Reference Limits for the Automated Haematology Analyser Sysmex XE-2100

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Reference limits for healthy individuals measured on the Sysmex XE-2100 automated haematology analyser were determined at the Kantonsspital Luzern in order to permit adequate discrimination between health and disease using this analyser. For this, a representative and heterogeneous test group was needed, which fulfilled the following conditions: Firstly, pre-analytical variables for the reference population were considered and controlled with respect to drugs, alcohol, intake of food, physical activity, emotional stress, posture and tourniquet application time. Secondly, all subjects felt healthy and had no known illnesses. Also, the test subjects represented equal number of males and females (88 each) and were between 17 to 100 years of age. The different age groups were represented equally.

In general, the reference limits determined were lower compared to bibliographical references. The reference limit determined for WBC was 1×10^9/L lower than today's accepted limit. However, already, experience from the haematology consultation at the Kantonsspital Luzern suggested a lower reference limit. A number of reasons can be advanced to explain this difference in values. In the future we will now only need to clarify all WBC results which are lower than 2.6×10^9/L. Additionally, we also had the same experience with the HGB results for males. As a result, cost can be reduced with the new reference limits, since some follow-up medical examinations may now be avoided.

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Key Words Automated Hematology Analyzer, XE-2100, Reference Limits

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INTRODUCTION

Clearly defined reference limits permit identification of results outside the reference range in health. Together with clinical examination, other laboratory findings and X-rays, the overall health of the patient may be judged. But what is meant by healthy? The WHO (World Health Organization) defines health as "A state of complete physical, spiritual and social well-being". The definition of absolute health is not given[1].

The haematology team of the Kantonsspital Luzern wanted to establish reference limits for the Sysmex XE-2100 because there were none available as yet. To determine reference limits, an adequate reference sample group has to be defined first.

MATERIALS AND METHODS

A representative and heterogeneous test group was needed, which had to fulfill the following conditions: First, the reference individuals should be comparable to the subjects for whom the reference limits would be used. The reference individuals required to be healthy with no known illnesses. Secondly, equal numbers of males and females (88 each) were selected of ages ranging from 17 to 100 years. The different age groups were represented equally (***Table 1***).

It is well recognised that life style specifications can have a significant effect on reference limits and reference ranges. Thus, the test group individuals selected were non-smokers (1 cigarette max. per day), drank no more than two glasses of wine per day and lived no higher than 2,000 meters above sea level. Female individuals were not pregnant and did not use hormonal contraceptives.

The taking of the blood samples was performed between 7:00 and 8:30 a.m. in the fasting state, no physical activity and 10 min. rest before venous sampling. The effect of different influences on each of the blood count parameters is shown in ***Table 2***[2-7].

Sarstedt EDTA-Monovettes were used as standard equipment for venous sampling[8].

All blood count parameters were measured on the Sysmex XE-2100.

Table 1 *Distribution of subjects in groups according to sex and age*

Age female	Number	Age male	Number
F 17 – 30 years old	n=14	M 17 – 30 years old	n=14
F 31 – 50 years old	n=25	M 31 – 50 years old	n=26
F 51 – 70 years old	n=28	M 51 – 70 years old	n=26
F 71 – 100 years old	n=21	M 71 – 100 years old	n=22
F 17 – 100 years old	n=88	M 17 – 100 years old	n=88

Table 2 *Types of influence on different blood count parameters*

Type of influence	WBC	RBC	HGB	HCT	MCV	MCH	MCHC	PLT	DIFF	RET
Physical activity	X							X		
Diurnal rhythm	X		X	X				X		
Body position			X							
Fasting before blood test	X									
Height above sea level			X	X						
Medication/ Medicaments										
Alcohol					X					
Nicotine	X		X	X						
Hormone treatment	X									
Pregnancy	X	X	X	X						
10 min. rest before blood test		X	X	X	X	X	X			

Table 3 *Reference ranges in health determined by Sysmex XE-2100 in comparison to literature*

