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Multi-centric determination of reference ranges for automated blood counts[1]

Thomas Nebe[1,*], Frank Bentzien[2], Mathias Bruegel[3], Georg Martin Fiedler[3], Kai Gutensohn[4], Hermann Heimpel[5], Nicole Krebs[3], Manfred Ossendorf[6], Peter-Schuff-Werner[7], Gudrun Stamminger[8] and Hannsjörg Baum[9] for the working party Laboratory of the German Society for Hematology and Oncology

[1] Onkologikum, Frankfurt am Main, Germany
[2] Institut für Transfusionsmedizin, Universitätsklinikum Hamburg-Eppendorf, Hamburg, Germany
[3] Institut für Laboratoriumsmedizin, Klinische Chemie und Molekulare Diagnostik (ILM) Universitätsklinikum Leipzig, Leipzig, Germany
[4] Aesculabor Hamburg, Hamburg, Germany
[5] Medizinische Klinik III des Zentrums der Inneren Medizin, Klinikum der Universität Ulm, Ulm, Germany
[6] Labor Limbach, Heidelberg, Germany
[7] Institut für Klinische Chemie und Laboratoriumsmedizin, Universität Rostock, Rostock, Germany
[8] Zentrum für Diagnostik GmbH am Klinikum Chemnitz, Chemnitz, Germany
[9] Institut für Laboratoriumsmedizin, Mikrobiologie, Blutdepot und Krankenhaushygiene, Ludwigsburg, Germany

Abstract

The analysis of blood cell count including differential, is the most frequent laboratory test and has still essential diagnostic value in the diagnosis of primary or secondary diseases of the hematopoietic system. The interpretation is based on a comparison with age matched reference intervals, despite the fact that a comparison with the patients own preceding values allows a more precise statement. In the present study, blood counts and differential were performed in 1158 healthy males and females with an age ranging from 16 to 75 years were investigated using current hematology systems. The donors were examined by personnel physicians and those examining blood donors in transfusion medicine and in addition were asked via standardized questionnaire. The study resulted in reference ranges for the complete blood count, the differential blood count and reticulocytes. For several analytes, age-, gender- or analyzer-specific reference ranges were obtained. We found an age dependent decrease of the erythrocyte concentration and an increase for MCH and MCV as well as for basophils. These findings could be important for the diagnosis of diseases of the elderly like like myeloproliferative diseases (MPN) and myelodysplasias (MDS). Gender specific changes could be shown for the hemoglobulin-, the erythrocyte- and reticulocyte concentration, as well as for hematocrit and MCHC, being reduced in females. On the other hand, females showed significant higher levels for platelets and leukocytes, based on an increase of neutrophils and lymphocytes. The leukocytosis of smokers was confirmed for neutrophils and lymphocytes but lower than one could expect from recent studies. A multicentric study shows a broader distribution compared to unicentric data but this probably better reflects the reality and improves the applicability of the reference ranges.

Keywords: reference ranges, hematology, complete blood count, blood differential, reticulocytes, hematology analyzer.

Introduction

Frequently the reference ranges laboratories use for blood count parameters are based on the results of older studies [1–4] or on outpatient groups whose health status has not been thoroughly examined. Furthermore, the equipment often used to analyze blood counts is based on obsolete measurement technologies and no longer meets today's standards. Analyzer-specific reference ranges are available only to a very limited degree [5–7]. There are publications that show the differences in blood count parameters depending on age [8–13], gender [14, 15], race [16–19], pregnancy [20] and the analyzer used. A meta-analysis yielded distinct differences in reference ranges between laboratories in Switzerland [5]. Nothing has been published about potential changes in blood count or differential blood count because of changed life styles over the course of the last decades, which calls into

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*Korrespondenz: Dr.med. Carl Thomas Nebe
Hämatologisches Speziallabor
Onkologikum Frankfurt
Gartenstraße 134
69596 Frankfurt (Main)
Tel.: 069 697 696 5-17
Fax: 069 697 696 5-19
E-Mail: Thomas.nebe@onkologikum-labor.de

question the transferability of old reference ranges to today's population. The transferability of such studies performed on subjects with Asian backgrounds or on groups of US army members with a high proportion of African-Americans onto Central Europeans must be called into question as well. Results from hospital patient groups (uncertain health status) [21, 22] or preselected blood donor groups must also be looked at with a critical eye. Additionally, data on smokers are very often lacking. Data on pregnancy-dependent effects on blood count parameters also are hardly ever available [23]. Many studies for determining reference ranges have continued to be performed with only one certain type of analyzer. However, results from external quality assessment schemes show clear analyzer-specific differences do exist [24–28].

Because of these limitations the normal value ranges resulting from different reference value studies are very variable and difficult to classify. Therefore the goal of this study was to determine standardized reference values for a complete and differential blood count while taking into account age, gender and type of equipment. Storage-dependent effects also were to be investigated. Since clear differences between the blood count analyses from various laboratories are known from inter-laboratory test results and from isolated studies, this project was designed as a multi-centric study.

Methods

This study project was approved through the various ethics commissions of the participating centers. It focused on adults between the ages of 18 to 65 and adhered to a balanced age distribution. We recruited healthy men and women within the framework of routine medical investigation of employees by medical staff or from blood donor centers (Table 1). Among blood donors only first-time donors were allowed, there were no financial incentives for participation in the study. The test subjects were given an informed consent form, including a privacy protection declaration. The standard questionnaire for determining their state of health included cardiovascular, renal, liver, gastrointestinal and lung diseases, bleeding tendency, thrombosis, consumption of alcohol, smoking, pregnancy as well as possible medications. The diseases listed and pregnancy were criteria for exclusion.

There were no separate blood collections, blood count analyses were performed from EDTA blood collected for routine diagnostic clarification within the framework of blood donor and personnel health checks. A blood count analysis was performed on the day the blood was collected; in some centers a further analysis was performed one day after blood collection in order to test the effects of storage. To be able to determine analyzer-specific reference ranges we used systems from various manufacturers, that meet current equipment standards (Advia 120 by Siemens, CellDyn 3500/3700/4000/Sapphire/CD 4000 by Abbott, LH750 by Beckman-Coulter, Pentra120 by ABX, XE-2100 by Sysmex). Prior to the study these firms serviced their respective analyzers and, where necessary, provided training.

