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Abstract

Objectives: Our aim was to derive reference intervals for all Sysmex XN hematology analyzer parameters. The rationale behind the study was the lack of reference intervals for the XN analyzer cell population data (CPD) and functional parameters.

Methods: Fresh fasting blood samples from 18,484 participants in the Dutch Lifelines study were analyzed using two automated XN analyzers. Structured health questionnaire data were used to select a subgroup of 15,803 apparently healthy individuals for inclusion in the reference population. The Latent Abnormal Values Exclusion (LAVE) approach was used to reduce the influence of latent diseases in the reference population on the resulting reference intervals. We applied analysis of variance to judge the need for partitioning of the reference intervals by sex or age.

Results: We report reference intervals for 105 XN analyzer hematological parameters with and without applying LAVE. Sex-related partitioning was required for red blood cells, (RBC, RBC-O), hemoglobin (HGB, HGB-O), hematocrit (HCT), mean corpuscular hemoglobin concentration (MCHC), reticulocyte production index (RPI), and side scattered light intensity of the red blood cell population in the RET channel (RBC-Z). Partitioning for age was not warranted. Body mass index (BMI) and smoking had moderate influence on a minority of the parameters.

Conclusions: We provide reference intervals for all Sysmex XN analyzer routine, CPD and functional parameters, using a direct approach in a large cohort in the Netherlands.

Keywords: cell population data (CPD); functional parameters; hematology; latent abnormal value exclusion (LAVE); reference interval (RI); Sysmex XN analyzer.

Introduction

Reference intervals (RIs) for routine hematological parameters are widely publicized. However, modern-day hematology analyzers can report more than just the routine hematological parameters. The flow cytometric technique employed by most of the modern-day hematology analyzers yields additional information that may reflect differences in developmental stage and functional status of subpopulations of red and white blood cells and thrombocytes. Over the past years these cell population parameters have gained interest as they may be correlated with specific diagnoses or immunological response patterns [1–7]. In this report we refer to these parameters collectively as cell population data (CPD) and functional parameters.

One of the drawbacks of many of these parameters is the lack of RIs. The laboratory of the University Medical Center Groningen (UMCG) is in a unique position to provide such RIs for the Sysmex XN hematology analyzer, as we provide the laboratory services for Lifelines, a large, three-generational cohort study that includes over 167,000 participants [8, 9]. For research purposes, we extended the analysis of the routine laboratory parameters to all XN parameters on blood drawn from Lifelines participants.

RIs can be derived through direct and indirect methods. Indirect methods apply statistical techniques to estimate RIs from laboratory datasets established for other purposes [10]. The direct methods encompass selection of reference individuals recruited using specific, well-defined criteria. The CLSI C28-A3 document [11] is a well-known protocol for the selection of subjects and proper statistical

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analysis. In recent years, the Committee on RIs and Decision limits (C-RIDL) of the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) has produced protocols and standard operating procedures for use in large, national, or international multicenter RI studies [12–14]. Both protocols rely on health questionnaires for the selection of healthy subjects for the reference population. Lifelines participants fill in extensive questionnaires at inclusion and as a part of their 5-yearly visits.

Health questionnaires will not reveal latent medical conditions, whereas it may be important to exclude subjects with such conditions as much as possible from RI studies [11]. As an example, latent anemia will not be reported in a health questionnaire, but when calculating RIs for mean corpuscular volume (MCV), one may reason that MCV results from subjects with a hemoglobin (HGB) concentration below its RI should not be included in the calculation. The C-RIDL working group has proposed the Latent Abnormal Value Exclusion (LAVE) approach as a possible solution to this problem [15, 16]. The LAVE algorithm iteratively refines the RIs by excluding the subjects with 'abnormal values' until the convergence criteria are met. A limited number of parameters which are deemed to be associated with latent clinical conditions, are chosen to serve as index parameters. In the first iteration all results are included in the calculation. By definition, five percent of the values for the index parameters are outside their calculated RIs. In the second iteration, results for all other parameters from subjects who have a result outside calculated RI of the index parameters, are excluded. In the anemic subject example, the HGB result is kept in the calculation for the HGB RI, but all other results, including the MCV result, are excluded from the calculation in the second iteration. Thus, the RI for HGB is not truncated in the second iteration, but the RIs for all other parameters will not be influenced by data from (latently) anemic subjects.

We report RIs with and without applying LAVE.

Materials and methods

Lifelines recruitment and sample acquisition

Lifelines is a multi-disciplinary prospective population-based cohort study examining in a unique three-generation design the health and health-related behaviors of 167,729 persons living in the North of the Netherlands. It employs a broad range of investigative procedures in assessing the biomedical, socio-demographic, behavioral, physical and psychological factors which contribute to the health and disease of the general population, with a special focus on multi-morbidity and complex genetics. Participants were recruited through general practitioners in three provinces in the northern part of The Netherlands: Groningen, Friesland and Drenthe [8]. Subjects could not take part if they had: (a) limited life expectancy (<5 years); (b) severe psychiatric or physical illness; or (c) were unable to read Dutch. After the informed consent form was signed, participants were asked to complete an extensive questionnaire to collect general health information and blood and urine samples were collected. For the present study, from January 2014 until January 2015 a total of 18,484 participants were included, aged 20–92 years.

The Lifelines cohort study is conducted according to the principles of the Declaration of Helsinki and in accordance with UMCG research code. The study was approved by the Medical Ethics Committee of the UMCG, The Netherlands (METc 2007/152).

A posteriori inclusion of lifelines participants for the RI study

Participants from the Lifelines cohort were excluded if they fulfilled one or more of the following criteria: active cancer or a history of cancer; history of stroke; diabetes mellitus (self-reported; HbA_{1c}≥47.5 mmol/mol; or fasting plasma glucose≥7.0 mmol/L); chronic liver disease; chronic kidney disease (self-reported; eGFR (CKD-Epi)<60 mL/min/1.73 m^2); renal failure; or currently pregnant. A total of 15,803 participants were eligible for inclusion in our dataset.