Parameter	Reference limits for Sysmex XE-2100	Reference limits used until now	Literature
WBC	2.6 – 7.8×10^9/L	4 – 10×10^9/L	2
PLT	130 – 330×10^9/L	150 – 400×10^9/L	3, 5
RBC	F 3.7 – 5.0×10^{12}/L	F 4.0 – 5.0×10^{12}/L	1
	M 4.1 – 5.4×10^{12}/L	M 4.5 – 5.5×10^{12}/L	2
HGB	F 115 – 148 g/L	F 120 – 160 g/L	1
	M 127 – 163 g/L	M 140 – 180 g/L	2
HCT	F 0.34 – 0.43L/L	F 0.38 – 0.46L/L	1
	M 0.37 – 0.46L/L	M 0.40 – 0.48L/L	2
MCV	80 – 97 fL	83 – 99 fL	1
MCH	27 – 34 pg	28 – 32 pg	1
MCHC	330 – 364 g/L	310 – 360 g/L	3, 2
RET	0.4 – 1.6 %	F 0.5 – 2.2%	1
		M 0.5 – 1.8%	6

Automat. Diff.	× 10^9/ L	%	× 10^9/ L	%	Literature
NEUT	0.9 – 4.5	36 – 68	1.5 – 7.50	37 – 75	4
LYMPH	1.0 – 3.0	18 – 51	0.5 – 5.0	12 – 50	4
MONO	0.0 – 1.0	3 – 14	0 – 1.2	0 – 12	4
EO	0.0 – 0.4	0 – 7	0 – 0.7	0 – 7	4
BASO	0.0 – 0.05	0 – 0.9	0 – 0.2	0 – 2	4

RESULTS

Statistical analysis was undertaken as follows: For each parameter the results were checked for outliers; the distribution curve was made and tested for a normal distribution. If a normal distribution ($p > 0.05$) was possible, the average value and reference limits (mean ± 2 standard deviations (s)) were determined. If a normal distribution was not feasible, then the median and 2.5- and 97.5-percentiles were used, i.e. 2.5% from the highest and the lowest results were deleted to determine the upper and lower reference limit.

The t-test for non-paired data was employed to examine the results for significant differences at this juncture (the same reference limit was used for both females and males) with the pre-condition that normal distribution ($p > 0.05$) and no significant differences between both average values (during spot check) were detected ($p < 0.05$)[9]. Correction for «multiple tests according to Bonferroni»: The p-value of 0.05 was divided by the number of measured, independent parameters. Independent parameters were: HGB, HCT, PLT, WBC, RBC and RET. If $p > 0.008$, there were no significant differences between both average values (during spot check). If $p < 0.008$, the clinical relevance decides whether results are conclusive only if the same reference limits for females and males are used.

For the RBC, HGB and HCT parameters, the clinic requires separate reference ranges for females and males. For PLT, one common reference range for males and females is employed.

Table 3 shows the reference ranges determined for the Sysmex XE-2100 in comparison to the values reported in the literature. The ***Figs. 1a-1c*** show the distribution

curves for the values of the individual parameters. Whenever common reference ranges were determined for male and female subjects, it was decided to display only one of the two comparable distribution curves.

*References referred to in **Table 3***
(stated reference values)

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A normal distribution ($p > 0.05$) was found for the following parameters: WBC, RBC, HGB, HCT, MCV, MCH, MCHC, RET, PLT, NEUT, LYMPH, MONO (female, male) and EO (male). The reference limits were calculated by the average value (x) ± 2 (s). As expected there was an abnormal distribution for EO (female) and BASO (female, male), since most of the values were near zero. In these cases, the median and 2. 5 - and 97. 5-percentiles were used to determine the upper and lower reference limit. There were non-significant differences