To guarantee the transferability of the recorded results the study was designed as a multi-centric study (Chemnitz, Hamburg, Heidelberg, Leipzig, Mannheim, Munich, Rostock). For the purpose of comparability of the donor groups in the various centers we established a common study branch in which analyses were conducted with the one type of analyzer (XE-2100 and XT-2000i by Sysmex) used in all laboratories. Additionally, we kept a record of equipment controls with control reagents to verify comparability.

The data were anonymized and the results were evaluated with SAS (SAS Institute) and displayed with WinSTAT for Excel (R. Fitch Software). The comparison of males and females as well as the evaluation of the age dependence of the respective measured parameters was performed based on the data from the XE-2100 system manufactured by Sysmex that was used in all participating centers exept of Hamburg.

An independent biometrician from the University of Heidelberg consulted during statistical evaluation. The manufacturing firms sponsored the study by providing the analyzers, reagents and service for this study at no cost. Remaining expenditures were borne by the Deutsche Gesellschaft für Hämatologie und Onkologie (DGHO) and the external quality asessment organization INSTAND.

Results

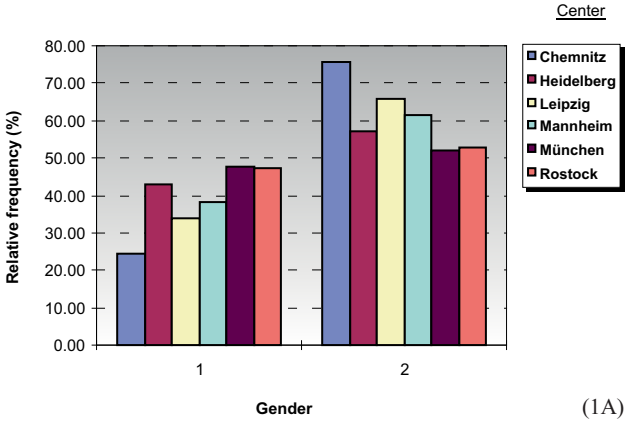
We could not achieve our goal of a balanced distribution of age and gender (see Figures 1A, 1B). Primary reasons were the uneven gender distribution in the personnel structure and the blood donor group as well as the exclusion of a great number of older test subjects, because they were taking medication or because they were ill. The portion of smokers in this study group is shown in Figure 1C. The comparability of the centers was examined and confirmed based on the measurement results of the standard XE2100 analyzer (see Figure 2E), except for Chemnitz, where the study had to be discontinued prematurely when the manufacturer took back the loan equipment, and the quicker speed with which younger persons could be recruited resulted in a lower age cross-section at that time (Figure 1). The charts of the measured hematologic values show only those parameters that resulted in differences or are of particular interest. In addition, we chose to show the 95% percentiles in tabular form to facilitate the use of the reference ranges for the reader. As far as the measurement parameters are concerned, the numbering of the tables corresponds to the superior numbers of the figures.

Erythrocyte concentration and hemoglobin

Adequate reference ranges for hemoglobin and erythrocyte concentration are the defining criteria of most particular importance for anemia. The detection of erythrocyte concentration – with the exception of the age-related factor in Chem-

Table 1 Age distribution of test subjects (see Figure 1C).

Analysis Variable : Age							
Center	n Obs	n	Mean	SD	Minimum	Median	Maximum
Chemnitz	189	189	28.4	12.1	16.9	20.0	57.2
Hamburg	309	309	34.0	10.5	18.0	34.0	61.0
Heidelberg	430	430	37.9	11.1	21.0	38.0	68.0
Leipzig	299	299	32.6	9.8	19.0	30.0	59.0
Mannheim	717	656	35.9	11.6	18.0	37.0	58.0
München	1697	1695	36.0	13.6	16.0	32.0	75.0
Rostock	495	495	38.1	11.1	19.0	36.0	65.0



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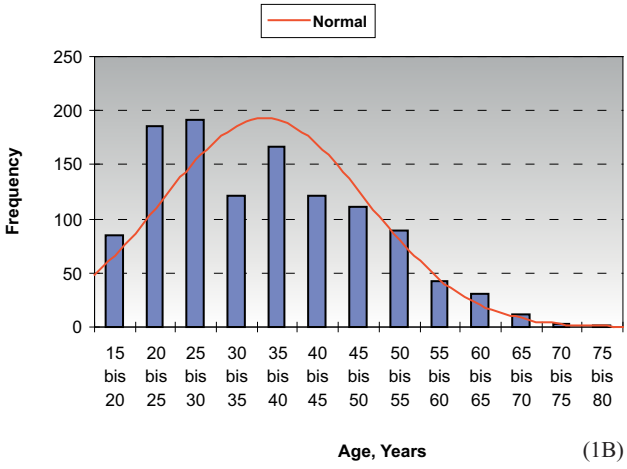

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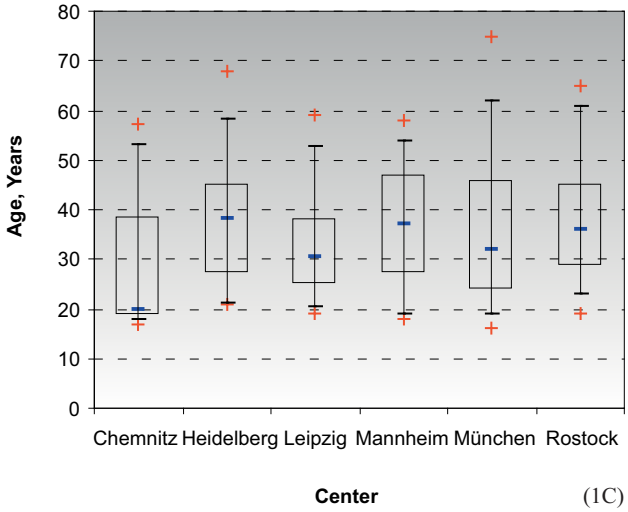