Sample analysis

Fasting blood samples were taken by venipuncture at one of the Lifelines research sites. The blood samples were kept at 4 °C and were transported to the Lifelines laboratory in Groningen. From there, the samples were transferred without delay to the UMCG central laboratory where the routine clinical chemistry and hematological tests were performed. The whole process was tightly controlled and monitored continuously [8]. The fresh EDTA anticoagulated blood samples were analyzed within 6 h after venipuncture on one of two automated hematology analyzers (XN-series, Sysmex, Kobe, Japan).

Quality control

Installation and regular calibration of the analyzers were performed according to the manufacturer's recommendations. Daily internal quality control was performed at normal and pathological concentrations prior to sample testing using three-level commercial quality controls (XN Check Control, Sysmex, Kobe, Japan). The laboratory participates in external quality control (EQC) schemes organized by the Dutch EQAS organization (SKML) for routine blood count parameters (HGB, RBC, MCV, HCT, WBC, neutrophils, eosinophils, lymphocytes, monocytes, and platelets). There were no performance issues in the EQC during the course of the study.

Statistical methods

The statistical methods used to derive the RIs and specifically the application of LAVE, have been described extensively by members of the C-RIDL working group [15, 16].

To evaluate the impact of lifestyle factors (BMI, current smoking and alcohol consumption), we performed multiple regression analysis

(MRA). The independent effect of each of these sources of variation was calculated as the standardized partial regression coefficient (rp). A cut-off value of ±0.2 was used to discriminate between a slight and moderate contribution to RIs, whereas a rp larger than ±0.4 was considered a prominent association [17].

Reported units

We have followed the 2016 ICSH recommendations for reporting units [18]. For data expressed in other customary units we refer readers to the Supplemental Material.

Derivation of RIs

RIs were derived parametrically after data transformation to a Gaussian distribution using a modified Box-Cox transformation [13]. Conformity of the data to the Gaussian distribution was assessed using visual inspection of histograms and probability plots before and after Box-Cox transformation (Figure 1A). Additionally, the following cut-off values were used to quantitatively judge normality after transformation: Skewness (<–1 or >1) and Kurtosis (<–2 or >2). The mean ± 1.96 SD were calculated to define the limits of the RI after power transformation. For parameters failing to achieve a near Gaussian distribution, RIs were calculated non-parametrically, using the 2.5 and 97.5% centiles in their distribution as limits.

Quantifying the impact of LAVE on RIs

RIs were derived with and without applying LAVE. In LAVE(–) RIs were calculated in a single iteration based on all results from all subjects. To further refine the RIs we chose HGB, MCV, red blood cells (RBC), reticulocyte count (RET#), neutrophil count (NEUT#), lymphocyte count (LYMPH#), monocyte count (MONO#), platelet count (PLT#), and mean platelet volume (MPV) as index parameters to identify individuals with possible, sub-clinical medical conditions such as anemia and (chronic) inflammation. LAVE abnormal 0, abbreviated as LAVE(+)Abn0 in this report, only accepts a subject's results if there are no (zero) results outside the calculated RIs for all index parameters. LAVE(+)Abn1 accepts one result outside the calculated RI of an index parameter in a single subject. LAVE(+)Abn2 accepts two results outside the calculated RIs in two index parameters. RIs converged after six iterations (Figure 1B).

