Short Communication

Wolfgang Hermann, Lorenz Risch, Chris Grebhardt, Urs E. Nydegger, Benjamin Sakem, Mauro Imperiali, Harald Renz and Martin Risch*

Reference intervals for platelet indices in seniors and frequency of abnormal results in a population-based setting: a comparison between directly and indirectly estimated reference intervals

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Abstract

Objectives: Mean platelet volume (MPV), platelet distribution width (PDW), and plateletcrit (PCT) possess diagnostic and prognostic capabilities in a variety of diseases. We aimed to establish reference intervals (RI) for platelet indices (PI) in seniors.

Methods: We established direct and indirect RI for MPV, PDW, and PCT in selected reference individuals aged 60 years and older. Abnormal PI were assessed in a population-based setting in the Principality of Liechtenstein, where 37.7% of the whole nation's population aged 60 years and older had PI determined by hematology analyzers from Sysmex (Horgen, Switzerland).

Results: Among 689 female and 542 male participants, MPV and PDW did not exhibit age- and gender-specific differences, whereas PCT in females also displayed no age-specific differences. Age- and sex-independent RI were 9.3–12.5 fl for MPV and 10.1–16.7% for PDW, whereas the age-independent RI for PCT in women was 0.18–0.37. In males, age-specific RI for PCT were 0.16–0.30 (age 60–69), 0.15–0.33 (age 70–79), and 0.14–0.33 (age 80 and older).

The population-based frequency of abnormal PI results was 0.8% (MPV), 1.1% (PDW), and 24.4% (PCT).

Conclusions: Applying novel RI for PI reveals that only approximately 1% of patients exhibit abnormal MPV and PDW. Abnormal PCT is observed much more frequently.

Keywords: abnormal results; elderly; geriatric; platelet indices; prevalence; reference interval; seniors.

Platelets belong to the most frequently ordered tests in the medical laboratory and primarily are assessed by flow cytometric methods in automated hematology analyzers. Hematology analyzers can enumerate platelets as well as other features of platelets, i.e., the mean platelet volume (MPV), the platelet distribution width (PDW), and the plateletcrit (PCT) [1]. These parameters are related to platelet morphology and activation [1, 2]. Platelet counts as well as platelet indices possess diagnostic and prognostic capabilities [3–7]. However, the exact role of morphological platelet indices remains unknown. In elderly patients, diseases with an influence on platelet indices are frequently encountered. However, to date, whether platelet indices in the elderly are influenced by age or sex and whether reference intervals specific for seniors are needed remain unknown. To the best of our knowledge, we are not aware of other such studies evaluating reference intervals with direct methods. Further, there is not much known about how frequently abnormal platelet indices are observed in the general elderly population. We therefore sought to determine reference intervals within the framework of the SENIORLAB study, a prospective study to evaluate age- and gender-specific reference intervals in seniors [8, 9]. For the present analysis, we included participants who were 60 years and older, residing in Switzerland, and in fasting state at the baseline examination.

*Corresponding author: Dr. med. Martin Risch, Zentrallabor, Kantonsspital Graubünden, 7000 Chur, Switzerland, Phone: +41 812566531, E-mail: martin.risch@ksgr.ch

Wolfgang Hermann, Private University in the Principality of Liechtenstein, Triesen, Liechtenstein

Lorenz Risch, Chris Grebhardt, Urs E. Nydegger, Benjamin Sakem and Mauro Imperiali, LMZ Dr. Risch, 3097 Liebefeld, Switzerland

Harald Renz, Institute of Laboratory Medicine, University Hospital Giessen and Marburg, Philipps University Marburg, Marburg, Germany

Because life expectancy in seniors differs according to age, we deliberately chose the following minimal survival criteria for exclusion from the present analysis: death at first follow-up for participants <80 years of age, death of at least three years from baseline between age 80 and age less than 85, death of at least two years from baseline between age 85 and less than 90 years, and death of at least one year from baseline for age 90 and older. Further exclusion criteria were polypharmacy (defined as the use of more than five pharmacologically active compounds) and circumstances known to affect platelet counts: ferritin <15 µg/L as an indicator of iron deficiency, vitamin B12 concentration <130 pmol/L as an indicator of vitamin B12 deficiency, red blood cell folate <340 nmol/L as an indicator of folate deficiency, and CRP >8.7 mg/L or white blood cell count >10.4 × 10⁹/L (as indicators of inflammation) [10]. Participant characteristics are shown in Table 1. To investigate the frequency of abnormal platelet index results (MPV, PDW, and PCT), we analyzed anonymized platelet index results obtained from a small European country with only one central medical laboratory, i.e., the Principality of Liechtenstein. Platelet indices were determined with analyzers from Sysmex (XE5000, XT 2100, XN 1000; Sysmex, Horgen, Switzerland). The study was performed in accordance with the declaration of Helsinki and was approved by the local Ethics Committee (KEK Bern, Switzerland; 166/08). A total of 542 male and 659 female participants were available for direct evaluation of reference intervals. We did not identify a significant correlation between age and mean platelet volumes and platelet distribution width (Spearman rank correlation), and no sex difference was noted for these two parameters (Mann-Whitney U-test). Females had a significantly higher plateletcrit than men (median 0.26 vs. 0.22% $p < 0.001$, Mann-Whitney U-test), and a significant inverse correlation between age and plateletcrit was noted in men ($r = -0.09$; Spearman rank correlation $p = 0.03$) but not in females. The platelet indices results according to age and sex are provided in Figure 1.