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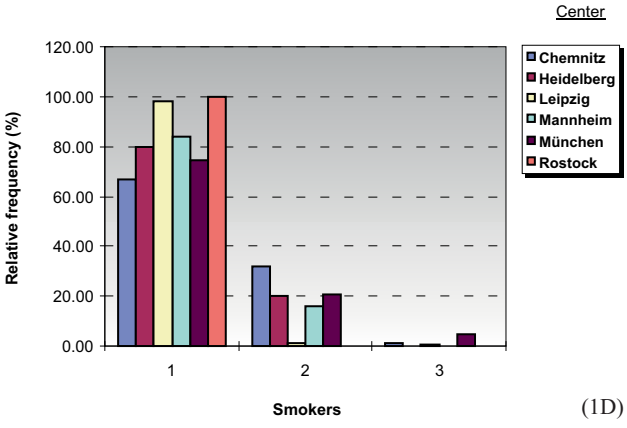


(1D)

Figure 1 (online only) Demographic data.
(A) Gender distribution of the study participants. (B) Age distribution of the study participants. (C) Age distribution of the test subjects in the study centers (see Table 1). (D) Smoker status of the study participants. 1 = non smoker, 2 = smoker, 3 = unknown.

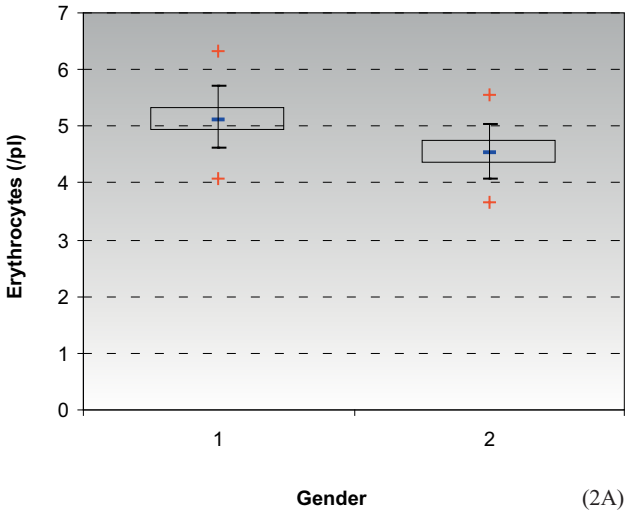
Table 2 Erythrocyte concentration.

Gender	Analyzer	n	Mean	SD	P 2.5%	P 97.5%
Male	Abbott CD 3200	58	5.115	0.351	4.492	5.796
Male	Abbott CD 3500/3700	109	5.060	0.344	4.520	5.858
Male	Abbott Sapphire/CD 4000	415	5.090	0.371	4.401	5.810
Male	Advia 120	258	5.190	0.352	4.570	5.980
Male	Coulter LH 750	101	5.200	0.325	4.490	5.830
Male	Horiba ABX Pentra 12	126	5.124	0.345	4.560	5.680
Male	Sysmex XE-2100	486	5.129	0.326	4.540	5.770
Male	Sysmex XT	203	5.234	0.365	4.530	5.920
Female	Abbott CD 3200	100	4.551	0.333	3.898	5.228
Female	Abbott CD 3500/3700	198	4.445	0.284	3.900	5.040
Female	Abbott Sapphire/CD 4000	494	4.501	0.333	3.920	5.170
Female	Advia 120	299	4.615	0.341	4.010	5.290
Female	Coulter LH 750	159	4.572	0.302	3.990	5.160
Female	Horiba ABX Pentra 12	161	4.546	0.314	3.940	5.130
Female	Sysmex XE-2100	671	4.539	0.302	3.960	5.160
Female	Sysmex XT	220	4.652	0.334	4.100	5.400

nitz – does not result in any center- or analyzer-specific differences (see Figures 2E and 3F). The measurement of hemoglobin also does not result in any equipment-specific differences, although there are distinct variations between the various centers that most likely might be attributed to differences in calibration (Figure 3A). There are no differences between smokers and non-smokers (Figures 2B, 3C). The known gender dependence with lower values in females could be confirmed (Figures 2A, 3B). For both genders a previously unreported association was a significant reduction of erythrocyte numbers and hemoglobin concentration with increasing age and more strongly pronounced in men (Figure 2D, 3E), which proved significant in the GLN procedure of SAS. As far as gender and analyzer differences are concerned, the behavior of the hematocrit is similar to that of the hemoglobin concentration (see Figure 4 and B), but demonstrates no significant age dependence (Figure 4C).

Erythrocyte indices and erythrocyte distribution width

Erythrocyte indices (MCV, MCH, MCHC) are important for the classification of anemias and their pathophysiologic classification. There are location- and analyzer-specific differences for the mean corpuscular volume (MCV) as a calibration-dependent parameter (see Figure 5A), so that each laboratory must define its own normal ranges. High demands must be placed on the MCV, since it is a directly measured parameter and this parameter plays a big role in differentiating between microcytic and macrocytic forms of anemia. The analyzer comparison in Figure 5A demonstrates the necessity for analyzer-specific reference ranges, whereas no differences exist between genders. For mean corpuscular hemoglobin (MCH) there are no significant differences specific to gender, location or analyzer (see Figure 6). The above described increase with advancing age is borne out for MCH and MCV [14]. There is a small, but significant gender- and analyzer-specific difference, but no age dependence, for the MCHC value (mean corpuscular hemoglobin concentration) (see Figures 7A) and B)). The use of the Cell-Dyn 3500 (Abbott) results in distinctly higher values for the width of erythrocyte distribution (RDW) as a measure for anisocytosis (see Figure 8). There are no differences with RDW in regard to center, age, gender, smoker or non-smoker (no Figure).