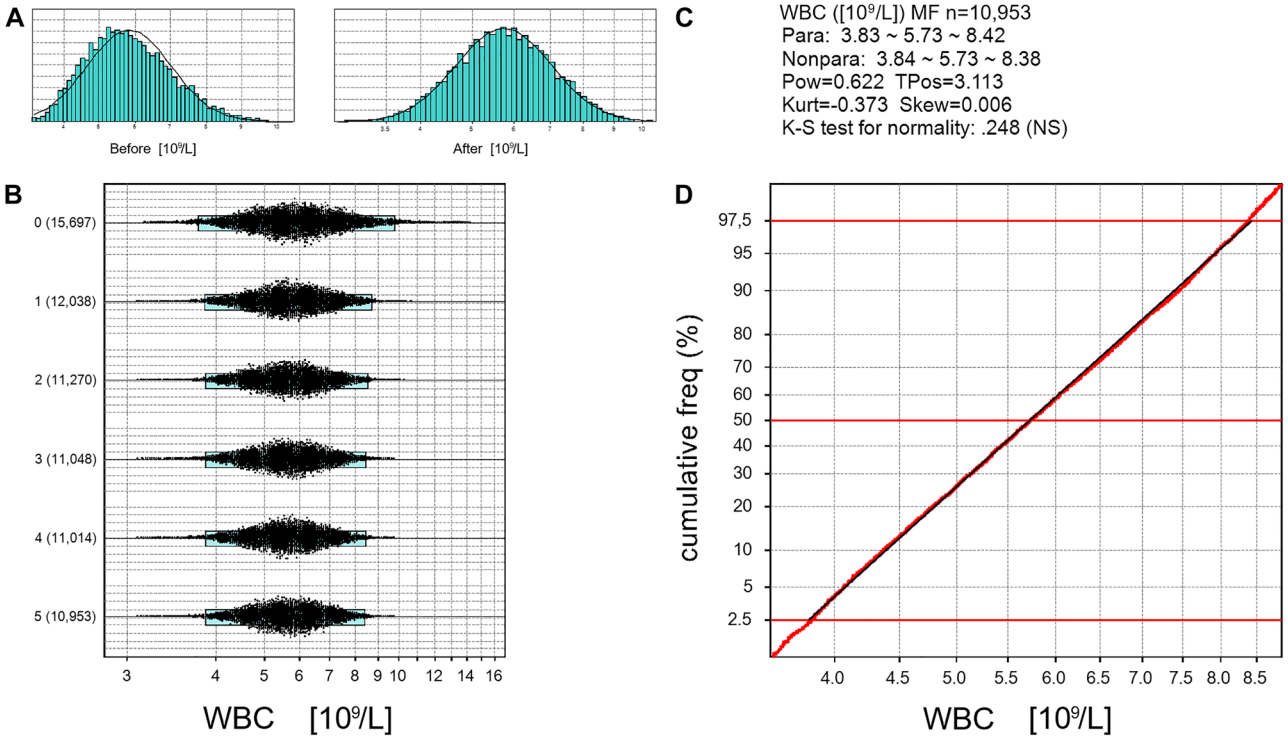

Figure 1: Box-Cox power transformation and LAVE iterations.
LAVE(+)Abn0 analysis of WBC [10^9/L] for the pooled cohort, males and females together, is demonstrated as an exemplary parameter. In the histograms in (A), the distribution of the analyzed parameter is depicted before (left) and after (right) Box-Cox power transformation. The serial changes of the RI width during the LAVE procedure are shown in (B). The box in the scattergram represents 95% interval. After the number of iterations, the number of values which remained in the calculation is indicated in brackets. In (C) outputs of RIs, by both parametric (Para) and non-parametric (Nonpara) methods are given together with predicted power (Pow), transform origin (TPos) to obtain Gaussian distribution, kurtosis (Kurt) and skewness (Skew) of the distribution, as well as kurtosis and skewness test (K-S test) for normality. (D) shows the probability plot for the data. The Y-axis represents cumulative frequency (%) and the x-axis represents test values in the power transformed scale. The linearity of the red cumulative frequency line between 10 and 90% indicates conformity of the distribution to the Gaussian form (Source: Ichihara K, Yamashita T. User Manual for RI-Master 2020).

The effect of each method on the RI width was assessed using the following equations:

$$\Delta LL\ ratio = |LL+ - LL-|/((UL- - LL-)/3.92)$$
$$\Delta UL\ ratio = |UL+ - UL-|/((UL- - LL-)/3.92)$$

where LL+ and UL+ are lower and upper reference limits with LAVE, while LL– and UL– represent reference limits determined without LAVE. The magnitude of differences in UL or LL of the RIs is expressed as their ratio to the standard deviation of the RI calculated without applying LAVE ((UL– – LL–)/3.92). We adopted the decision limit for significant change of 0.25 from Ozarda et al. [19].

Stratification criteria

To judge the need for stratification by sex and age, the magnitude of between-subgroup variations of RIs was estimated as a standard deviation ratio (SDR) by analysis of variance (ANOVA). Initially, a two-way nested ANOVA for combined analysis of the two factors was done, followed by a one-way ANOVA for age, performed separately for each sex. We categorized the subjects in the following age-groups: 20–29; 30–39; 40–49; 50–59; 60–69; and 70–92 years. An SDR ≥ 0.4 was regarded as an indication to consider partition of an RI [15, 20].

Statistical software

Summary statistics and MRA were performed using Stata MP13.1 (Stata Corp., Texas, USA). A software named 'RI-Master' developed by Kiyoshi Ichihara was employed to derive RIs based on the LAVE approach.

Results

A total of 15,803 subjects, aged 20–92 years, were selected to serve as a reference population after a posteriori exclusion of 2,681 subjects from the original 18,484 Lifelines participants in the dataset. Table 1 summarizes the baseline characteristics of the study population. The abbreviations for the XN analyzer parameters are explained in Table 2.

Lifestyle related sources of variation

MRA (Supplemental Table 1) revealed moderate (rp≥0.2 or ≤–0.2) BMI-related changes for RET#/%, MFR, LFR, IRF and RPI in both sexes, for RBC and RBC-O in females, and for HFR and RBC-X in males. Moderate smoking-related changes were observed for RE-LYMP# in both sexes and for TNC, WBC, WBC-D, WBC-P, NEUT# and LYMPH# in males. Alcohol consumption was not associated with significant change in any of the parameters, bearing in mind that chronic liver disease and diabetes were among the exclusion criteria.

Reference intervals

We have compiled RIs for 105 Sysmex XN parameters, calculated without and with the application of LAVE (Supplemental Table 2 in electronic format for ease of transcription if transfer to a laboratory information system is desired). Additionally, LAVE(+)Abn1 RIs are compiled in Table 3. We calculated the SDR for sex and age to indicate the need for partitioning. The SDRage was below the threshold for all parameters. In the erythrocytic cell lineage the SDRsex was ≥0.4 for RBC, RBC-O, HGB, HGB-O, HCT, MCHC, MacroR (LAVE(+)Abn1 and LAVE(+)Abn0), HYPER-H*e*, RPI, and RBC-Z; these values were partitioned except for MacroR and HYPER-H*e* because the differences between resulting RIs would be small and clinically irrelevant (Table 3A). In the leucocytic (Table 3B) and thrombocytic (Table 3C) cell lineages none of the parameters had an SDRsex ≥ 0.4. Plots of sex-related differences in RIs are shown in Figure 2 and RIs calculated with and without partitioning for sex in Supplemental Table 2.

Impact of LAVE

Exclusion of latently abnormal values reduces the width of the calculated RIs. Columns W – AB in Supplemental Table 2, summarize the relative changes of LL and UL of the RIs due to the application of LAVE. A relative change ≥0.25 SD (of the RI calculated without LAVE), was considered significant [19]. LAVE(+)Abn0 induced a relative change ≥0.25 SD in 48/105 (45.7%) of the parameters; the effect of LAVE(+)Abn1 was ≥0.25 SD in 18/105 (17.1%) of the parameters; the effect of LAVE(+)Abn2 was ≥0.25 SD in 7/105 (6.7%) of the parameters. The effect of LAVE on the RI of the WBC count is shown graphically in Figure 3.