The direct reference intervals for the different platelet indices are given in Table 2. Here we stratified the PCT in analogy to the stratification of platelet count reference intervals from the SENIORLAB cohort, as published elsewhere [10].

The reference limits obtained from the direct evaluation (Table 2) were confirmed by an indirect evaluation of the reference limits in routine results from 25,648 clinical patients (Table 3), as the 90% CI from the directly evaluated reference limits overlapped with the permissible uncertainty of the reference limits in the indirect evaluation [12]. No overlap could be observed in only two indirect reference

Table 1: Participant characteristics in the direct reference interval determination.

Characteristics, units	Median [IQR]/number
Participants, male/female	1,201 (659/542)
Age, years	71 [66, 77]
BMI, m ² /kg	25 [22.8, 27.5]
eGFR _{CKD-EPI CreaCysC} , ml/min/1.73 m ²	83 [72, 93]
HbA _{1c} , %	5.8 [5.6, 6.1]
Ferritin, µg/L	114 [69, 184]
Vitamin B12, pmol/L	240 [195, 306]
Red blood cell folate, nmol/L	922 [702, 1214]
TSH, U/L	1.8 [1.2, 2.3]
CRP, mg/L	1.3 [0.7, 2.4]
Hemoglobin, g/L	142 [135, 150]
Reticulocytes, × 10 ¹² /L	0.04 [0.03, 0.05]
White blood cells, × 10 ⁹ /L	5.4 [4.7, 6.4]
Platelet count, × 10 ⁹ /L	225 [196, 360]
Mean platelet volume, fl	10.6 [10.1, 11.2]
Platelet distribution width, %	12.5 [11.5, 13.6]
Plateletcrit, %	0.24 [0.21, 0.28]

Data are given as counts or medians and interquartile range [IQR]. Laboratory methods with method imprecision are provided in Ref [8]. IQR, interquartile range; BMI, Body Mass Index; CRP, C-reactive Protein; eGFR_{CKD-EPI CreaCysC}, CKD-EPI glomerular filtration rate estimate with cystatin C and creatinine; HbA_{1c}, glycated hemoglobin; TSH, thyroid stimulating hormone. All measurements were done in LMZ Dr. Risch.

limits (i.e., lower reference limit PDW and upper reference limits PCT in men aged 60–69). However, the two ranges were adjacent in both cases.

In the indirect RI determination we could also demonstrate that the RI for MPV and PDW in seniors are comparable to that in younger adults. For PCT we could show that the age trend of the lower reference limit in males also projects to younger adults. During the period from 1st January 2013 to 31st December 2019, a total of 12,897 individuals (corrected for 1,613 death cases) aged 60 or older were living in the country. Of these, 4,866 (37.7% 95% CI [36.9, 38.6] of the entire population) individuals (2,388 males/2,478 females) had at least one determination of platelet indices available with a total of 19,062 determinations available. The protocol for this part of the study was verified by the responsible ethics committee (KEK Zürich, Switzerland; BASEC 2020-00918), and informed consent was waived. The frequency of abnormal MPV in the population-based setting was 143/19,062 samples (0.8%, 95% confidence interval, CI, [0.6, 0.9]; 105 with decreased MPV, 38 with increased MPV). The proportion of abnormal PDW was observed in 214/19,062 samples (1.1% 95% confidence interval, CI, [1.0, 1.3]; 163 with decreased PDW, 51 with increased PDW). The proportion of abnormal plateletcrit was 4,654/19,062 (24.4% 95% CI

Figure 1: Mean platelet volume, MPV. (A), platelet distribution width, PDW, (B), and plateletcrit, PCT, (C), stratified according to age and gender.

Table 2: Direct reference intervals for platelet indices in seniors.