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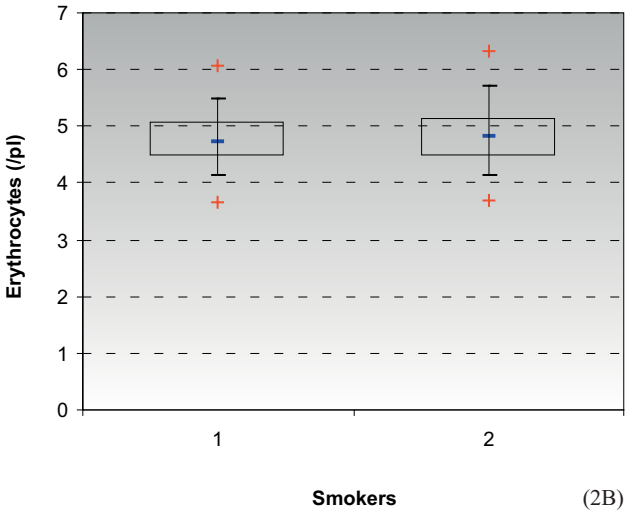

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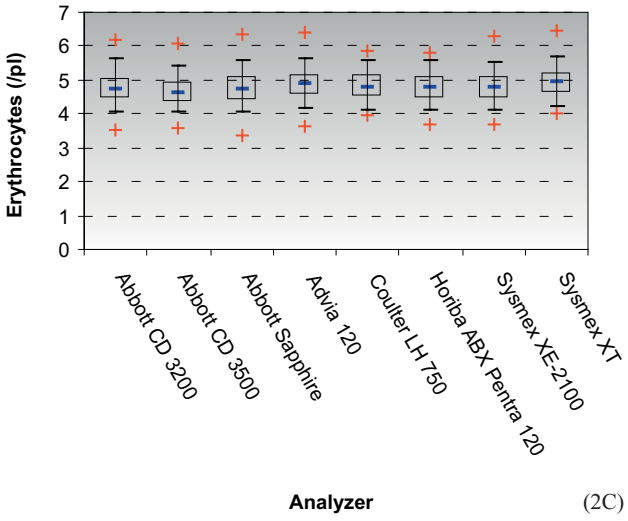

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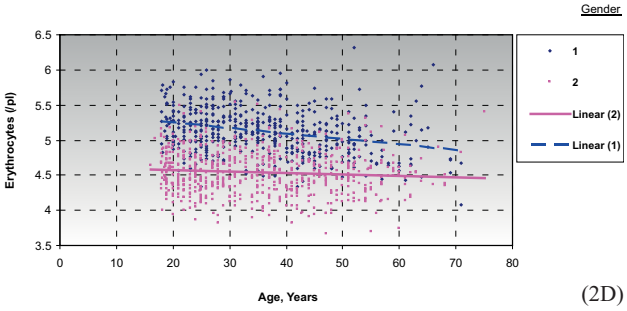

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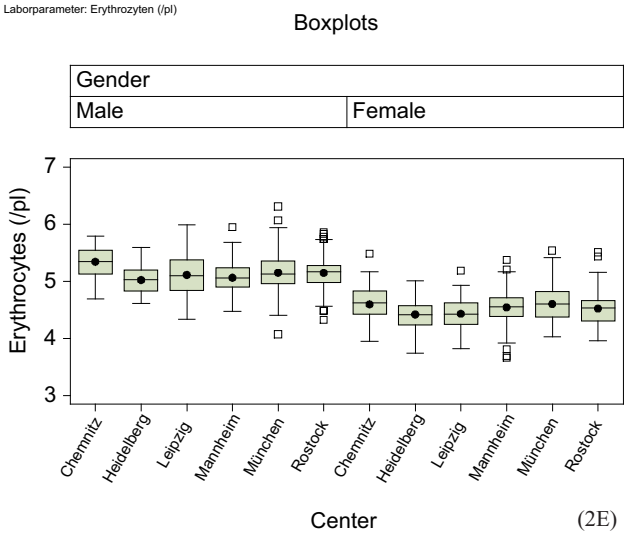


(2E)

Figure 2 (online only) Erythrocyte concentration (red blood count, RBC)
(A) Comparison of males (1) and females (2). (B) Comparison of non-smokers (1) and smokers (2). (C) Comparison of analyzers. (D) Investigation of age dependence in relation to gender. (E) Comparability of the centers in relation to erythrocyte numbers.

Table 3 Hemoglobin concentration (Hb).

Gender	Analyzer	n	Mean	SD	P 2.5%	P 97.5%
Male	Abbott CD 3200	58	155.301	8.702	140.004	171.967
Male	Abbott CD 3500/3700	109	152.992	8.520	135.927	167.782
Male	Abbott Sapphire/CD 4000	415	155.559	9.428	137.000	174.000
Male	Advia 120	258	157.763	9.921	139.000	177.000
Male	Coulter LH 750	101	157.608	10.039	133.630	177.000
Male	Horiba ABX Pentra 12	126	152.929	8.056	138.000	167.000
Male	Sysmex XE-2100	486	154.960	10.140	135.000	175.490
Male	Sysmex XT	203	158.448	9.908	139.000	178.000
Female	Abbott CD 3200	100	137.723	9.938	117.168	156.211
Female	Abbott CD 3500/3700	198	132.888	9.089	111.738	149.984
Female	Abbott Sapphire/CD 4000	495	137.530	10.938	120.000	156.000
Female	Advia 120	299	138.986	9.372	124.000	161.000
Female	Coulter LH 750	159	137.068	9.362	118.000	154.560
Female	Horiba ABX Pentra 12	161	134.516	8.474	119.000	149.000
Female	Sysmex XE-2100	671	134.964	9.588	115.920	154.560
Female	Sysmex XT	220	140.132	9.343	124.000	161.000

Reticulocytes

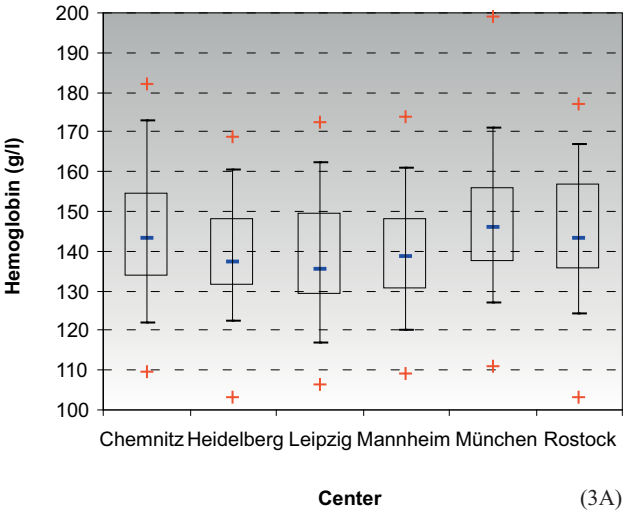
Reticulocytes are of critical importance in the differentiation of the causes of blood count changes. Other than for medical indications reticulocyte detection continues to be used in screening for so-called blood doping with recombinant erythropoietin by top athletes. This requires standardized reference ranges. The percentage of reticulocytes in the erythrocyte total is independent of gender (Figure 9A), but not their absolute concentration per µl (Figure 9C). The relative as well as the absolute reticulocyte count clearly showed analyzer-specific differences (Figure 9C), so that a change of method (analyzer change) must be taken into account in the follow-up evaluation. Whereas with the analyzers the upper 95% percentiles lie below 2.2%, values of above 3% occur in a few of the 2.5% of healthy individuals above these percentiles, without a previous dosage of EPO. There is no evidence of age dependence (Figure 9E). In fact, the clinically relevant measured parameter is the reticulocyte production index (RPI), since reference values only apply to healthy persons and reticulocytes play a role in the differentiation of the causes of anemia. In healthy individuals it is 1 [27].