Discussion

In this study, we established RIs for all Sysmex XN analyzer routine, CPD and functional parameters in the large, well-defined Lifelines cohort at the UMCG laboratory in the Netherlands.

We performed our calculations with and without LAVE. Applying LAVE revealed which parameters bear a relation to others and which parameters may be regarded as physiologically independent. Although HGB, RBC#, MCV, MCH, MCHC are all physiologically connected, the changes in RIs with and without LAVE were neither very large in relative nor in absolute numbers. For instance, LAVE(+)Abn0 raised the LL for HGB in males with 0.29 SD

Table 1: Baseline characteristics of Lifelines cohort participants.

	Males	Females
Total number of participants enrolled	7,553 (40.9%)	10,931 (59.1%)
Exclusion criteria		
Aged blood sample	<10 (0.05%)	<10 (0.06%)
Active cancer or history of cancer	355 (4.7%)	691 (6.3%)
History of stroke	112 (1.5%)	110 (1.0%)
Diabetes mellitus (self-reported)	363 (4.8%)	364 (3.3%)
$HbA_{1c} \geq 47.5$ mmol/mol	312 (4.1%)	263 (2.4%)
Fasting plasma glucose ≥7.0 mmol/L	314 (4.2%)	261 (2.4%)
Chronic liver disease	108 (1.4%)	168 (1.5%)
Chronic kidney disease (self-reported)	47 (0.6%)	72 (0.7%)
eGFR (CKD-Epi) <60 mL/min/1.73 m2	151 (2.0%)	199 (1.8%)
Renal failure	13 (0.2%)	15 (0.1%)
Currently pregnant	<10 (0%)	<10 (0.04%)
Number of remaining participants in the reference population	6,424 (40.7%)	9,379 (59.3%)
Country of birth		
Europe	6,389 (99.5%)	9,332 (99.5%)
Other	34 (0.5%)	48 (0.5%)
Smoking behavior		
Current smoker	988 (15.4%)	1,327 (14.1%)
Ex-smoker	1838 (28.6%)	2,648 (28.2%)
Never smoked	2,769 (43.1%)	4,433 (47.3%)
Data not available	829 (12.9%)	971 (10.4%)
Alcohol consumption		
Mild drinker [m: 1 alc-d/d, f: 1 alc-d/d]	626 (9.8%)	1735 (18.5%)
Moderate drinker [m: 2–4 alc-d/d, f: 2–3 alc-d/d]	3,045 (47.4%)	3,435 (36.6%)
Heavy drinker [m: >4 alc-d/d, f: >3 alc-d/d]	663 (10.3%)	429 (4.6%)
Data not available	2090 (32.5%)	3,780 (40.3%)
Blood pressure		
Diastolic blood pressure, mmHg (median)	77	71
Systolic blood pressure, mmHg (median)	131	123
Body weight		
Body mass index, kg/m^2 (median)	25.9	25
Underweight	10 (0.17%)	89 (0.95%)
Normal weight	2,404 (37.4%)	4,520 (48.2%)
Overweight	3,171 (49.4%)	3,272 (34.9%)
Obese	837 (13.0%)	1,493 (15.9%)
Data not available	<10 (0.03%)	<10 (0.05%)
Blood glucose, mmol/L (median)	5.1	4.9
HbA_{1c}, mmol/mol (median)	35.5	35.5
Sex distribution		
Age, years (median)	50	49
20–29 years	212 (3.3%)	417 (4.4%)
30–39 years	968 (15.1%)	1,291 (13.8%)
40–49 years	1,988 (31.0%)	3,096 (33.0%)
50–59 years	2,142 (33.3%)	3,113 (33.2%)
60–69 years	811 (12.6%)	1,085 (11.6%)
70–92 years	303 (4.7%)	377 (4.0%)

Data are presented separately for males and females. Participants were excluded if they fulfilled one or more of the listed exclusion criteria. Participants were categorized into six age groups. Alc-d/d, alcoholic drinks per day.

of the RI calculated without LAVE, but in absolute numbers the LL only changed from 133 to 136 g/L. LAVE(+)Abn0 raised the LL of MCV with 0.26 SD from 82.4 to 83.3 fl in both genders.

The effect of LAVE on other parameters was more pronounced. LAVE(+)Abn0 lowered the UL of WBC by 0.90 SD from 9.8 to $8.4 \cdot 10^9$/L. LAVE(+)Abn0 raised the LL of the platelet count (PLT-I) by 0.26 SD from 160 to $174 \cdot 10^9$/L. In

Table 2: Sysmex XN analyzer parameters.

