Within-day biological variation and hour-to-hour reference change values for hematological parameters

Citation for published version (APA):

Hilderink, J. M., Klinkenberg, L. J. J., Aakre, K. M., de Wit, N. C. J., Henskens, Y. M. C., van der Linden, N., Bekers, O., Rennenberg, R. J. M. W., Koopmans, R. P., & Meex, S. J. R. (2017). Within-day biological variation and hour-to-hour reference change values for hematological parameters. *Clinical Chemistry and Laboratory Medicine*, 55(7), 1013-1024. <https://doi.org/10.1515/cclm-2016-0716>

Document status and date:

Published: 01/07/2017

DOI:

[10.1515/cclm-2016-0716](https://doi.org/10.1515/cclm-2016-0716)

Document Version:

Publisher's PDF, also known as Version of record

Document license:

Taverne

Please check the document version of this publication:

- A submitted manuscript is the version of the article upon submission and before peer-review. There can be important differences between the submitted version and the official published version of record. People interested in the research are advised to contact the author for the final version of the publication, or visit the DOI to the publisher's website.
- The final author version and the galley proof are versions of the publication after peer review.
- The final published version features the final layout of the paper including the volume, issue and page numbers.

[Link to publication](#)

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal.

If the publication is distributed under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license above, please follow below link for the End User Agreement:

www.umlib.nl/taverne-license

Take down policy

If you believe that this document breaches copyright please contact us at:

repository@maastrichtuniversity.nl

providing details and we will investigate your claim.

Download date: 26 Apr. 2023

Judith M. Hilderink, Lieke J.J. Klinkenberg, Kristin M. Aakre, Norbert C.J. de Wit, Yvonne M.C. Henskens, Noreen van der Linden, Otto Bekers, Roger J.M.W. Rennenberg, Richard P. Koopmans and Steven J.R. Meex*

Within-day biological variation and hour-to-hour reference change values for hematological parameters

DOI 10.1515/cclm-2016-0716

Received August 10, 2016; accepted November 1, 2016; previously published online December 21, 2016

Abstract

Background: Middle- and long-term biological variation data for hematological parameters have been reported in the literature. Within-day 24-h variability profiles for hematological parameters are currently lacking. However, comprehensive hour-to-hour variability data are critical to detect diurnal cyclical rhythms, and to take into account the ‘time of sample collection’ as a possible determinant of natural fluctuation. In this study, we assessed 24-h variation profiles for 20 hematological parameters.

Methods: Blood samples were collected under standardized conditions from 24 subjects every hour for 24 h. At each measurement, 20 hematological parameters were determined in duplicate. Analytical variation (CV_A), within-subject biological variation (CV_I), between-subject biological variation (CV_G), index of individuality (II), and reference change values (RCVs) were calculated. For the parameters with a diurnal rhythm, hour-to-hour RCVs were determined.

Results: All parameters showed higher CV_G than CV_I . Highest CV_G was found for eosinophils (46.6%; 95% CI, 34.9%–70.1%) and the lowest value was mean corpuscular hemoglobin concentration (MCHC) (3.2%; 95% CI,

2.4%–4.8%). CV_I varied from 0.4% (95% CI, 0.32%–0.42%) to 20.9% (95% CI, 19.4%–22.6%) for red cell distribution width (RDW) and eosinophils, respectively. Six hematological parameters showed a diurnal rhythm.

Conclusions: We present complete 24-h variability profiles for 20 hematological parameters. Hour-to-hour reference change values may help to better discriminate between random fluctuations and true changes in parameters with rhythmic diurnal oscillations.

Keywords: biological variation; diurnal rhythm; hematology; reference change value.

Introduction

When interpreting changes in consecutive laboratory test results, health-care professionals rely on their intuition and clinical experience to distinguish ‘real changes’ from physiological fluctuations. A more objective interpretation of changes in consecutive laboratory results can be achieved by calculating the ‘reference change value’ (RCV), i.e. the required threshold for a change between consecutive measurements to be statistically significant [1]. For a change to become significant, the difference between two measurements must exceed the total inherent variation. The calculated RCV can provide information to prevent random fluctuations from being interpreted as true changes.

