

Reference intervals for a complete blood count on an automated haematology analyser Sysmex XN in healthy adults from the southern metropolitan area of Barcelona

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ABSTRACT

Background

The examination of peripheral blood is routinely used as a basic test in daily medical practice. Reliable reference intervals are necessary to avoid misdiagnoses, and the establishment of those intervals is an important task for clinical laboratories.

The aim of the present study was to establish the reference intervals for complete blood count (CBC) on a Sysmex XN haematology analyser in healthy adults from the southern metropolitan area of Barcelona (Spain).

Methods

A total of 213 apparently healthy adults who received a general health examination at Hospital Universitari de Bellvitge were recruited to this study. Blood samples collected in K₃EDTA tubes were analysed on a Sysmex XN. Statistically relevant gender based partition was assessed, outliers removed, and the reference intervals calculated in concordance with Clinical

and Laboratory Standards Institute (CLSI) EP28-A3C guidelines.

Results

The CBC reference intervals were established in 191 adults (64 men and 127 women) who fulfilled all of the inclusion criteria. Significant gender-dependent differences in red blood cells, haematocrit, haemoglobin and platelets were found. The rest of the CBC reference intervals were obtained from the overall data.

Conclusions

We report CBC reference intervals established on a Sysmex XN analyser, a widely used automated analyser for which reference intervals were previously lacking in the literature. However, these reference intervals we recommend should be validated by individual laboratories for the local population as recommended by CLSI.



INTRODUCTION

One of the main aims of clinical laboratories is to provide accurate results as well as appropriate reference intervals for a better interpretation of a patient's results. The reference intervals of a biological parameter depend on the origin of the population and its measurement procedure. Metrological variability is the main reason why different measurement procedures provide different results.

Some international organisations, such as the International Federation of Clinical Chemistry (IFCC), recommend that all clinical laboratories should develop their own biological reference values (1). Moreover, the standard ISO 15189:2012 requires that biological reference intervals be reviewed periodically and whenever there is any change in laboratory technology (2). However, few laboratories currently follow

these recommendations because of its difficulty, large time consumption and high costs.

A complete blood count (CBC) including differential leukocyte count is widely used in clinical practice. Recently developed automated haematology cell counters incorporate technologic improvements including, new parameters and the ability to determining the CBC with better accuracy.

The information from manufacturers about the reference intervals for the CBC is often very limited which highlights the need for laboratories to establish references intervals, that are based on the use of their own equipment and routines. The establishment of local reference intervals for a CBC provides useful data to a laboratory because it reflects the population for which the tests are targeted.

The Sysmex XN-series system (Sysmex, Kobe, Japan) is a recently launched automated haematology analyser with new methods of measurement. Sysmex XN aims to improve the quality of the results of the CBC.

The impedance method is the basis for red blood cell and platelet counts. Some channels count red blood cells and platelets using the sheath flow direct current detection method. The cell signals are sensitively captured because of innovations in the unique digital waveform processing technology.

The haemoglobin concentration is measured using sodium lauryl sulfate (SLS). This reagent haemolysis the red blood cell membrane and SLS-haemoglobin shows absorption at a wavelength of 555 nm.

Using a semiconductor laser, flow cytometry counts and classifies cells by irradiating them with a 633 nm laser beam and analysing their forward scattered light (FSC), side scattered light (SSC) and side fluorescent light (SFL). The intensity of the two types of scattered light (FSC

and SSC) reflects cell surface structure, particle shape, nucleus form, refractive index and reflectivity of the cells. In general, the FSC signal is stronger for larger cells, and the SSC signal becomes stronger as the intracellular structures become more complex. The intensity of the SFL mainly reflects the type and amount of nucleic acids and cell organelles. These three signals are used to differentiate and count white blood cells, nucleated red blood cells, reticulocytes, and platelets, and to detect abnormal cells and immature cells with the help of unique digital technology and algorithms (3, 4).

The aim of the present study was to establish reference intervals for the following haematology parameters using the Sysmex XN-series analyser in an local adult population: red blood cells (RBC), haematocrit, haemoglobin, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), erythrocyte distribution width, white blood cells (WBC), neutrophils, lymphocytes, monocytes, eosinophil and basophil counts and differentials, platelets, mean platelet volume (MPV), reticulocyte counts and differentials and erythroblast counts.

MATERIALS AND METHODS

Subjects and samples

Our study protocol followed the recommendations of the Clinical and Laboratory Standards Institute (CLSI: former National Council of Clinical Laboratory Services) (5-7) and the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) (1). The study was performed in accordance with the ethical standards laid down in the 1964 Helsinki Declaration.