Platelet count and platelet volume

For the first time we were able to show gender-specific differences in platelet counts with higher numbers for females (Figure 10A) [4]. There is no evidence of age dependence (Figure 10D). Any effects caused by smoking also cannot be demonstrated (Figure 10B). However, clear differences in platelet counts result from the use of different analyzer systems (Figure 10C) and determining platelet volume has similar results (MPV, see Figure 11). Gender-specific differences, however, do not exist with MPV (Figure 10B).

Leukocytes

No analyzer, age or gender dependence was found for the leukocyte count and the neutrophilic and lymphocytic subpopulation (Figures 12A, B, 13A, B, 14A, B). When using the peroxidase method in the Advia 120, the monocyte count is systematically lower (see Figure 15C), since the peroxidase-negative large cells are identified separately as so-called "large unstained cells" (LUC) and amount to approximately 1 to 4% in healthy individuals. In line with expectations smokers have higher leukocyte counts (Figure 12B), and this



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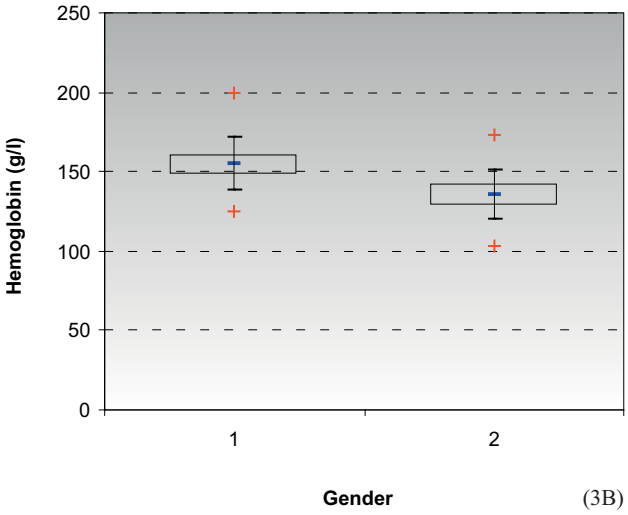

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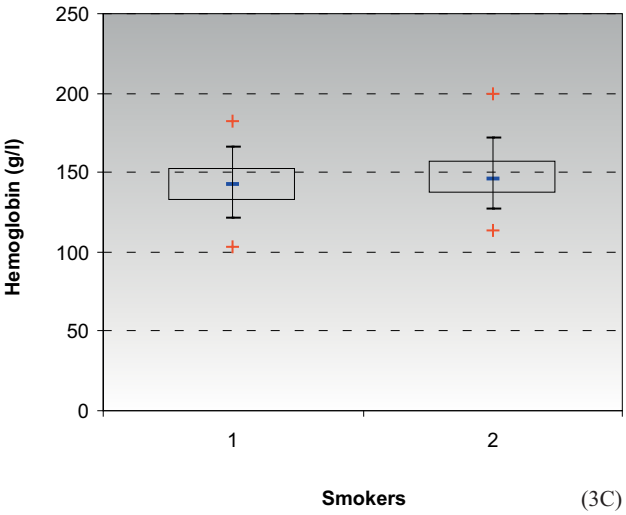

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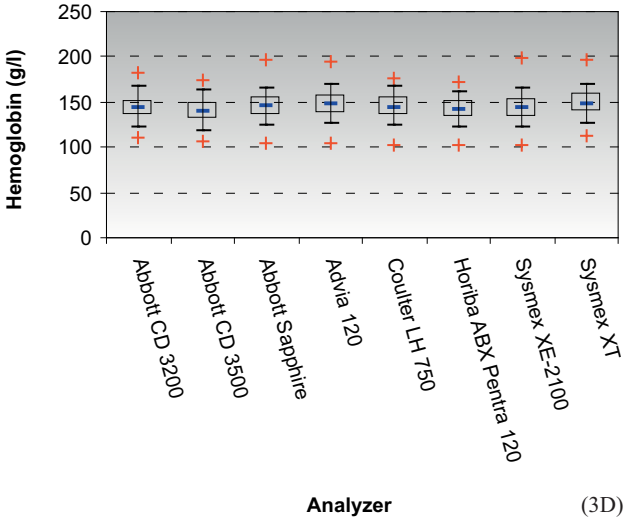

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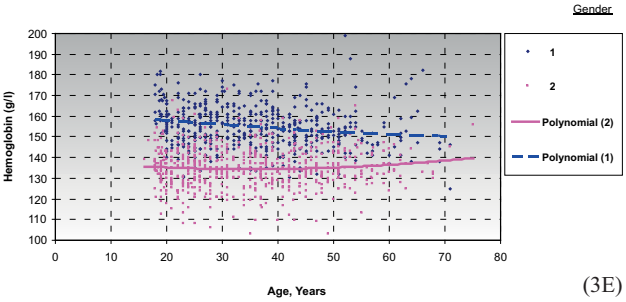