Two factors can influence the RCV: the analytical variation (CV_A) and the within-subject biological variation (CV_I). In laboratories that meet contemporary quality and precision specifications, CV_I is the strongest determinant of test result variability [2]. Studies of biological variation can provide insight in the natural fluctuations within and between subjects for a given parameter. Extensive biological variation studies are laborious, and most studies of hematological parameters have only assessed biological variation between days [3–8]. A few studies report within-day variation with a limited number of measurements

*Corresponding author: Steven J.R. Meex, Department of Clinical Chemistry, Cardiovascular Research Institute Maastricht (CARIM), Maastricht University Medical Center+ (MUMC+), P.O. Box 5800, 6202 AZ Maastricht, The Netherlands, Phone: +31-(0)43-3874694, Fax: +31-(0)43-3874692, E-mail: steven.meex@mumc.nl

Judith M. Hilderink, Lieke J.J. Klinkenberg, Norbert C.J. de Wit, Yvonne M.C. Henskens, Noreen van der Linden and Otto Bekers: Department of Clinical Chemistry, Central Diagnostic Laboratory, Maastricht University Medical Center+, Maastricht, The Netherlands
Kristin M. Aakre: Laboratory of Clinical Biochemistry, Haukeland University Hospital, Bergen, Norway

Roger J.M.W. Rennenberg and Richard P. Koopmans: Department of Internal Medicine, Maastricht University Medical Center+, Maastricht, The Netherlands

[8–11]. Complete 24-h variability profiles for hematological parameters are currently lacking. Within-day hour-to-hour variability data are, however, critical to detect diurnal cyclical rhythms and take into account the ‘time of sample collection’ as a possible determinant of natural fluctuation. According to laboratory statistics from Maastricht University Medical Center+, the assessment of repeated within-day hematological testing is common in hospitalized patients (up to 10%).

The present study was conducted to provide comprehensive 24-h variation profiles for 20 hematological parameters in 24 subjects. Specifically, within-day components of variation and index of individuality (II) were assessed. The II is the ratio between the CV_G and CV_I . It determines the utility of conventional population-based reference values [12]. Finally, within-day RCVs were calculated for parameters with random variability over the day, whereas hour-to-hour RCVs were calculated for hematological parameters with rhythmic oscillations during the day.

Materials and methods

This study conforms to the principles of the Declaration of Helsinki and was approved by the institutional review board and the Ethics Committee at Maastricht University Medical Center. This study was registered at clinicaltrials.gov as NCT02091427. The current study meets the critical appraisal checklist criteria for studies of biological variation as proposed by Bartlett et al. [13].

The routine hematological parameters we studied were leukocytes, lymphocytes, neutrophils, monocytes, eosinophils, basophiles, erythrocytes, reticulocytes (percentage), reticulocytes (count), hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), mean platelet volume (MPV), and platelets. Research hematological parameters included immature platelet fraction (IPF), plateletcrit (PCT), and platelet distribution width (PDW).

Study population

The study population consisted of 24 volunteers (19 men and 5 women). These subjects were previously described in the study of Klinkenberg et al. [14]. All participants provided written informed consent.

The average age was 72 years (range 58–82 years). Baseline characteristics of the study population are shown in Table 1. Exclusion criteria were change in medication use in the 3 months prior to the inclusion and a history of severe cardiovascular problems (acute myocardial infarction or stroke) within 12 months prior to inclusion. All participants were free from acute diseases at the time of the study.

Table 1: Participants’ baseline characteristics.

n	24
Age, years	72 ± 7
Male, gender	19 (79%)
BMI, kg/m ²	27 ± 5
Systolic blood pressure, mmHg	140 ± 15
Diastolic blood pressure, mmHg	68 ± 8
Anti-hypertensive medication ^a	10 (42%)
Type 2 Diabetes Mellitus ^b	7 (29%)
Fasting glucose ^c , mmol/L	5.9 (5.2–6.7)
HbA _{1c} ^c , %	5.7 (5.3–6.5)
Oral blood glucose lowering medication ^a	7 (29%)
Total cholesterol ^c , mmol/L	3.7 (4.8–5.4)
HDL cholesterol ^c , mmol/L	1.3 (1.6–1.9)
LDL cholesterol ^c , mmol/L	1.7 (2.5–3.0)
Triglycerides ^c , mmol/L	1.2 (0.8–1.8)
Lipid lowering medication ^a	12 (50%)
eGFR ^{c,d} , mL/min/1.73 m ²	75 (61–92)

Data is presented as n (%), mean ± SD or median (IQR). BMI, body mass index; eGFR, estimated glomerular filtration rate. ^aMedication status was stable for at least 3 months prior to inclusion, and continued as normal during the entire study period, including the test-days. ^bThe diagnosis type 2 Diabetes Mellitus was defined as fasting plasma glucose ≥ 7.0 mmol/L and/or HbA_{1c} ≥ 6.5% (= 48 mmol/mol) [15]. ^cValue at 8:30 a.m. ^dAccording to CKD-EPI Creatinin-cystatin C equation [16].