The Hospital Universitari de Bellvitge (HUB) is a 700-bed teaching hospital that specialises in adult patient care. It provides assistance to a population of 300,000 inhabitants, and it is

also a reference centre for more than 2 million people from the southern metropolitan area of Barcelona. The Clinical Laboratory of the HUB is accredited according to the ISO standard 15189.

To produce the reference values for the haematological parameters, blood samples were obtained from apparently healthy adults who received a general health examination at the HUB. In total, 213 subjects (70 men and 143 women), ranging from 18 to 70 years of age, were enrolled in this study. The different age groups were represented equally. To exclude unhealthy individuals, blood and serum biomarkers from liver and renal function, inflammation and iron status and CBC were evaluated. The following parameters were assessed: CBC, differential leukocyte count and the fraction number of reticulocytes, catalytic concentration of alanine aminotransferase, aspartate aminotransferase, gamma glutamyl transferase and alkaline phosphatase: substance concentration of urea and creatinine and glomerular filtration rate (CKD-EPI) and erythrocyte sedimentation rate (ESR) and mass concentration of ferritin.

Individuals were excluded if they had a result outside of the reference interval for any parameter used in our clinical laboratory. Blood was drawn by venipuncture with the Vacuette® blood collection system (Vacuette Greiner Bio-One GmbH, Frickenhausen, Germany) and BD Vacutainer® system (Beckton Dickinson, San Jose, California, USA). For CBC and ESR, tri-potassium ethylenediaminetetraacetate (K3-EDTA) was used as an anticoagulant, and serum was collected in serum gel tubes for all biochemical parameters.

The CBC and differential leukocyte count was initially performed in the current laboratory haematology analyser, a Pentra DF120 analyser (HORIBA® ABX SAS, Montpellier, France). The

erythrocyte sedimentation rate (ESR) was measured using a microagglutination method on TEST 1 (Alifax, Padova, Italy). Biochemical parameters were measured on a Cobas c 711 analyser (Roche Diagnostics®). The measurement method for all parameters was spectrometry molecular absorption, except for the mass concentration of ferritin, which was determined by immunoturbidimetry.

All samples were then processed in the Sysmex XN-2000 analyser within six hours of venipuncture, following International Council for Standardization in Haematology (ICSH) guidelines (8-9).

Quality control

Three-level commercial quality controls (XN-Check Control) were processed daily on the Sysmex XN-2000 system during the study in order to evaluate bias and the between-day analytical imprecision, which fulfilled the metrological requirements.

Statistical analysis

All calculations to determine reference intervals were based on the CLSI guidelines (former National Council of Clinical Laboratory Services) document EP28-A3C (5).

Differences between genders were evaluated using the Harris and Boyd criteria as suggested by CLSI (10). Outliers were detected and excluded from the study by Tukey's boxplot.

Overall and gender stratified data were evaluated for a normal distribution using the Anderson-Darling test. The reference ranges for each parameter were calculated according to the CLSI guidelines document EP28-A3C (5).

All statistical analyses were carried out using Analyse-It software (Analyse-It software Ltd., Leeds, UK) for Microsoft Excel. *P* values of 0.05 or lower were considered to be statistically significant.

RESULTS

Of the 213 subjects who were recruited, 191 (64 men and 127 women) fulfilled all of the inclusion criteria and were selected for the reference interval calculation.

The mean age of all participants at study entry was 42 (± 15) years. Based on gender, the mean age was 42 (± 14) and 41 (± 15) years for women and men, respectively.

Table 1 shows selected haematology reference intervals for our population. Reference intervals for: RBC, haematocrit, haemoglobin and platelets were defined according to gender as dictated by the Harris and Boyd's Test for partitioning the reference values.

DISCUSSION

Sysmex XN modular system is a new automated haematology analyser that is often used in clinical laboratories in our country. The lack of information from manufacturers about the reference ranges for the CBC parameters highlights the need for laboratories to establish reference intervals for the CBC, using their own equipment and routines. The importance of this study relies on the usefulness of these results for other laboratories with European populations that use this analyser system.

There are previous studies on reference intervals using the Sysmex analysers (XE-Class analysers) (11, 12); however, for the first time, we report reference intervals for parameters on the new Sysmex XN-Class analysers on Spanish population.