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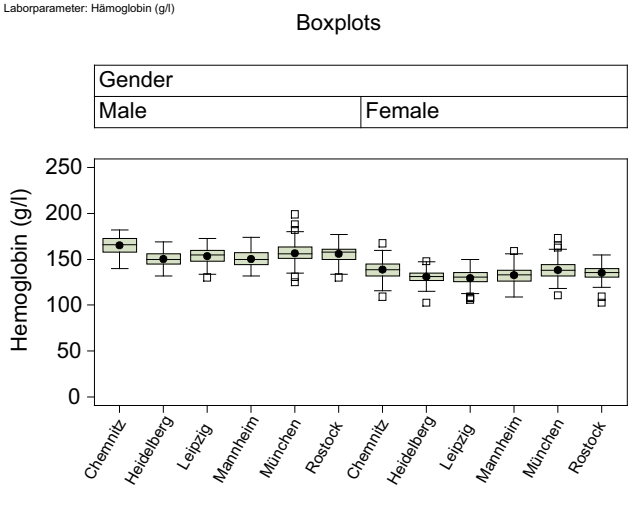


(3F)

Figure 3 (online only) Hemoglobin concentration (Hb). (A) Comparison of centers based on one analyzer (XE-2100). (B) Comparison of males (1) and females (2). (C) Comparison of non-smokers (1) and smokers (2). (D) Comparison of analyzers. (E) Investigation of age dependence by gender. (F) Comparison of centers based on the Sysmex XE-2100 analyzer

Table 4 Hematokrit (HCT).

Gender	Analyzer	n	Mean	SD	P 2.5%	P 97.5%
Male	Abbott CD 3200	58	0.449	0.030	0.402	0.514
Male	Abbott CD 3500/3700	109	0.442	0.024	0.394	0.487
Male	Abbott Sapphire/CD 4000	415	0.456	0.031	0.395	0.521
Male	Advia 120	258	0.449	0.029	0.396	0.518
Male	Coulter LH 750	101	0.453	0.028	0.390	0.504
Male	Horiba ABX Pentra 12	126	0.457	0.029	0.402	0.514
Male	Sysmex XE-2100	486	0.448	0.029	0.396	0.506
Male	Sysmex XT	203	0.461	0.028	0.410	0.526
Female	Abbott CD 3200	100	0.404	0.028	0.354	0.453
Female	Abbott CD 3500/3700	198	0.387	0.025	0.334	0.434
Female	Abbott Sapphire/CD 4000	494	0.405	0.030	0.345	0.465
Female	Advia 120	299	0.403	0.028	0.354	0.463
Female	Coulter LH 750	159	0.403	0.025	0.350	0.449
Female	Horiba ABX Pentra 12	161	0.401	0.025	0.353	0.452
Female	Sysmex XE-2100	671	0.399	0.028	0.346	0.453
Female	Sysmex XT	220	0.417	0.027	0.371	0.477

applies to all subpopulations with the exception of basophils. In contrast to previously existing data, however, this smoker effect is clearly less. When determining basophils, there are distinct analyzer-specific differences. It is a known fact that, besides the detection of polymorphous monocytes, the automated detection of basophiles presents the greatest problems. This stems particularly from the small measurement differences or from unsuitable criteria for delimitation against the remaining leukocyte subgroups. The eosinophil count shows a clear dispersion into the area of higher values (Figure 16A–D). This illustrates the difficulty of defining health, since atopic individuals who are free of complaints usually classify themselves as healthy. We did not specifically ask about allergies and they were not a criterion for exclusion. In both the charts and the tables we deliberately did not include percentages for the leukocyte subgroups, since the concentrations only represent the clinically relevant parameter.

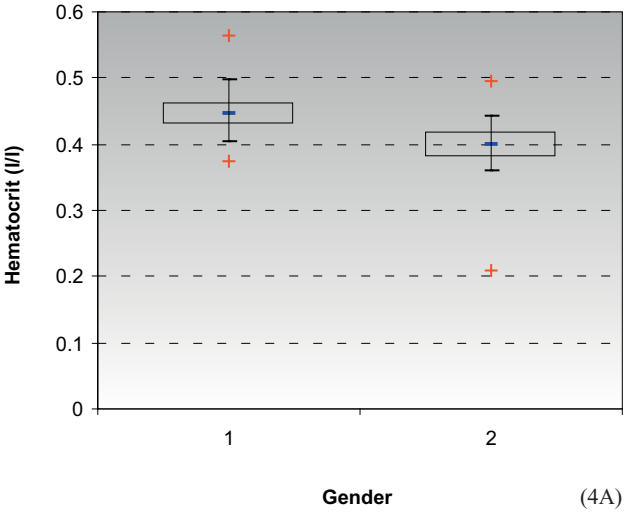
Classification of influencing variables

Influencing variables are the differences in age and gender distribution as well as the differences in the proportion of smokers between the centers. The waiting period for the participants with completion of the questionnaire and the examination of the test subjects represent a resting period before blood collection and were intended to compensate for the previously described fluctuations of blood count parameters in outpatients.

Reference ranges

Testing the parameters examined in this study for normal distribution for continuous measured parameters with the Kolmogorov-Smirnov test showed that all measured parameters do not follow a Gaussian normal distribution. The chi-squared test for discrete variables arrives at the same result. The box and whisker plots are designed as 95% percentiles and are not premised on normal distribution. This type of chart can be explained as follows: The short line within the rectangle represents the median value of the variable. The upper and lower edges indicate the 25^{th} or the 75^{th} percentiles of the data set. 50% of data fall within the rectangle and 50% are outside it. The whiskers mark the 5th and 95th percentiles. Finally, the minimum and maximum data values of the sample are marked with a + symbol.

At this time we do not believe that a separate reference table for smokers is indicated, especially since the relationship between the amount of tobacco consumption (cigarettes/day) and the associated blood count changes has not been investigated.