Study design

After an overnight fast, participants arrived at 8 a.m. at the laboratory. Participants were asked to refrain from heavy physical labor and exercise training 2 days before the test day. They were sampled every hour by using an indwelling venous cannula in an antecubital vein during a time span of 25 h. The participants spent the day seated in a chair or couch.

Standardized meals were consumed at 8:30 a.m. (breakfast), 12:30 p.m. (lunch), and 5:30 p.m. (dinner). The participants went to bed at 11:30 p.m. and got up the next morning at 7:00 a.m. At night, all subjects received a polyethylene coiled extending line (Vygon) that was attached to the indwelling venous catheter to obtain the blood samples and to minimize sleep disturbance.

Laboratory measurements

Blood sampling was performed under standardized conditions in order to minimize sources of pre-CV_A. Blood samples were collected in ethylenediaminetetraacetic acid (EDTA)-containing tubes (4 mL) every hour over a time span of 25 h. Within 1 h after collection, samples were analyzed in duplicate on a Sysmex XE-5000 analyzer (Sysmex Corporation, Kobe, Japan).

Statistical analysis

The outlier method of Burnett was used to check for analytical outliers [17]. As suggested by Fraser and Harris [18], we tested for

Figure 1: (A–C) Subject-specific concentrations over 24 h with hourly sampling. Parameters were shown as absolute ranges (min–max).

homogeneity in the analytical- and within-subject biological variances using the method described by Cochran. Briefly, analytical variances were tested for homogeneity by examining the ratio of the maximum variance to the sum of the variances and compare

this with a critical value [19, 20]. Subjects were excluded until homogeneity of variance was achieved (data not shown) [21]. Finally, the criteria of Reed were used to identify between-subject outliers [20, 22, 23].

Figure 1 (continued)

Between-subject (CV_G), CV_P , and CV_A were calculated using a balanced analysis of variance (ANOVA) with a nested random design in two levels. Verified assumptions for using this model include a normally distributed error term of the means and constant variances [24]. 95% confidence intervals (CI) of these variance components

were calculated using the method described by Burdick and Graybill [24, 25]. The II was calculated by the method described by Petersen et al. [26], and the RCVs were calculated according to the method of Fraser and Harris [18, 27]. The residuals of the parameters followed a Gaussian distribution on the analytical and within-subject

Figure 1 (continued)

level. Therefore, no transformation into the natural logarithm was required.

$$\Pi = \frac{\sqrt{CV_A^2 + CV_I^2}}{CV_G}$$

$$RCV = \sqrt{2} * Z * \sqrt{CV_A^2 + CV_I^2}$$

In the RCV formula, Z represents the number of standard deviations appropriate for the desired level of statistical significance for a bidirectional change (e.g. 1.96 for $p=0.05$ and 2.58 for $p=0.01$ [3]). For the RCV calculations in this study, a Z -score of 1.96 was used.

In parameters with rhythmic diurnal variation, the assumption of constant variances is violated, precluding the calculation of an 'overall' within-day RCV. Instead, hour-to-hour RCVs were calculated for parameters with rhythmic diurnal oscillations, taking into account the systematic increase or decrease at all time-points [18, 28, 29]. Briefly, the average systematic change for each time point (as a percentage from the baseline value) was subtracted from the original individual data. Hence, variation due to rhythmic diurnal oscillations was eliminated and an artificial 'steady-state' concentration with random fluctuation over the day was created.

With these adjusted data, assumptions for ANOVA were fulfilled, and components of biological variation could be calculated. Finally, the percentage hourly changes (β) were added again to the individual time points. To calculate adjusted asymmetric RCVs that take into account rhythmic diurnal oscillations, the following formulas were used [28]:

$$RCV_{\text{upper limit}} = \beta + \sqrt{2} * 1.96 * \sqrt{CV_A^2 + CV_I^2}$$

$$RCV_{\text{lower limit}} = \beta - \sqrt{2} * 1.96 * \sqrt{CV_A^2 + CV_I^2}$$

All statistical calculations were performed using SPSS version 20 (IBM SPSS Statistics, IBM Corporation, Armonk, NY, USA) and Matrix Laboratory (Matlab & Simulink Student Suite R2015b).