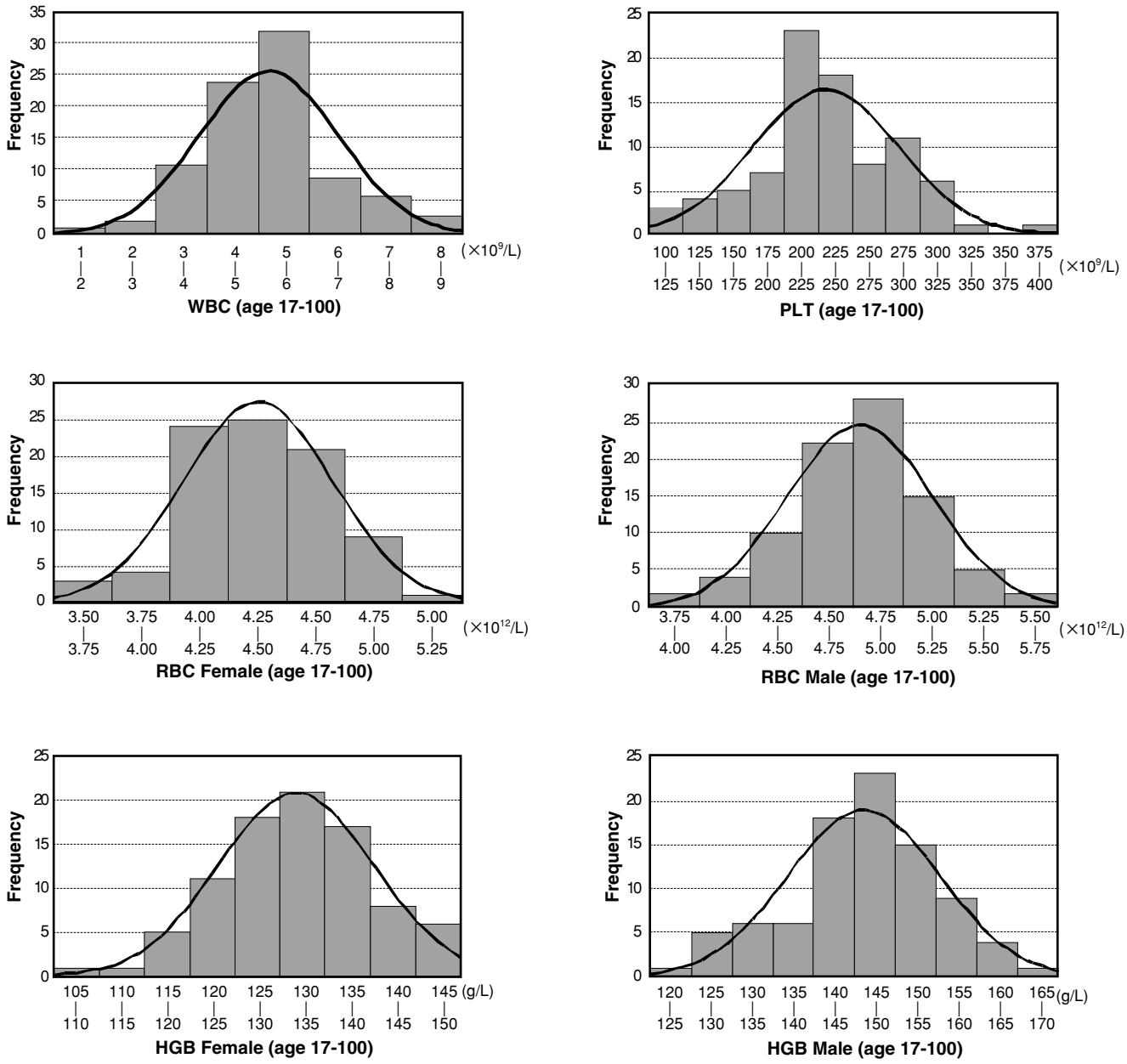
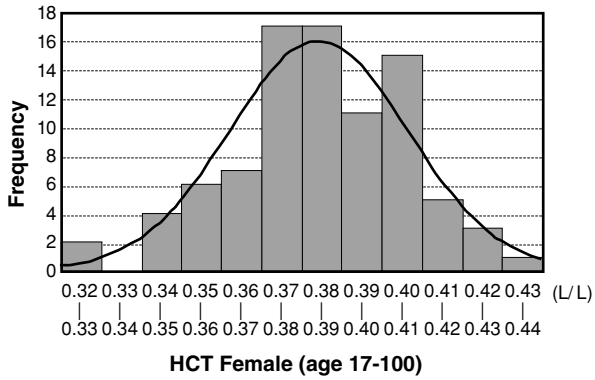
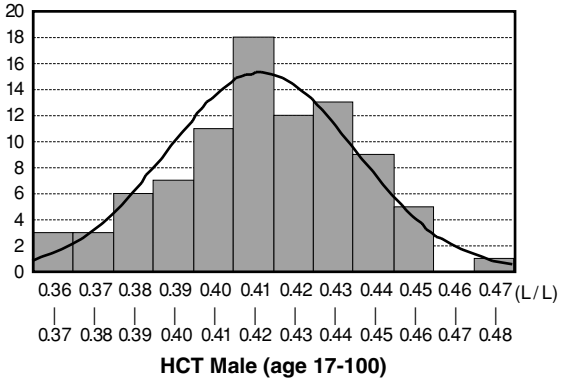