Parameter	n	LRL [95% CI]	URL [95% CI]
Mean platelet volume, fl	1,197	9.3 [9.3, 9.3]	12.5 [12.4, 12.6]
Platelet distribution width, %	1,190	10.1 [10.0, 10.2]	16.7 [16.5, 17.0]
Plateletcrit, %			
Females	649	0.18 [0.18, 0.18]	0.37 [0.36, 0.37]
Males, 60–69 years	240	0.16 [0.15, 0.16]	0.30 [0.29, 0.31]
Males, 70–79 years	203	0.15 [0.14, 0.16]	0.33 [0.32, 0.35]
Males, ≥80 years	88	0.14 [0.13, 0.15]	0.33 [0.31, 0.35]

Direct evaluation of double sided 95% reference intervals was performed according the Clinical Laboratory Standards Institute (CLSI) guideline EP28-A3c using a robust method [11]. Outliers were eliminated by Tukey’s method [11]. Reference intervals were calculated with Medcalc Version 18.3 (MedCalc Software bvba, Ostend, Belgium; <http://www.medcalc.org>; 2019). n, included reference subjects; CI, confidence interval. reviation1; LRL, lower reference limit; URL, upper reference limit.

[23.8, 25.0]; 748 with decreased plateletcrit and 1,670 with increased plateletcrit). Our data present direct reference intervals for platelet indices in seniors, which have been verified by indirect methods. We revealed no age and sex dependency of the reference intervals in mean platelet volume and platelet distribution width. In contrast, the plateletcrit exhibits an age and sex dependency of the reference intervals. Since the plateletcrit is calculated by multiplying the platelet count with the mean platelet volume and platelet counts also have age- and sex-specific reference intervals in seniors, it can be assumed that the platelet count is causative for age- and sex-specific differences [15]. Our reference intervals are comparable to other investigations also employing Sysmex hematology analyzers [16, 17]. This is important to note as measurement of platelet indices is not yet standardized [16, 17]. The present study also shows that reference intervals for MPV and PDW in younger individuals can also be extended to seniors [16]. Our investigation further shows that platelet indices are a well suited example to demonstrate that direct and indirect evaluation of reference intervals can provide comparable reference limits [11]. Finally, in the population-based patient collective from the Principality of Liechtenstein, we demonstrated that the MPV and PDW are platelet indices with low frequencies of abnormal results. It can therefore be assumed that platelet indices could be specific but insensitive markers for several diseases [17, 18]. In contrast to the rare occurrence of abnormal test results, a plateletcrit beyond the reference limits is observed rather frequently, as one in four patients displays an abnormal

Table 3: Indirect reference intervals for platelet indices in seniors.

Parameter	n	LRL [pU-LRL]	URL [pU-URL]
Mean platelet volume, fl			
Age 18–60 years	61,576	9.1 [8.8, 9.5]	12.5 [12.1, 13.0]
Age ≥60 years	25,648	9.1 [8.7, 9.4]	12.4 [12.0, 12.8]
Platelet distribution width, %			
Age 18–60 years	61,576	9.7 [9.3, 10.2]	16.2 [15.5, 16.9]
Age ≥60 years	25,648	9.5 [9.0, 9.9]	16.2 [15.4, 17.0]
Plateletcrit, %			
Females, 18–60 years	46,633	0.19 [0.18, 0.20]	0.39 [0.37, 0.42]
Females, ≥60 years	14,912	0.18 [0.17, 0.19]	0.39 [0.37, 0.41]
Males, 18–60 years	14,933	0.17 [0.16, 0.18]	0.35 [0.33, 0.37]
Males, 60–69 years	5,430	0.16 [0.15, 0.17]	0.34 [0.32, 0.36]
Males, 70–79 years	3,860	0.15 [0.14, 0.16]	0.36 [0.34, 0.38]
Males, ≥80 years	1,446	0.13 [0.13, 0.14]	0.33 [0.31, 0.35]

Indirect evaluation of double sided 95% reference intervals was performed with the indirect calculation of reference limits derived from the routine platelet indices results measured on Sysmex hematology analyzers in the LMZ Dr. Risch. From each patient, the last available result was used for this analysis. The “Reference Limit Estimator” software released by the decision limits working group of the German Association of Clinical Chemistry and Laboratory Medicine (DGKL) was employed [13]. To minimize contamination of the nondiseased subgroup with samples from diseased individuals, the samples violating the following criteria were excluded: hemoglobin <130 g/L or >170 g/L in males and <120 g/L or >160 g/L in females; leucocytes <3.8 or >10.4 × 10⁹/L [14]. For comparison, indirect RI were also for younger adults. The responsible ethics committee (KEK Bern, Switzerland; BASEC 2020-00139) approved this verification. n, included patients; LRL, lower reference limit; URL, upper reference limit; pU-LRL, permissible uncertainty of the LRL; pU-URL, permissible uncertainty of the URL.

result. Further studies investigating the association of platelet indices with disease states and prognosis are needed to further increase the usefulness of platelet indices in routine clinical practice and research projects.