XN parameter	Explanation
AS-LYMP	Antibody-synthesizing lymphocytes
AS-LYMP, %WBC	Antibody-synthesizing lymphocytes as a percentage of all white blood cells
AS-LYMP, %LY	Antibody-synthesizing lymphocytes as a percentage of all lymphocytes
BA-WX	Fluorescent light distribution width of the basophil population in the WNR channel
BA-WY	Forward scattered light distribution width of the basophil population in the WNR channel
BA-X	Mean fluorescent light intensity of the basophil population in the WNR channel
BA-Y	Mean forward scattered light intensity of the basophil population in the WNR channel
BASO	Basophils
BASO-D	Basophils as measured in the WDF channel
Delta-H*e*	Difference of hemoglobin equivalent between RET and RBC
Delta-HGB	Difference of hemoglobin concentration between HGB (RBC/PLT channel) and HGB-O (RET channel)
EO	Eosinophils
EO-WX	Side scattered light distribution width of the eosinophil population
EO-WY	Fluorescent light distribution width of the eosinophil population
EO-WZ	Forward scattered light distribution width of the eosinophil population
EO-X	Mean side scattered light intensity of the eosinophil population
EO-Y	Mean fluorescent light intensity of the eosinophil population
EO-Z	Mean forward scattered light intensity of the eosinophil population
FRC	Fragmented red blood cells
HCT	Hematocrit
HFLC	High fluorescent lymphocyte count
HFR	High fluorescent reticulocytes
HGB	Hemoglobin concentration as measured in the RBC/PLT channel
HGB-O	Hemoglobin concentration as measured in the RET channel
H-IPF	High fluorescent immature platelet fraction
HYPO-H*e*	Red blood cells with a low (hypochromic) hemoglobin equivalent
HYPER-H*e*	Red blood cells with a high (hyperchromic) hemoglobin equivalent
IG	Immature granulocytes
IPF	Immature platelet fraction
IRF	Immature reticulocyte fraction
IRF-Y	Mean forward scattered light intensity of the immature reticulocyte fraction
LFR	Low fluorescent reticulocytes
LYMPH	Lymphocytes
LY-WX	Side scattered light distribution width of the lymphocyte population
LY-WY	Fluorescent light distribution width of the lymphocyte population
LY-WZ	Forward scattered light distribution width of the lymphocyte population
LY-X	Mean side scattered light intensity of the lymphocyte population
LY-Y	Mean fluorescent light intensity of the lymphocyte population
LY-Z	Mean forward scattered light intensity of the lymphocyte population
MacroR	Macrocytic red blood cells
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration as measured in the RBC/PLT channel
MCHC-O	Mean corpuscular hemoglobin concentration as measured in the RET channel
MCV	Mean corpuscular volume
MFR	Medium fluorescent reticulocytes
MicroR	Microcytic red blood cells
MONO	Monocytes
MO-WX	Side scattered light distribution width of the monocyte population
MO-WY	Fluorescent light distribution width of the monocyte population
MO-WZ	Forward scattered light distribution width of the monocyte population
MO-X	Mean side scattered light intensity of the monocyte population
MO-Y	Mean fluorescent light intensity of the monocyte population
MO-Z	Mean forward scattered light intensity of the monocyte population
MPV	Mean platelet volume
NEUT	Neutrophils
NEUT-GI	Neutrophil granularity intensity; formerly NEUT-SSC

Table 2: (continued)

XN parameter	Explanation
NEUT-RI	Neutrophil reactivity intensity; formerly NEUT-SFL
NE-WX	Side scattered light distribution width of the neutrophil population
NE-WY	Fluorescent light distribution width of the neutrophil population
NE-WZ	Forward scattered light distribution width of the neutrophil population
NE-Z	Mean forward scattered light intensity of the neutrophil population; formerly NE-FSC
NRBC	Nucleated red blood cells
PCT	Plateletcrit
PDW	Platelet distribution width
P-LCR	Platelet large cell ratio
PLT	Platelet count
PLT-F	Platelet count as measured in the PLT-F channel
PLT-F-X	Mean fluorescent light intensity of the platelet population in the PLT-F channel
PLT-F-Y	Mean forward scattered light intensity of the platelet population in the PLT-F channel
PLT-F-Z	Mean side scattered light intensity of the platelet population in the PLT-F channel
PLT-I	Platelet count as measured in the RBC/PLT channel
PLT-O	Platelet count as measured in the RET channel
RBC	Red blood cell count as measured in the RBC/PLT channel
RBC-H*e*	Red blood cell hemoglobin equivalent
RBC-O	Red blood cell count as measured in the RET channel
RBC-X	Mean fluorescent light intensity of the red blood cell population in the RET channel
RBC-Y	Mean forward scattered light intensity of the red blood cell population in the RET channel
RBC-Z	Mean side scattered light intensity of the red blood cell population in the RET channel
RDW-CV	Red blood cell distribution width – coefficient of variation
RDW-SD	Red blood cell distribution width – standard deviation
RE-LYMP	Reactive lymphocytes
RE-LYMP, %WBC	Reactive lymphocytes as a percentage of the total white blood cell population
RE-LYMP, %LY	Reactive lymphocytes as a percentage of the total number of lymphocytes
RE-MONO	Reactive monocytes
RE-MONO, %WBC	Reactive monocytes as a percentage of the total number of white blood cells
RE-MONO, %MO	Reactive monocytes as a percentage of the total number of monocytes
RET	Reticulocytes
RET-H*e*	Reticulocyte hemoglobin equivalent
RET-Y	Mean forward scattered light intensity of the reticulocyte population
RPI	Reticulocyte production index
TNC	Total number of nucleated cells as counted in the WDF channel
WBC	White blood cell count as measured in the WNR channel
WBC-D	White blood cell count as measured in the WDF channel
WBC-P	White blood cell count as measured in the WPC channel
%	Percentage of a cell population
#	Absolute cell count
ch	Channel
FI	Fluorescence intensity
SI	Scatter intensity

the red cell lineage LAVE(+)Abn0 changed the MCH LL from 26.2 to 27.4 pg, a change of 0.73 SD. The LL of RBC-H*e*, the equivalent parameter from the RET channel, showed a similar increase of 26.6 to 27.9 pg. The small differences reflect the different techniques; the RBC-H*e* is an estimation of the MCH in the RET channel of the analyzer, based on an empirical calculation factor [21]. In reticulocytes, LAVE(+)Abn0 changed the RET-H*e* from 28.5 to 30.1 pg, a change of 0.94 SD. Remarkably, LAVE did not have a significant effect on the absolute number of reticulocytes.

To judge the need for stratification by sex and age, we used the magnitude of between-subgroup variation of RIs expressed as the SDR and regarded an SDR ≥ 0.4 as an indication for partitioning [15, 20]. For those parameters with well-known differences between the sexes, SDRsex were well above this limit: HGB: 1.25; RBC: 1.05; HCT: 1.16.

Table 3: Reference intervals for all XN analyzer parameters.