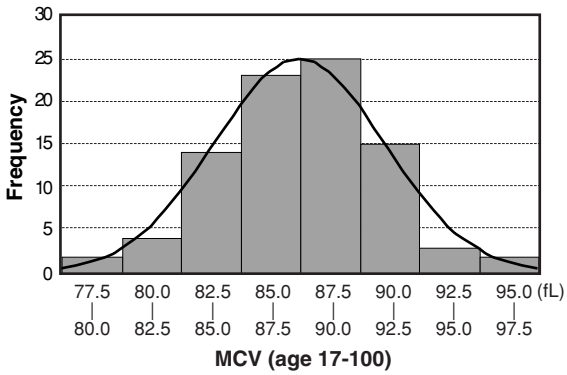
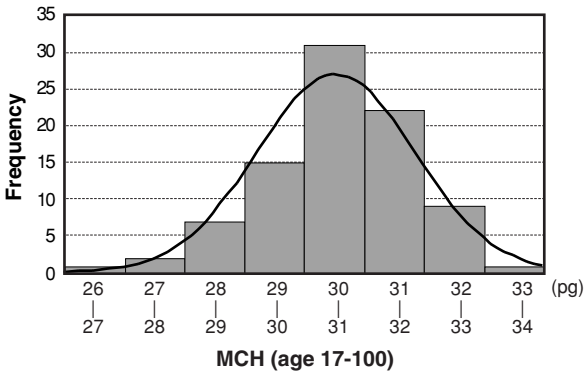