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Informed consent: Informed consent was obtained from all individuals included in the SENIORLAB-study. Informed consent has been waived for the determination of indirect reference interval determination.

Ethical approval: The study was performed in accordance with the declaration of Helsinki and was verified by the local Ethics Committees (KEK Bern, Switzerland, 166/08a

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References

1. Briggs C, Harrison P, Machin SJ. Continuing developments with the automated platelet count. *Int J Lab Hematol* 2007;29:77–91.
2. Vagdatli E, Gounari E, Lazaridou E, Katsibourlia E, Tsikopoulou F, Labrianou I. Platelet distribution width: a simple, practical and specific marker of activation of coagulation. *Hippokratia* 2010;14: 28–32.
3. Budzianowski J, Pieszko K, Burchardt P, Rzezniczak J, Hiczkiewicz J. The role of hematological indices in patients with acute coronary syndrome. *Dis Markers* 2017;2017:3041565.
4. Weymann A, Ali-Hasan-Al-Saegh S, Sabashnikov A, Popov AF, Mirhosseini SJ, Nombela-Franco L, et al. Platelets cellular and functional characteristics in patients with atrial fibrillation: a comprehensive meta-analysis and systematic review. *Med Sci Mon Basic Res* 2017;23:58–86.
5. Pogorzelska K, Kretowska A, Krawczuk-Rybak M, Sawicka-Zukowska M. Characteristics of platelet indices and their prognostic significance in selected medical condition – a systematic review. *Adv Med Sci* 2020;65:310–5.
6. Budak YU, Polat M, Huysal K. The use of platelet indices, plateletcrit, mean platelet volume and platelet distribution width in emergency non-traumatic abdominal surgery: a systematic review. *Biochem Med* 2016;26:178–93.
7. Matowicka-Karna J. Markers of inflammation, activation of blood platelets and coagulation disorders in inflammatory bowel diseases. *Postepy Hig Med Dosw* 2016;70:305–12.
8. Risch M, Nydegger U, Risch L. SENIORLAB: a prospective observational study investigating laboratory parameters and their reference intervals in the elderly. *Medicine (Baltim)* 2017;96: e5726.
9. Risch M, Sakem B, Risch L, Nydegger U. The SENIORLAB study in the quest for healthy elderly patients. *J Lab Med* 2019;42:109–12.
10. Hermann W, Risch L, Grebhardt C, Nydegger UE, Sakem B, Imperiali M, et al. Reference intervals for platelet counts in the elderly: results from the prospective SENIORLAB study. *J Clin Med* 2020;9:2856.
11. Clinical and Laboratory Standards Institute (CLSI). Defining, establishing, and verifying reference intervals in the clinical laboratory; approved guideline, 3rd ed. CLSI document C28-A3c. Wayne PA: Clinical and Laboratory Standards Institute; 2008.
12. Haeckel R, Wosniok W, Gurr E, Peil B. Permissible limits for uncertainty of measurement in laboratory medicine. *Clin Chem Lab Med* 2015;53:1161–71.
13. Arzideh F, Wosniok W, Haeckel R. Reference limits of plasma and serum creatinine concentrations from intra-laboratory data bases of several German and Italian medical centres: comparison between direct and indirect procedures. *Clin Chim Acta* 2010;411: 215–21.
14. Adeli K, Higgins V, Nieuwesteeg M, Raizman JE, Chen Y, Wong SL, et al. Complex reference values for endocrine and special chemistry biomarkers across pediatric, adult, and geriatric ages: establishment of robust pediatric and adult reference intervals on the basis of the Canadian health measures survey. *Clin Chem* 2015;61:1063–74.

15. Biino G, Santimone I, Minelli C, Sorice R, Frongia B, Traglia M, et al. Age- and sex-related variations in platelet count in Italy: a proposal of reference ranges based on 40,987 subjects' data. *PloS One* 2013;8:e54289.
16. Latger-Cannard V, Hoarau M, Salignac S, Baumgart D, Nurden P, Lecompte T. Mean platelet volume: comparison of three analysers towards standardization of platelet morphological phenotype. *Int J Lab Hematol* 2012;34:300–10.
17. Maluf CB, Barreto SM, Vidigal PG. Standardization and reference intervals of platelet volume indices: insight from the Brazilian longitudinal study of adult health (ELSA-BRASIL). *Platelets* 2015; 26:413–20.
18. Korniluk A, Koper-Lenkiewicz OM, Kaminska J, Kemona H, Dymicka-Piekarska V. Mean platelet volume (MPV): new perspectives for an old marker in the course and prognosis of inflammatory conditions. *Mediat Inflamm* 2019;2019:9213074.