A	Parameter	Unit	Males and females			Males			Females		
			LL	Me	UL	LL	Me	UL	LL	Me	UL
Routine parameters	RBC	10^{12}/L				4.4	5.1	5.7	4.0	4.5	5.2
	HGB	g/L				134	152	170	118	136	152
	HCT	L/L				0.41	0.45	0.50	0.37	0.41	0.46
	MCV	fL	82.5	90.3	97.4						
	MCH ([a])	pg	26.8	30.0	32.6						
	MCHC	g/L				317	336	352	311	330	346
	RDW-SD	fL	37.9	42.5	48.3						
	RDW-CV	%	11.8	12.8	14.3						
	RET	10^9/L	32.8	57.8	97.7						
	HFR ([a])	%	0.00	0.60	2.33						
	MFR	%	2.5	6.3	11.9						
	LFR	%	86.2	93.1	97.6						
	IRF	%	2.7	6.9	13.8						
	IRF-Y	ch	16.8	18.1	18.9						
	NRBC ([a])	10^9/L	0.00	0.00	0.01						
	RET-He ([a])	pg	29.3	32.8	35.4						
	RBC-He ([a])	pg	27.2	30.2	32.5						
	DELTA-He ([a])	pg	1.2	2.6	3.6						
	DELTA-HGB	g/L	−7	0	6						
	MicroR	%	0.3	1.1	3.3						
	MacroR	%	3.1	3.6	4.5						
	HYPO-He ([a])	%	0.0	0.1	0.4						
	HYPER-He ([a])	%	0.4	0.6	0.8						
CPD and functional parameters	RBC-O	10^{12}/L				4.4	5.0	5.7	4.0	4.5	5.1
	HGB-O	g/L				135	152	170	119	136	153
	MCHC-O	g/L	312	333	352						
	FRC ([a])	10^{12}/L	0.0000	0.0000	0.0029						
		%	0.00	0.00	0.06						
	RPI					0.7	1.3	2.4	0.5	0.9	1.7
	RBC-X	ch	15.8	17.5	19.5						
	RBC-Y	ch	162	172	179						
	RBC-Z	ch				28.0	31.5	34.7	26.4	30.4	33.7
	RET-Y	ch	170	180	188						

B	Parameter	Unit	Males and females		
			LL	Me	UL
Routine parameters	TNC	10^9/L	3.7	5.8	9.3
	WBC	10^9/L	3.7	5.8	9.2
	WBC-D	10^9/L	3.8	5.8	9.3
	WBC-P	10^9/L	3.7	5.8	9.2
	NEUT	10^9/L	1.6	3.1	5.8
	LYMPH	10^9/L	1.1	1.9	3.3
	MONO	10^9/L	0.3	0.5	0.8
	EO	10^9/L	0.05	0.16	0.53
	BASO	10^9/L	0.02	0.04	0.10
	BASO-D	10^9/L	0.01	0.04	0.08
	IG	10^9/L	0.01	0.03	0.07
	NEUT-RI	FI	42.0	46.1	50.6
	NEUT-GI	SI	142.5	149.4	157.0
	RE-LYMP	10^9/L	0.03	0.06	0.17
		%WBC	0.4	1.1	2.5
		%LY	1.3	3.3	7.8
	AS-LYMP ([a])	10^9/L	0.00	0.00	0.00
		%WBC	0.0	0.0	0.0
		%LY	0.0	0.0	0.0

Table 3: (continued)

B	Parameter	Unit	Males and females		
			LL	Me	UL
	RE-MONO ([a])	10^9/L	0.00	0.01	0.02
		%WBC	0.0	0.2	0.4
		%MO	0.0	2.0	4.4
CPD and functional parameters	HFLC ([a])	10^9/L	0.00	0.00	0.02
	NE-Z	ch	85.5	91.2	97.4
	NE-WX		291	317	345
	NE-WY		550	597	651
	NE-WZ		589	775	911
	LY-X	ch	74.6	77.7	80.8
	LY-Y	ch	63.5	68.6	74.2
	LY-Z	ch	58.5	61.0	63.2
	LY-WX		455	531	614
	LY-WY		752	870	1,011
	LY-WZ		465	647	800
	MO-X	ch	115	118	121
	MO-Y	ch	99	109	118
	MO-Z	ch	64.2	68.4	72.4
	MO-WX		224	264	301
	MO-WY		534	689	861
	MO-WZ		478	780	935
	EO-X	ch	182	194	203
	EO-Y	ch	33.4	35.8	38.7
	EO-Z	ch	97	113	127
	EO-WX		121	203	261
	EO-WY		383	497	623
	EO-WZ		97	472	764
	BA-X	ch	176	189	199
	BA-Y	ch	150	164	182
	BA-WX		13	117	195
	BA-WY		13	102	300
C	**Parameter**	**Unit**	**Males and females**		
			LL	**Me**	**UL**
Routine parameters	PLT-I	10^9/L	164	254	369
	PLT-O	10^9/L	154	235	344
	PLT-F	10^9/L	167	256	377
	IPF	10^9/L	3.1	7.9	18.7
		%	1.2	3.1	8.9
	PDW	fL	10.0	12.8	17.4
	MPV	fL	9.3	10.7	12.7
	P-LCR	%	19.3	31.2	47.1
	PCT ([a])	L/L	0.002	0.003	0.004
CPD and functional parameters	H-IPF	%	0.3	0.9	3.0
	PLT-F-X	ch	69.7	78.7	87.7
	PLT-F-Y	ch	47.8	59.4	72.2
	PLT-F-Z	ch	39.4	44.6	50.7

The demonstrated medians (Me), lower (LL) and upper limits (UL) are calculated with LAVE(+)Abn1. The parameters are clustered in the (A) erythrocytic, (B) leucocytic and (C) thrombocytic cell lineages. (A) If SDRsex≥0.4 the results are given for males and females separately. (B) and (C) SDRsex is less than 0.4 for all parameters, thus results are given for males and females together. Distinction between routine parameters and CPD and functional parameters is demonstrated. The ([a]) indicates parameters that do not have a normal distribution even after log transformation.