Results

Sample collection was incomplete for four subjects. Two subjects left the study prematurely, with, respectively 12 and 4 h of consecutive missing data (subjects 1 and 8, respectively). Since the missing values were not 'at random', a balanced design was required [30]. In order to maintain a balanced design, in line with the statistical conditions for a balanced ANOVA with a nested random design in two levels, these subjects were excluded from all analyses. In two other individuals (subjects 15 and 23, respectively), night samples were missing due to technical problems with the intravenous cannula. Analyses in these subjects were restricted to the samples obtained between 8:30 a.m. and 11:30 p.m. Subject 20 became sick during the test day due to a lack of a stable situation, this subject was excluded from all analyses.

Figure 2: Diurnal variation of posture-dependent parameters.

Values represent mean $\pm$ SEM of posture-dependent parameters. At 11:30 p.m., there was a transition from upright position to sleep position and at 07:00 a.m., posture changed from sleep to upright position.

Outliers and excluded subjects per parameter are listed in Supplemental Table 1. Concentration ranges of all hematological parameters are shown in Figure 1.

Posture-dependent changes of hematological parameters

Concentrations of seven parameters (hemoglobin, hematocrit, erythrocytes, reticulocytes, platelets, and plateletcrit) were posture-dependent with an acute concentration drop at 11:30 p.m. (during transition from upright position to sleep position) and a prominent rise at 7 a.m. (change from sleep to upright position) [31]. Figure 2 shows the average concentrations during the day of the parameters with posture-dependent changes. For these parameters, variation components were analyzed separately for the day and night. Since biological variation within both time-frames was similar (data not shown), only day-variation components (08:30 a.m.–11:30 p.m. and 07:30 a.m. the next morning) are listed in Table 2 for these parameters.

24-Hour variation components

Variation components (CV_A , CV_I , and CV_G), indexes of individuality and RCVs are listed in Table 2. The uncertainties regarding the calculations are reflected in the 95% CI given for all CV values [24, 25].

All parameters showed higher CV_G than CV_I values. Highest CV_G was found for eosinophils (46.6%; 95% CI, 34.9%–70.1%) and the lowest value was MCHC (3.2%; 95% CI, 2.4%–4.8%). CV_I was much lower and varied from 0.4% (95% CI, 0.32%–0.42%) to 20.9% (95% CI, 19.4%–22.6%) of RDW and eosinophils, respectively. Duplicate measurements of all parameters offered the advantage of CV_A estimation at the same level as subject samples [20], and yielded an CV_A range from 0.17% (95% CI, 0.16%–0.18%) to 21.2% (95% CI, 19.9%–22.7%) (of MCV and basophils, respectively).

All parameters, except hematocrit, basophils, and monocytes, showed marked individuality, with individuality indexes <0.6 . The lowest calculated individuality index was 0.1 for MCV and RDW, and the highest

Table 2: Analytical variation, biological variation, indexes of individuality and reference change values for hematological parameters.

	n	Measured unit	Mean	CV_G , %	CV_I , %	CV_A , %	$RCV_{95\%}$, %	II
Routine hematological parameters								
Leukocytes	17	$10^9/L$	6.6	13.7 (10.2–21.0)	7.0 (6.6–7.6)	1.4 (1.3–1.5)	^b	0.5
Neutrophils	18	$10^9/L$	3.8	22.3 (16.7–33.5)	11.1 (10.3–11.9)	1.9 (1.8–2.0)	^b	0.5
Lymphocytes	18	$10^9/L$	1.9	26.0 (19.4–36.1)	15.0 (14.1–16.1)	2.7 (2.5–2.9)	^b	0.6
Eosinophils	18	$10^9/L$	0.2	46.6 (34.9–70.1)	20.9 (19.4–22.6)	9.3 (8.7–9.9)	^b	0.5
Basophils	19	$10^9/L$	0.03	32.2 (24.2–47.7)	8.8 (5.4–11.4)	21.2 (19.9–22.7)	^b	0.7
Monocytes	18	$10^9/L$	0.6	16.8 (12.5–25.3)	12.3 (11.4–13.3)	6.2 (5.8–6.6)	^b	0.8
Erythrocytes ^c	20	$10^{12}/L$	4.4	8.1 (6.1–11.8)	2.9 (2.7–3.1)	0.65 (0.61–0.71)	8.2	0.4
Reticulocytes, count ^c	21	$10^9/L$	57.2	23.5 (17.9–33.9)	5.1 (4.5–5.7)	4.7 (4.4–5.0)	19.2	0.3
Reticulocytes, percentage ^c	20	%	1.3	23.3 (17.7–34.1)	4.0 (3.5–4.6)	4.6 (4.3–4.9)	16.9	0.3
Hemoglobin ^c	20	mmol/L	8.5	7.2 (5.4–10.5)	2.7 (2.5–2.9)	0.55 (0.51–0.59)	7.6	0.4
Hematocrit ^c	17	L/L	0.4	4.1 (3.0–6.3)	3.0 (2.8–3.3)	0.6 (0.55–0.65)	8.5	0.8
MCV	17	fL	90.7	4.1 (3.1–6.3)	0.45 (0.42–0.48)	0.17 (0.16–0.18)	1.3	0.1
MCH	19	fmol	1.9	4.0 (3.0–5.9)	0.8 (0.78–0.89)	0.17 (0.16–0.17)	2.4	0.2
MCHC	19	mmol/L	21.3	3.2 (2.4–4.8)	0.8 (0.7–0.9)	0.8 (0.77–0.88)	3.2	0.4
MPV	18	fL	10.1	7.2 (5.4–10.8)	1.9 (1.7–2.1)	1.4 (1.3–1.5)	6.4	0.3
RDW	17	%	13.2	4.2 (3.1–6.3)	0.37 (0.32–0.42)	0.47 (0.44–0.50)	1.7	0.1
Platelets ^c	19	$10^9/L$	228	20.2 (15.2–29.8)	3.2 (2.9–3.5)	1.5 (1.4–1.6)	9.8	0.2
Research hematological parameters								
IPF	17	%	2.1	42.3 (31.4–64.5)	12.8 (11.6–14.0)	10.2 (9.5–10.9)	45.2	0.4
Plateletcrit ^c	16	%	0.2	20.1 (14.9–31.2)	3.5 (3.1–4.0)	2.8 (2.6–3.1)	12.6	0.2
PDW	18	fL	11.6	14.6 (10.9–21.9)	3.7 (3.2–4.2)	3.8 (3.5–4.2)	14.7	0.4

