 (0.2)	56.5 (7.8/51-62)	2 (0.2)	2 (0.2)
Antiphospholipid syndrome	2 (0.2)	296.5 (41.7/267 326)	0 (0)	0 (0)
Acute viral hepatitises	1 (0.1)	120	1 (0.1)	1 (0.1)
Systemic lupus erythematosus	1 (0.1)	31	1 (0.1)	1 (0.1)
Heparin induced thrombocytopenia	1 (0.1)	112	1 (0.1)	1 (0.1)
With no cause of thrombocytopenia	659 (71.9)	269.6 (125.8/191-323)	67 (7.3/5.7-9.2)	37 (4/2.8-5.5)
Total	917 (100)	238.6 (132.4/160-301)	197 (21.5/18.9-24.3)	156 (17/14.6-19.6)

*The possible cause of thrombocytopenia in this table does not necessarily reflect the main diagnosis at discharge reported in Table 1 because many patients had more than one disease. °With at least another cytopenia in addition to thrombocytopenia.

Table 3. Prevalence of thrombocytosis in investigated patients.

	No. of patients (%)	Mean Platelet count (SD/25th-75th)	No. of patients with thrombocytosis (% of all patients/95% C.I.)	
			Traditional reference range	Personalized reference range
With a possible cause of thrombocytosis*	724 (78.9)	247.2 (140.7/161-312)	79 (8.6/6.9-10.6)	106 (11.6/9.6-13.8)
Inflammation	353 (38.5)	228.8 (129.1/151-293)	28 (3.1)	44 (4.8)
Infection	126 (13.7)	259.6 (118.6/174-324)	16 (1.7)	17 (1.9)
Cancer	123 (13.4)	499.4 (333.9/272-681)	10 (1.1)	17 (1.9)
Iron deficiency	70 (7.6)	261.5 (135.2/172-335)	10 (1.1)	13 (1.4)
Hemorrhage	24 (2.6)	246.5 (147.6/166.5-290)	3 (0.3)	3 (0.3)
Myeloproliferative disorders	15 (1.6)	591.2 (334.5/376-681)	9 (1)	9 (1)
Hemolysis	8 (0.9)	314 (108.9/247-381.5)	2 (0.2)	2 (0.2)
Tissue damage	5 (0.6)	269.6 (90.1/249-275)	1 (0.1)	1 (0.1)
With no cause of thrombocytosis	193 (21.1)	206.5 (87.8/154-258)	4 (0.4/0.1-1.1)	4 (0.4/0.1-1.1)
Total	917 (100)	238.6 (132.4/160-301)	83 (9.1/7.3-11.1)	110 (12/10-14.3)

*The possible cause of thrombocytosis in this table does not necessarily reflect the main diagnosis at discharge reported in Table 1.

opoiesis, behaves as an acute phase reactant and increases in parallel with CRP.[5] Furthermore, a large proportion of subjects with reactive thrombocytosis have increased levels of both thrombopoietin and CRP.[6]

Descriptive statistics were computed as means, standard deviations and 25th-75th percentiles. Prevalence of thrombocytopenia and thrombocytosis, along with 95% confidence intervals, was estimated according to the traditional and personalized reference ranges, both on the overall sample and by the presence/absence of a possible cause for disease. Data management, quality control and statistical analyses were performed by STATA 11 (College Station, TX, USA).

The institutional review board of the hospital approved the protocol, and at admission all patients gave written informed consent for their data to be analyzed for research purposes in the future.

Table 1 describes the main characteristics of the investigated population, showing that it was mainly composed of elderly subjects (mean age 74.2±14.3 years), and included 476 men and 441 women. According to the main diagnoses at discharge, which were obtained from clinical records, the majority of patients had disorders of the cardiovascular or respiratory system, or neoplastic diseases.

Application of the new personalized reference intervals instead of the traditional one of 150-450x10⁹ platelets/L, resulted in relevant differences in the number of patients classified as thrombocytopenic or affected by thrombocytosis.

The prevalence of thrombocytopenia in the whole cohort of patients was high with both the traditional and the personalized reference range, however, it decreased

from 21.5% with the former to 17% with the latter (Table 1). The degree of thrombocytopenia was mild or moderate in most cases, and only 5.6% and 1.7% of patients had less than 50 and 20x10⁹ platelets/L, respectively.