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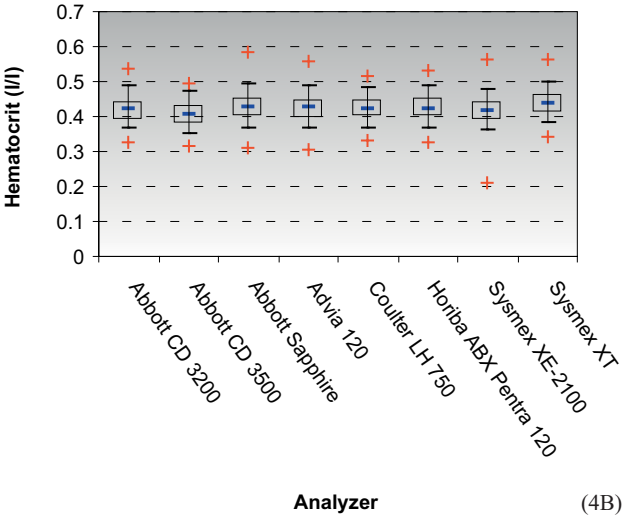

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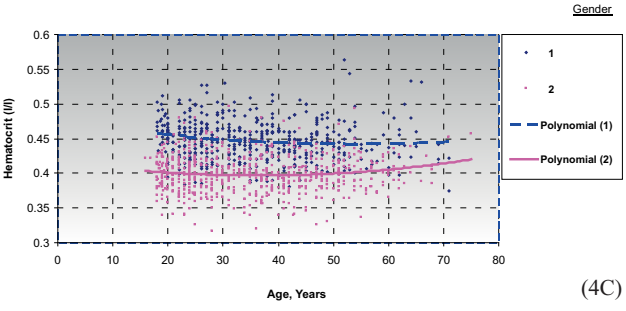
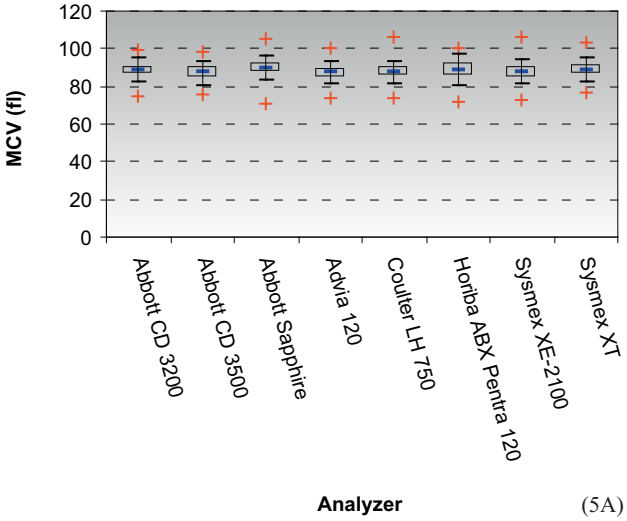
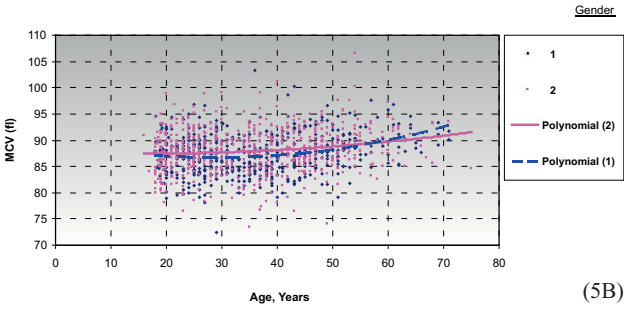


(4C)

Figure 4 (online only) Hematocrit (HCT).
(A) Comparison of males (1) and females (2). (B) Comparison of analyzers. (C) Investigation of age dependence by gender (1 = males, 2 = females)



(5A)



(5B)

Figure 5 (online only) Mean corpuscular volume (MCV) of erythrocytes.
(A) Comparison of analyzers. (B) Investigation of age dependence by gender (1 = males, 2 = females)

Calibration and control material

It is obvious that the control material made available by the companies for their respective analyzers does not lead to the fact that all locations with the same analyzers and the same control material generate the same values. The most likely cause is the deviation between the mean value of the control period and the target value of the control material (incorrectness).

Conclusions

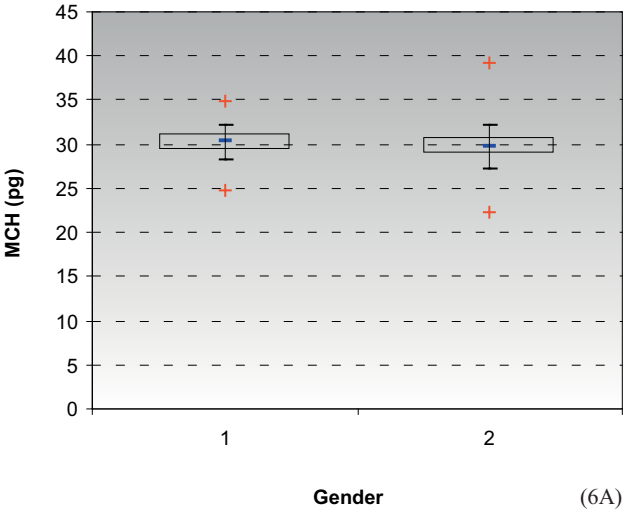
Although a blood count is the most frequently performed laboratory test, reference values that are specific to the analyzer and can be transferred to today's population are available only to a very limited degree. However, a comparison with standardized reference ranges is truly essential for recognizing disease-associated blood count changes. Frequently, violations of the standard ranges defined by a laboratory, lead to automated flagging through the laboratory information systems or the internal software systems of the analyzer that

Table 5 Mean corpuscular volume of erythrocytes (MCV).

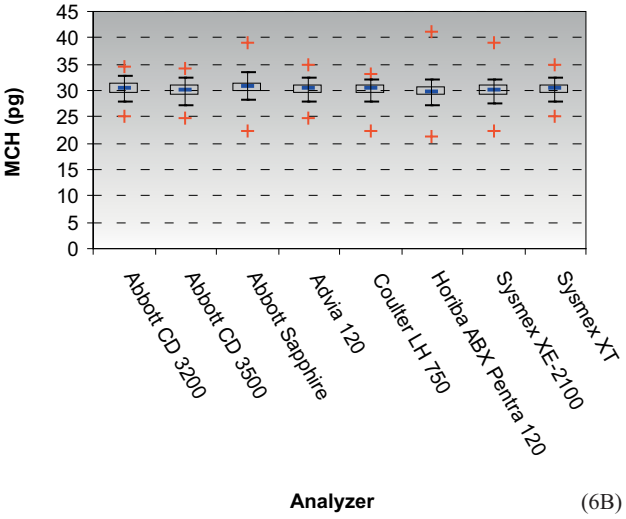
Analyzer	n	Mean	SD	P 2.5%	P 97.5%
Abbott CD 3200	179	88.586	3.793	81.190	96.240
Abbott CD 3500/3700	328	87.347	3.887	78.700	94.060
Abbott Sapphire/CD 4000	928	89.907	3.880	82.532	97.570
Advia 120	558	87.111	3.816	80.100	95.300
Coulter LH 750	260	87.790	3.843	80.600	95.300
Horiba ABX Pentra 12	287	88.822	4.708	80.000	98.000
Sysmex XE-2100	1157	87.757	4.014	80.000	95.500
Sysmex XT	423	89.010	3.895	81.500	97.300

Table 6 Mean corpuscular hemoglobin (MCH).