The 95% CIs intervals are shown in brackets. ^aOn the basis of duplicate measurements. ^bThis parameter has a diurnal rhythm and therefore, hour-to-hour RCVs are calculated (see Table 3 and Supplemental Table 1). ^cConcentrations are posture-dependent, hence only data from 08:30 a.m. to 11:30 p.m. and 07:30 a.m. were used. n, number of subjects after removing outliers; CV, coefficient of variation; CV_G , between-subject biological variation; CV_I , within-subject biological variation; CV_A , analytical variation; II, index of individuality; RCV, reference change value; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MPV, mean platelet volume; RDW, red cell distribution width; IPF, immature platelet fraction; PDW, platelet distribution width.

calculated II was 0.8 for hematocrit and monocytes. The RCVs ranged from 1.3% (MCV) to 45.2% (IPF).

Rhythmic diurnal variation in hematological parameters

Six hematological parameters showed a diurnal rhythm unrelated to posture-changes in blood volume: leukocytes, neutrophils, lymphocytes, eosinophils, basophils and monocytes. These intrinsic diurnal rhythms were previously described by Druzd et al. [32], Lasselin et al. [33], and Sennels et al. [10].

Rhythmic diurnal oscillations precluded calculation of overall variation components and RCVs. Instead, hour-to-hour RCVs were calculated, which take into account the structural change according to the diurnal rhythm. Hour-to-hour RCVs facilitate accurate interpretations of changes between two consecutive laboratory measurements within 24 h, taking into account the rhythmic diurnal oscillations. Hour-to-hour RCVs of neutrophils range from – 41% to 46%, depending on the time points chosen (Table 3). Hour-to-hour RCVs for other parameters with a diurnal rhythm are presented in Supplemental Table 2A-E.

Physiological basis of rhythmic diurnal variation

Rhythmic oscillations of parameters can be either intrinsic or in response to circadian variation of other parameters such as cortisol. As an illustration, we measured cortisol in all subjects of this study and plotted diurnal cortisol concentrations and lymphocytes together in Supplemental Figure 1. In this figure, exactly the opposite rhythmic oscillations are evident. This is in line with the established physiological effects of cortisol on lymphocyte levels [34, 35].

Discussion

This study presents novel 24-h variability profiles for 20 hematological parameters. We have calculated variation components (CV_A , CV_I , and CV_G), indexes of individuality and RCVs of these parameters in order to better differentiate true changes from random fluctuations when interpreting changes between consecutive laboratory measurements within a day. Repeated within-day testing is customary in various clinical settings, e.g. the evaluation of therapeutic interventions, or when monitoring the

disease course. The availability of comprehensive, 24-h variability data in the scientific literature represents a major gap for most laboratory parameters.