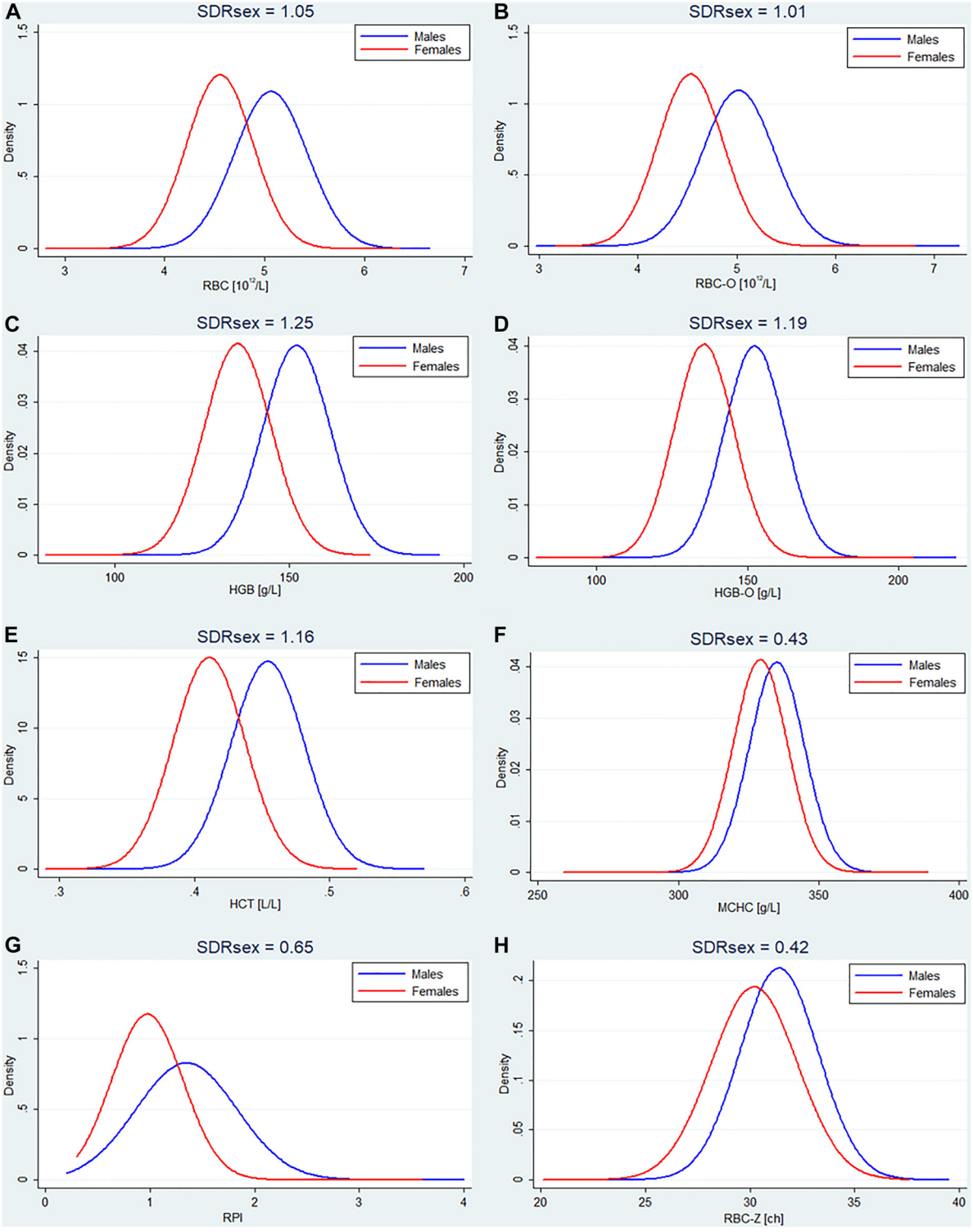

Figure 2: Sex-related differences in RIs distribution.
Illustration of distribution curves for each hematological parameter where sex-related statistically significant differences were determined with SDRsex ≥ 0.4 when LAVE(–) was applied: (A) RBC, (B) RBC-O, (C) HGB, (D) HGB-O, (E) HCT, (F) MCHC, (G) RPI, and (H) RBC-Z. Males are denoted in blue and females in red.

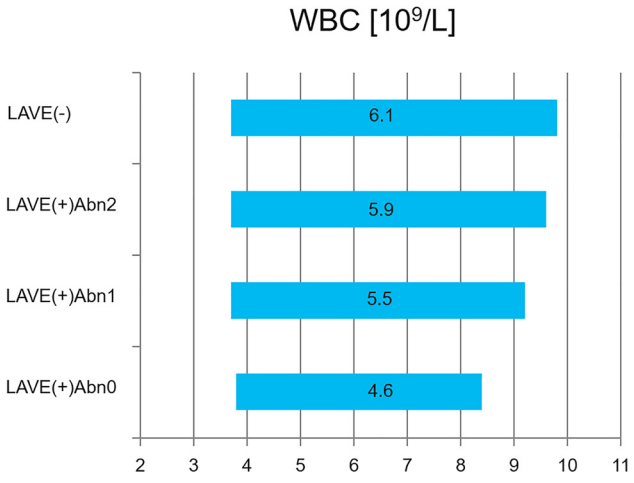

Figure 3: Effect of LAVE on RIs.
Graphical comparison of RI widths for WBC. LAVE(–): RIs derived without applying LAVE. LAVE(+)Abn2: two abnormal values among index parameters allowed for RI derivation. LAVE(+)Abn1: one abnormal value among index parameters allowed for RI derivation. LAVE(+)Abn0: no abnormal values among index parameters allowed for RI derivation. The box in the scattergram represents 95% confidence interval. The number in the bar indicates the numerical value of the RI width.