Analysis of clinical records revealed that, independently from the main diagnosis at discharge, 258 out of 917 patients (28.1%) had one of the possible causes of thrombocytopenia reported in Table 2. Of these 258 subjects, 130 (50.4%) were classified as thrombocytopenic by the traditional reference range, and 119 (46.1%) by the personalized reference range. Thus, by using the personalized reference intervals instead of the traditional one the number of thrombocytopenic subjects was reduced by 8.5%.

Much more relevant was the variation induced by the personalized reference range in the subgroup of subjects with unexplained thrombocytopenia, in that their number decreased from 67 (10.2% of the 659 subjects with no cause of thrombocytopenia) to 37 (5.6%). Thus, the personalized range reduced the number of subjects with unexpectedly low platelet counts by 44.8%. The observation that apparently healthy adults may have a mild reduction in platelet count, which remains without explanation despite appropriate investigation, is not recent. Notably, a prospective study of 217 subjects with incidentally discovered platelet counts between 100 and 150x10⁹/L found that, in most cases, the degree of thrombocytopenia remained stable during a 5-year follow up without the appearance of any disease.[7] The authors concluded that these subjects were affected by a mild form of immune thrombocytopenia, but our data suggest the possibility that some of them actually had platelet counts appropriate to their sex, age and ethnicity, and were therefore healthy.

The application of the new reference range also reduced the prevalence of thrombocytopenia in subjects with diseases that may be theoretically responsible for reduced platelet count, such as chronic liver disorders. However, the change was minimal, with the number of thrombocytopenic subjects decreasing from 130 (14.2% of all patients) to 119 (13%). Of note, none of these patients who switched from thrombocytopenic to non-thrombocytopenic had bleeding tendency (*data not shown*), and changing the diagnosis would have had no practical effect on their management.

In conclusion, using personalized ranges instead of the traditional one had the main effect of reducing the number of subjects with unexplained thrombocytopenia.

Concerning thrombocytosis, its prevalence was lower than that of thrombocytopenia, affecting 9% of all patients using the traditional reference range, and 12% with the personalized range (Table 1). The degree of thrombocytosis was nearly always mild, with only 1.4% and 1.1% of patients presenting with more than 750 and 1,000x10⁹ platelets/L, respectively.

Based on clinical records, 724 out of 917 subjects (78.9%) had clinical conditions that may cause thrombocytosis (Table 3), but only 79 (8.6%) actually had platelet counts higher than normal according to the traditional reference range, and 106 (11.6%) according to the personalized range. The new reference intervals, therefore, increased the percentage of thrombocytosic subjects by 34.2%. All switches from normal platelet count to thrombocytosis have been observed in patients with potential causes of high platelet count. The majority of changes occurred in subjects classified as being affected by 'inflammation', as they did not have one of the specific conditions reported in Table 3, but instead presented with CRP levels more than three times higher than the upper limit of normal range due to a variety of other illnesses. Thus, classifying patients as having increased platelet count instead of

normal platelet count would have had no consequences on their diagnosis or management.

One flaw in our study was that the population of hospitalized patients we investigated was mainly composed of older people, with only a limited proportion of young adults and no children at all. Thus, our findings are representative of the outcome of personalized ranges in older adults, and the analysis of other classes of patients is therefore required in order to evaluate its efficacy in young adults and children. Another limitation is that our results are pertinent to the Italian population and we have no evidence indicating that the reference intervals we tested can also be used advantageously in other countries. However, literature data show that the mean value of platelet count observed in Italy is similar to that observed in other Caucasian populations,[4,8] and it is therefore possible that the new reference intervals are also appropriate for other Western countries. Further studies are required to test this hypothesis.

In conclusion, we observed that the most notable effect of using personalized reference intervals for platelet count in elderly medical patients was a reduction by nearly half of the proportion of subjects with an unexplained form of thrombocytopenia. Introducing the new range into clinical practice is therefore expected to prevent many subjects from receiving a series of unnecessary and expensive tests, thus benefiting both the people involved and the health system.

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