Comprehensive 24-h sampling allowed to investigate diurnal cyclical rhythms and, if present, to take into account the ‘time of sample-collection’ as a determinant of natural fluctuation. For the six parameters with a detectable diurnal rhythm, we present for the first time hour-to-hour RCVs that may help to objectively evaluate changes between any two time-points of the day.

Hour-to-hour RCVs

RCV values describe the maximum non-significant hour-to-hour changes that might be seen in a ‘steady state situation’. In a parameter with a diurnal rhythm, there is no steady state situation, because the level of the parameter changes randomly as well as systematically over time. If the systematic component is ignored, the CV_I ’s will be inflated and the RCVs will be systematically biased up or down depending on the hour. As a result, the RCVs will not properly reflect the bandwidth within which values are to be expected with a 95% probability.

To calculate unbiased RCVs, we calculated the β value for each time point as a percentage from the first result. The data was then adjusted according to the β value, and an adjusted steady state was achieved. After calculating the CV_I ’s and CV_A ’s, the β ’s were combined with the CV_I ’s and CV_A ’s in order to get an asymmetrical hour-to-hour RCV with an upper and lower limit. This method was first described by Aakre et al. [28].

By implementing the β ’s the way we did, we were able to fully correct for any variation in parameter levels during the day. The advantage of our approach is that it yields unbiased results, but the downside is that it requires a substantial number of betas to be estimated on the data, potentially increasing estimation error. Other approaches, such as fitting a (co)sinus function to the data, make stronger assumptions about the nature of the fluctuations in the analyte during the day, which allows them to be implemented with fewer betas to estimate and, accordingly, reduced estimation error. However, if the inherent assumptions about the nature of the fluctuations are not correct, they yield biased results. In this trade-off, we chose to avoid making additional assumptions and, with that, to avoid introducing bias, at the cost of estimating more betas. What approach is optimal in the context of diurnal parameter rhythms is to our knowledge still an open question, and may vary per parameter. However, for parameters such as neutrophils, any of these approaches

is an improvement over the old assumption of a constant diurnal level.

The asymmetry of the calculated hour-to-hour RCVs in Table 3 shows how different they can be from standard symmetrical RCVs and underlines the clinical importance of letting go of the restricting assumption of a constant diurnal level.

Influence of fasting vs. non-fasting

To the best of our knowledge, fasting has no strong effect on the hematological parameters studied. However, since mealtimes (including breakfast) were standardized across all subjects, we were unable to explore the effect of fasting and mealtimes.

Comparison of CV_i values to those in previous studies

In general, the CV_i values of this study were comparable to those in previous biological variation studies with hematological parameters. Overall, previous within day biological variation studies reported slightly higher CV_i values, except for MCV and PDW [9, 10]. However, slightly lower values were found in previous studies when focusing on the between-day biological variation. As an example, the study of Fraser et al. reported CV_i values of 9.4 for lymphocytes, compared to 15.0 (14.1–16.1) in our study [36].

In Supplemental Table 3, CV_i values of the present study are listed, together with results from previous biological variation studies.

Gradual decrease of hemoglobin and hematocrit during the day

A remarkable finding is the gradual decrease of hemoglobin, hematocrit, and erythrocytes during the day, before the ‘acute concentration drop’ due to the transition from an upright to lying position in the evening. The other parameters that were posture-dependent did not show such a gradual decrease during the day. A dilution effect for the gradual decrease is unlikely since other parameters such as platelets are unaffected.

Hemoglobin concentration decreased from 8.2 mmol/L in sitting position to 7.9 mmol/L in lying position (–3.8%) and increased from 8.0 to 8.7 mmol/L (+8.8%) during the transition from a lying to an upright position. Parallel changes were found for hematocrit,

0.37 L/L–0.38 L/L (–2.6%) and from 0.37 L/L to 0.40 L/L (+8.1%), respectively.

Index of individuality

Parameters with individuality indexes of >1.4 show very little individuality and, therefore, the use of reference values is considered appropriate because unusual values for almost all subjects will lie outside the reference limits [18, 26, 37]. For parameters with individuality indexes of <0.6 however, population based reference values have limited utility, and the use of RCVs may have added value. Analysis of the samples obtained from participants in this study revealed that all parameters, except hematocrit, basophils, and monocytes, showed substantial individuality indexes below 0.6 (Table 2). Consequently, for almost all of the studied parameters, calculated RCVs may offer substantial benefit when monitoring a patient within a day.