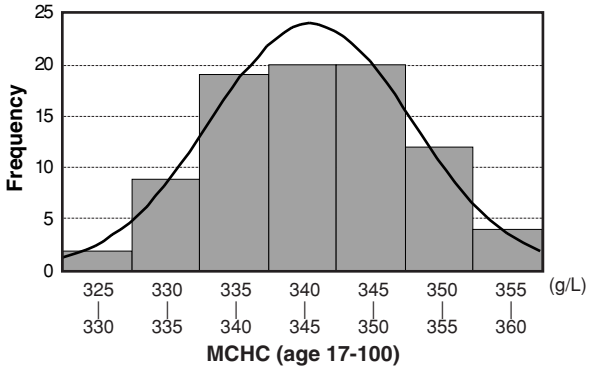
Fig. 1a *Distribution curves showing the individual parameters' values determined by Sysmex XE-2100 (—normal)*

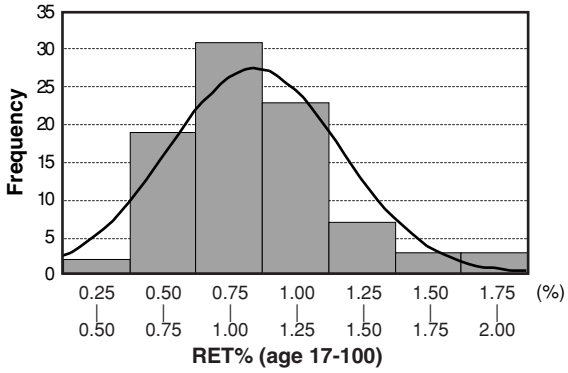


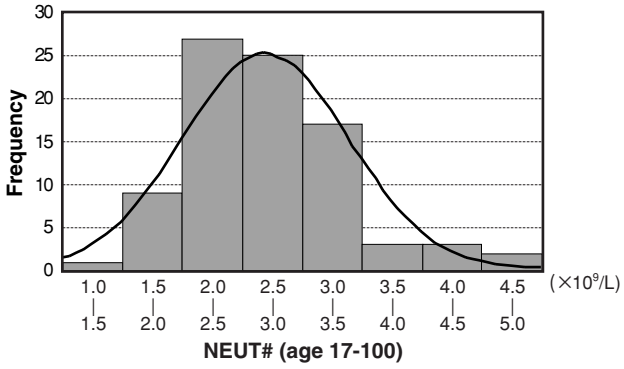












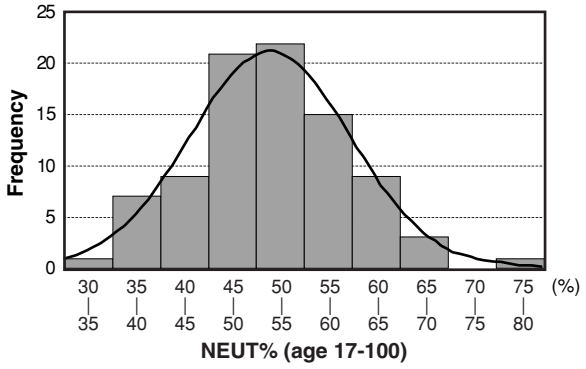


Fig. 1b *Distribution curves showing the individual parameters' values determined by Sysmex XE-2100 (* ▬ *normal)*