We studied blood samples from 191 healthy adults using the Sysmex-XN-2000 analyser, and we calculated reference intervals for the CBC, including leukocyte differential and reticulocytes. We confirmed that there are relevant gender differences for RBC, haematocrit and platelet counts. However, none of the other parameters

Table 1 References intervals for all haematology parameters stratified by gender when it was mandatory

Parameters	Units	n (men/women)	Men	Women
Red blood cells (RBC)	x10 ¹² /L	64/126	[4.3-5.6]	[3.9-5.1]
Haematocrit	%	59/124	[40-50]	[36-45]
Haemoglobin	g/L	59/126	[137.4-164.7]	[120.0-146.8]
MCV	fL	188	[83.6-97.0]	
MCH	pg	188	[27-32]	
MCHC	g/L	185	[314-319]	
Erythrocyte distribution width	%	189	[11.6-14.3]	
Leukocytes	10 ⁹ /L	188	[3.9-9.5]	
Neutrophils	10 ⁹ /L	188	[1.5-5.7]	
Neutrophils	%	189	[37.1-68.4]	
Lymphocytes	10 ⁹ /L	187	[1.3-3.4]	
Lymphocytes	%	190	[21-50]	
Monocytes	10 ⁹ /L	191	[0.31-0.92]	
Monocytes	%	188	[5.1-11.2]	
Eosinophils	10 ⁹ /L	181	[0.03-0.39]	
Eosinophils	%	188	[0.4-6.6]	
Basophils	10 ⁹ /L	190	[0.01-0.09]	
Basophils	%	189	[0.2-1.3]	
Platelets	10 ⁹ /L	63/124	[149-303]	[153-368]
MPV	fL	187	[9.7-13.2]	
Reticulocytes	10 ⁹ /L	113	[34-102]	
Reticulocytes	%	113	[0.68-1.86]	
Erythroblasts	/100 Leukocytes	190	0-0.01	

showed significant variations by gender, which was in accordance with most of the published data (13-15).

We calculated the reference values for haemoglobin, but we recommend the use of the cut-off value for anaemia defined by the World Health Organization (WHO) (10, 11). The reference interval concept describes the ranges found in studied populations, and WHO definition of anaemia represents a value based on a medical decision. The lower reference value must always be higher than the cut-off for anaemia because it is calculated with healthy subjects that are not anaemics.

The finding of higher values for haemoglobin, haematocrit and erythrocytes in males compared to females may be partly due to the influence of androgens on erythropoiesis, menstrual loss and gravidity. As found in other similar studies, platelet counts in women were higher than men. It seems to reflect different hormonal profiles or a compensatory mechanism associated with menstrual blood loss (15, 16). In the present study, we found a great gender difference in the upper reference limit ($303 \times 10^9/L$ for men vs $368 \times 10^9/L$ for women). This was in concordance with the study directed by Pekelharing et al. (11), which was carried out with the Sysmex XE-5000 analyser; more marked differences were observed in the upper reference limit ($308 \times 10^9/L$ and $390 \times 10^9/L$ for men and women, respectively).

As expected, the presented reference intervals are similar to previous published data using Sysmex XE-5000 (11) on a population in the Netherlands. Those findings seem logical because the analysers were similar and in both studies the population was European. However, it is mandatory to establish reference intervals when there is a change on routine analyser. It is worth mentioning that the reference range for platelets using the Sysmex

technology seems to be lower than most textbook data. Pfaeffli et al. (17) showed that reference limits for Sysmex XE-2100 were $130\text{--}330 \times 10^9/L$, which is lower than $150\text{--}400 \times 10^9/L$ from the published literature.

Based on the use of the impedance method with the Sysmex technology, the results for platelets do not seem to be interchangeable with the results from other measuring systems that also use the impedance method (18).

At the moment, there are few publications that establish reference ranges for CBC in adult population using the Sysmex-XN analyser and that also exhibit significant differences by gender for haemoglobin, RBC, haematocrit and platelet count (19). One of these studies was carried out using samples from an Italian population, and the results were comparable. The results of a second study have been obtained in a Korean population (19). There is enough literature describing relevant differences when comparing reference values in different populations, which emphasises the need for population specific reference intervals. Factors like the environment and nutritional status may play an important role (12).

One limitation of our study was the small number of blood samples analysed. However, the method was robust enough to establish reference intervals following the CLSI guidelines when the number of samples was fewer than 120. Also, it has to be considered that age-related differences were not assessed for the establishment of reference intervals due to the limited sample size.

In summary, we established reference intervals for CBC in apparently healthy adults from the southern metropolitan area of Barcelona using the Sysmex-XN analyser. Establishing such intervals is recommended by international guidelines whenever there is a change in any analyser in a clinical laboratory.

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