Study limitations

Some limitations in the study population merit attention. (1) We have included 16–21 subjects (depending on the parameter) in biological variation analyses. Although the number of excluded subjects was rather high for some parameters (i.e. PCT, eight subjects), 16 subject still afforded sufficient power to make reliable estimations for the various components of variation [24]. (2) Due to disproportional inclusion of both sexes (men $n=19$, women $n=5$), we were unable to assess the appropriateness of partitioning CV_i and CV_g by sex. (3) The age range of the subjects was 58–82 years. Although this age category corresponds to the age of the average hospitalized patient, the results of this study cannot be extrapolated to other age categories. (4) RCV calculations in this study were based on the CV_A values reported in Table 2. However, CV_A is to some degree concentration-dependent and RCVs may vary according to the concentration of the parameter. However, for the majority of the routine hematological parameters (65%), CV_A was $\leq 1/2 CV_i$, which diminishes the contribution of CV_A to the overall variation to less than 12% [20].

Conclusions

This is the first study that assesses the within-day biological variation of most of the relevant hematological parameters by using hourly blood samples. Moreover, it is the

first to study the hour-to-hour fluctuations in parameter levels that occur for certain parameters during the day. It presents CV_G , CV_I , CV_A , indexes of individuality and RCVs for 20 hematological parameters, and where appropriate adjusts the RCVs for diurnal rhythms. Twenty-four-hour biological variation profiles are an important part of the scientific basis for better interpretation of consecutive within day laboratory measurements.

Acknowledgments: The skillful technical assistance of the laboratory research analyst V. W. Kleijnen is gratefully acknowledged.

Author contributions: All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.

Research funding: This study was supported by an Academic Incentive Grant from Maastricht University Medical Center to S.J.R.M.

Employment or leadership: None declared.

Honorarium: None declared.

Competing interests: The funding organization(s) played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.

References

- Fraser CG. Reference change values. *Clin Chem Lab Med* 2012;50:807–12.
- Bailey D, Bevilacqua V, Colantonio DA, Pasic MD, Perumal N, Chan MK, et al. Pediatric within-day biological variation and quality specifications for 38 biochemical markers in the caliper cohort. *Clin Chem* 2014;60:518–29.
- Nunes LA, Brenzikofer R, de Macedo DV. Reference change values of blood analytes from physically active subjects. *Eur J Appl Physiol* 2010;110:191–8.
- Maes M, Scharpe S, Cooreman W, Wauters A, Neels H, Verkerk R, et al. Components of biological, including seasonal, variation in hematological measurements and plasma fibrinogen concentrations in normal humans. *Experientia* 1995;51:141–9.
- Dot D, Miro J, Fuentes-Arderiu X. Within-subject biological variation of hematological quantities and analytical goals. *Arch Pathol Lab Med* 1992;116:825–6.
- Pineda-Tenor D, Laserna-Mendieta EJ, Timon-Zapata J, Rodelgo-Jimenez L, Ramos-Corral R, Recio-Montealegre A, et al. Biological variation and reference change values of common clinical chemistry and haematologic laboratory analytes in the elderly population. *Clin Chem Lab Med* 2013;51:851–62.
- Rudez G, Meijer P, Spronk HM, Leebeek FW, ten Cate H, Kluft C, et al. Biological variation in inflammatory and hemostatic markers. *J Thromb Haemost* 2009;7:1247–55.
- Statland BE, Winkel P, Harris SC, Burdsall MJ, Saunders AM. Evaluation of biologic sources of variation of leukocyte counts and other hematologic quantities using very precise automated analyzers. *Am J Clin Pathol* 1978;69:48–54.
- Yang D, Zhou Y, Yang C. Daytime biological variation of hematological parameters in a healthy chinese population. *Int J Lab Hematol* 2016 [Epub ahead of print].
- Sennels HP, Jorgensen HL, Hansen AL, Goetze JP, Fahrenkrug J. Diurnal variation of hematology parameters in healthy young males: the bispebjerg study of diurnal variations. *Scand J Clin Lab Invest* 2011;71:532–41.
- Zhang P, Tang H, Chen K, Chen Y, Xu D. Biological variations of hematologic parameters determined by unice1 dxh 800 hematology analyzer. *Arch Pathol Lab Med* 2013;137:1106–10.
- Harris EK. Statistical aspects of reference values in clinical pathology. *Prog Clin Pathol* 1981;8:45–66.
- Bartlett WA, Braga F, Carobene A, Coskun A, Prusa R, Fernandez-Calle P, et al. A checklist for critical appraisal of studies of biological variation. *Clin Chem Lab Med* 2015;53:879–85.
- Klinkenberg LJ, Wildi K, van der Linden N, Kouw IW, Niens M, Twerenbold R, et al. Diurnal rhythm of cardiac troponin: consequences for the diagnosis of acute myocardial infarction. *Clin Chem* 2016;62:1602–11.
- American Diabetes A. Diagnosis and classification of diabetes mellitus. *Diabetes Care* 2004;27(Suppl 1):S5–10.
- Inker LA, Schmid CH, Tighiouart H, Eckfeldt JH, Feldman HI, Greene T, et al. Estimating glomerular filtration rate from serum creatinine and cystatin c. *N Engl J Med* 2012;367:20–9.
- Burnett RW. Accurate estimation of standard deviations for quantitative methods used in clinical chemistry. *Clin Chem* 1975;21:1935–8.
- Fraser CG, Harris EK. Generation and application of data on biological variation in clinical chemistry. *Crit Rev Clin Lab Sci* 1989;27:409–37.
- IUPAC. Protocol for the design, conduct and interpretation of method performance studies. *Pure Appl Chem* 1995;67:331–43.
- Fraser CG. Biological variation: from principles to practice. Washington DC: AACC Press, 2001.
- Carlsen S, Petersen P, Skeie S, Skadberg O, Sandberg S. Within subject biological variation of glucose and HbA1c in healthy persons and in type 1 diabetes patients. *Clin Chem Lab Med* 2011;49:1501–7.
- Reed AH, Henry RJ, Mason WB. Influence of statistical method used on the resulting estimate of normal range. *Clin Chem* 1971;17:275–84.
- Dixon WJ. Processing data for outliers. *Biometrics* 1953;9:74–89.
- Roraas T, Petersen PH, Sandberg S. Confidence intervals and power calculations for within-person biological variation: effect of analytical imprecision, number of replicates, number of samples, and number of individuals. *Clin Chem* 2012;58:1306–13.
- Burdick RK, Graybill FA. Confidence intervals on variance components. New York: Marcel Dekker, 1992.
- Petersen PH, Fraser CG, Sandberg S, Goldschmidt H. The index of individuality is often a misinterpreted quantity characteristic. *Clin Chem Lab Med* 1999;37:655–61.
- Harris EK, Yasaka T. On the calculation of a “reference change” for comparing two consecutive measurements. *Clin Chem* 1983;29:25–30.
- Aakre KM, Roraas T, Petersen PH, Svarstad E, Sellevoll H, Skadberg O, et al. Weekly and 90-minute biological variations in cardiac troponin t and cardiac troponin i in hemodialysis patients and healthy controls. *Clin Chem* 2014;60:838–47.