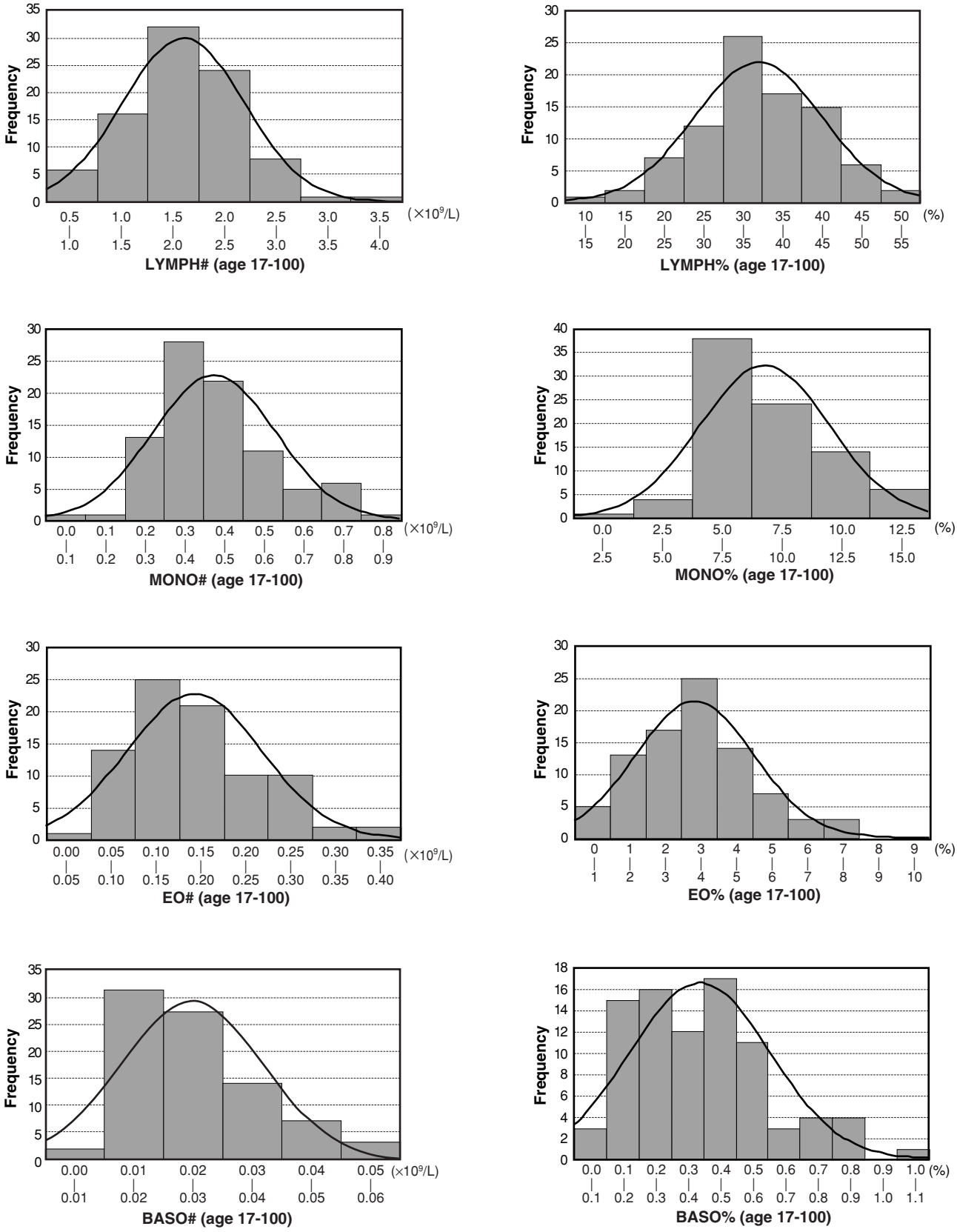


Fig. 1c *Distribution curves showing the individual parameters' values determined by Sysmex XE-2100 (—normal)*

between the average values for females and males throughout the t-test for non-paired data for the following parameters: WBC, MCV, MCH, MCHC and RET. A comparison of the average values for the automated differential parameters was omitted, since the reference limits for females and males were identical. There was a significant difference between average values, even after correction for «multiple tests according to Bonferroni» for RBC, HGB, HCT and PLT. The clinical relevance decides whether results are conclusive only if the same reference limits for females and males are used. There was no clinically relevant reason to employ separate reference limits for females and males for PLT. There is clear evidence for the need to calculate separate reference limits for females and males for RBC, HBG and HCT.

DISCUSSION

In general the reference limits determined are lower than the bibliographical references. The reference limit determined for WBC is 1×10^9/L lower than today's accepted limit. But already experience from the haematology consultation at the Kantonsspital Luzern had suggested a lower reference limit. Based on these results from the XE-2100 in the future we will now only need to clarify all WBC results which are lower than 3×10^9/L. Additionally, we also had the same experience with the HGB results for males. As a result, cost can be reduced with the new reference limits, since some follow-up medical examinations may now be avoided.

An attempt must be made to explain why the new reference limits are generally lower than the limits described in the literature. The XE-2100 was perfectly calibrated at the start of the study and remained so throughout as determined by appropriate internal quality control and external quality assessment. It seems unlikely that the reference population differs from other local populations of the same ethnic mix and geographic/environmental influences particularly since such differences have not been detected using other blood count analysers. The likeliest explanation relates to the stringency of the inclusion criteria avoiding conditions known to influence counts, e.g. food intake, exercise, smoking, etc. This is particularly so coupled with the timing of venous sampling in the early morning when diurnal rhythms indicate that leukocytes and platelets are at their lowest levels.

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