For MCHC the SDRsex was 0.43. For the RPI, which is a calculated parameter that corrects for reticulocyte maturation in severe anemia, intended to support differentiation between decreased production and increased loss of red blood cells [22, 23], SDRsex was 0.65. Since hematocrit is one of the prime parameters in the RPI equation, the SDR, which is rather high, may be a reflection of the difference between male and female hematocrit.

Beside the pre-analytical and analytical variability, the intra- and inter-individual variation should be considered when applying RIs [24]. Partitioning can reduce the inter-individual variation. We investigated the influence of some lifestyle-related factors and found that BMI and smoking (but not alcohol consumption) can have a moderate impact on the RIs of some parameters. Thus, theoretically, partitioning for these factors and others could further reduce the inter-individual component of variation in our RIs. Future investigations should also assess the intra-individual variation, although time-series analysis is a difficult requisite to meet.

The primary motive for initiating this study was scientific interest on the part of Lifelines researchers and the laboratory. This coincided with the laboratory's objective to report some of the newer parameters as a part of the clinical laboratory service. The ISO 15189 requirements for medical laboratories mandates that RIs should be available for all reported parameters [25]. Governing bodies also have recognized the importance of well-established RIs. The 1997 European Directive on *in vitro* diagnostics (IVDD) stated that manufacturers are obliged to provide RIs for all their CE-marked tests. Its successor, the European Regulation on *in vitro* diagnostics (IVDR) in 2017 has repeated this obligation and has set out to enforce stricter adherence through notified bodies in the member states. This study provided the opportunity for Sysmex to comply with the emergence of these stricter regulations in the European market.

The IFCC C-RIDL committee has worked for years to shape the protocols [12] and the statistical methods [13, 14, 16] that allow for a harmonized way to conduct multi-center, national or international RI studies [17, 19, 26–31]. The aim of the C-RIDL endeavor is regional, national or international harmonization of RIs for standardized and harmonized tests. For calculating the RIs in our study, we used the statistical methods that C-RIDL had developed in full acknowledgement of the fact that our single center study was never meant to be part of the C-RIDL initiative. We are aware that by definition manufacturer-specific parameters cannot be harmonized, and that none of the XN parameters, except hemoglobin, are formally standardized. However, we chose to apply C-RIDL's LAVE approach in our data analysis as we believe that it is the best way of reducing the influence of potential abnormal results from individuals with latent disease on RIs.

RIs were calculated with LAVE(–), LAVE(+)Abn2, LAVE(+)Abn1, and LAVE(+)Abn0 for each parameter. We chose the RIs calculated with LAVE allowing one abnormal result, based on published decisions by members of C-RIDL [17, 19, 29, 32]. The main argument for this choice is the large reduction in the total number of results available for deriving RIs if LAVE(+)Abn0 is applied. In our study, the data reduction was roughly 30% when LAVE(+)Abn0 was applied and roughly 8% when LAVE(+)Abn1 was applied.

The LAVE(+)Abn1 RIs presented in this report were established in a group of participants from the same ethnic background and living conditions as the patients that generally use our hospital services. Moreover, they were established with the hematology analyzers that we use in our daily practice, and we applied our regular internal and external quality control measures. These RIs could therefore be regarded as the ultimate set of RIs for our hospital and may at least be valuable for the laboratories and hospitals that use the same hematology analyzers and serve the same population. However, our laboratory needs to compare these RIs to other studies prior to implementation. The Dutch Society for Clinical Chemistry and Laboratory

Medicine (NVKC) has established RIs for some common standardized chemistry analytes in the NUMBER project [33]. The second NUMBER project (manuscript in preparation) aims to establish nationwide RIs for common harmonized hematology parameters. The preliminary results show that the RIs for the common RBC parameters are virtually the same. For WBC the UL of the RI is substantially higher in NUMBER-2 than in our study. Although, there is a need for a laboratory to comply with national harmonization efforts, local studies, like ours significantly complement the RIs generated by national studies, notably as the latter do not cover the CPD and functional parameters.

The European IVD regulation mandates that IVD manufacturers provide RIs for all CE-marked commercial tests, but the question is whether the RIs in this report would be applicable in other laboratories. Tight quality control would be one of the prerequisites. Independent external quality assessment organizations generally do not offer control material that can be used to monitor the performance of manufacturer specific hematology parameters. Sysmex offers all clients access to a worldwide quality control system which is based on the use of the same commercial internal control material in most laboratories. Also, all laboratories are encouraged to follow the same standardized maintenance and calibration programs and use vendor-specific calibrators. These human blood-based calibrators are traceable to acknowledged reference methods for hemoglobin and cell counts. They are used as well for the calibration of the scatter and fluorescent signals that constitute the basis for the CPD and functional parameters. As the routine hematology parameters from our analyzers perform well in our national external quality assessment organizations system, it may be that the Sysmex CPD and functional parameters may also have attained some form of harmonization through the company's calibration and quality control scheme. Comparing our results to a study in South Korea [34], it is remarkable that the differences are relatively small if differences in sex and age partitioning are ignored.

Doctors and their patients expect and assume the results of laboratory tests to be interchangeable between laboratories. The rapid introduction of electronic health records (EHR) over the past years has increased the urgency for harmonization [35–37]. Many EHR visually emphasize laboratory results that fall outside their RIs. RIs have unintentionally moved from a reference that can help a doctor to interpret a laboratory result, to almost informal clinical decision limits, defining disease rather than health [38]. The results in this study are not meant to become decision limits; they are meant to serve as a reference for interpreting results of Sysmex hematology parameters. If adopted by other laboratories, their origin should be communicated with the laboratory users for proper interpretation.

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Informed consent: Informed consent was obtained from all individuals included in this study.
Ethical approval: The Lifelines cohort study is conducted according to the principles of the Declaration of Helsinki and in accordance with UMCG research code. The study was approved by the Medical ethics committee of the UMCG, The Netherlands (METc 2007/152).

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