29. Solvik UO, Petersen PH, Monsen G, Stavelin AV, Sandberg S. Discrepancies in international normalized ratio results between instruments: a model to split the variation into subcomponents. *Clin Chem* 2010;56:1618–26.
30. McDonald JH. *Handbook of biological statistics*, 3rd ed. Baltimore, Maryland: Sparky House Publishing, 2014.
31. Felding P, Tryding N, Hyltoft Petersen P, Horder M. Effects of posture on concentrations of blood constituents in healthy adults: practical application of blood specimen collection procedures recommended by the scandinavian committee on reference values. *Scand J Clin Lab Invest* 1980;40:615–21.
32. Druzd D, de Juan A, Scheiermann C. Circadian rhythms in leukocyte trafficking. *Semin Immunopathol* 2014;36:149–62.
33. Lasselin J, Rehman JU, Akerstedt T, Lekander M, Axelsson J. Effect of long-term sleep restriction and subsequent recovery sleep on the diurnal rhythms of white blood cell subpopulations. *Brain Behav Immun* 2015;47:93–9.
34. Mazzocchi G, Corra M, Bianco G, De Cata A, Balzanelli M, Giuliani A, et al. Age-related changes of neuro-endocrine-immune interactions in healthy humans. *J Biol Regul Homeost Agents* 1997;11:143–7.
35. Mazzocchi G, Carughi S, Sperandio M, Paziienza V, Giuliani F, Greco A. Alteration of circadian rhythmicity of cd3+cd4+ lymphocyte subpopulation in healthy aging. *J Biol Regul Homeost Agents* 2011;25:405–16.
36. Fraser CG, Wilkinson SP, Neville RG, Knox JD, King JF, MacWalter RS. Biologic variation of common hematologic laboratory quantities in the elderly. *Am J Clin Pathol* 1989;92:465–70.
37. Iglesias N, Petersen PH, Ricos C. Power function of the reference change value in relation to cut-off points, reference intervals and index of individuality. *Clin Chem Lab Med* 2005;43:441–8.

Supplemental Material: The online version of this article (DOI: 10.1515/cclm-2016-0716) offers supplementary material, available to authorized users.