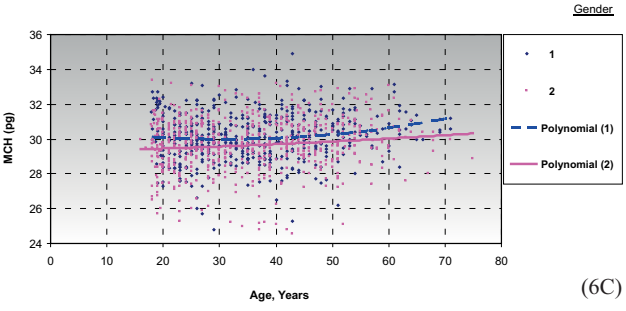
Gender	Analyzer	n	Mean	SD	P 2.5%	P 97.5%
Male	Abbott CD 3200	58	30.411	1.260	27.656	32.704
Male	Abbott CD 3500/3700	108	30.285	1.330	26.779	32.461
Male	Abbott Sapphire/CD 4000	415	30.625	1.436	28.000	33.640
Male	Advia 120	258	30.437	1.320	27.600	33.200
Male	Coulter LH 750	101	30.337	1.133	28.200	32.200
Male	Horiba ABX Pentra 12	126	29.926	1.694	27.000	32.500
Male	Sysmex XE-2100	486	30.240	1.334	27.600	32.836
Male	Sysmex XT	203	30.316	1.343	27.700	33.000
Female	Abbott CD 3200	100	30.316	1.712	26.592	33.632
Female	Abbott CD 3500/3700	198	29.920	1.571	26.174	33.180
Female	Abbott Sapphire/CD 4000	494	30.612	1.747	27.500	34.000
Female	Advia 120	299	30.171	1.535	27.000	32.900
Female	Coulter LH 750	159	30.013	1.467	25.600	32.500
Female	Horiba ABX Pentra 12	161	29.644	1.765	25.700	32.800
Female	Sysmex XE-2100	671	29.780	1.543	26.108	32.600
Female	Sysmex XT	220	30.163	1.347	27.600	32.700



(6A)

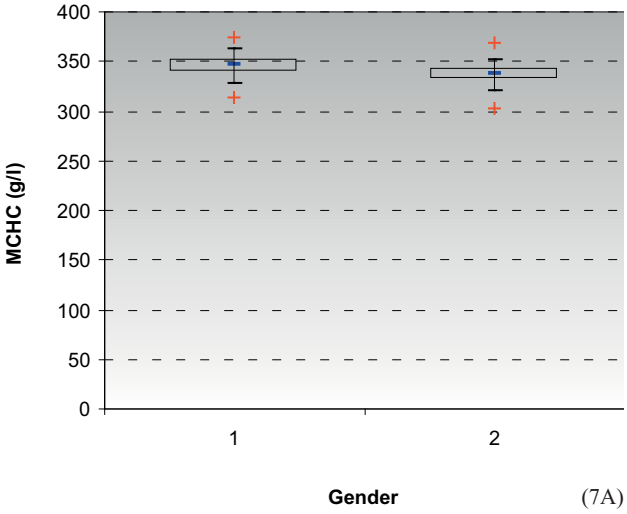


(6B)

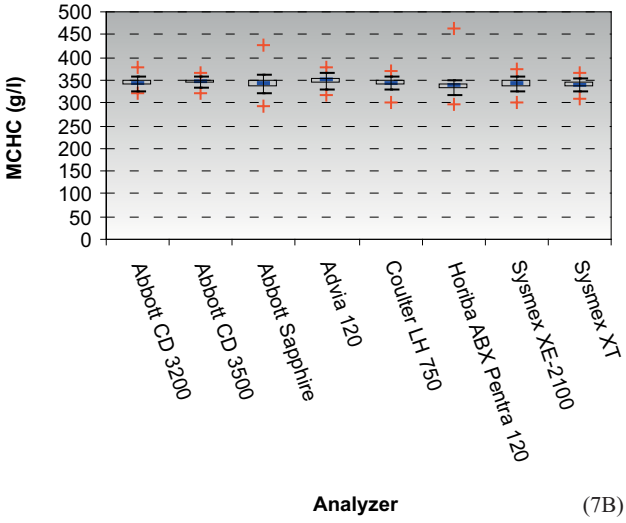


(6C)

Figure 6 (online only) Mean corpuscular hemoglobin volume (MCH).
(A) Comparison of males (1) and females (2). (B) Comparison of analyzers. (C) Investigation of age dependence by gender (1 = males, 2 = females)



(7A)



(7B)

Figure 7 (online only) Mean corpuscular hemoglobin concentration (MCHC).
(A) Comparison of males (1) and females (2). (B) Comparison of analyzers

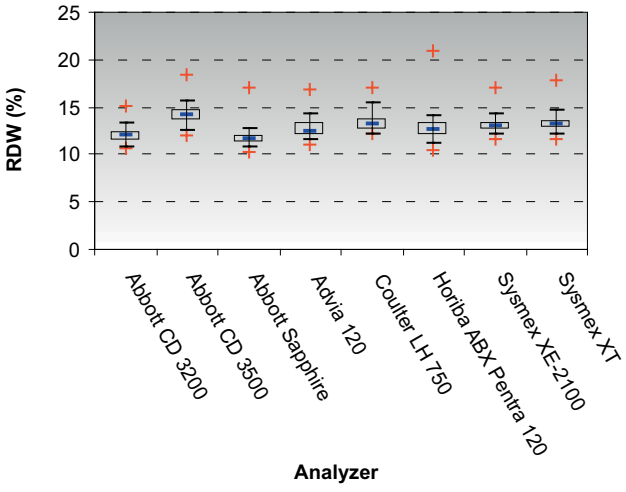


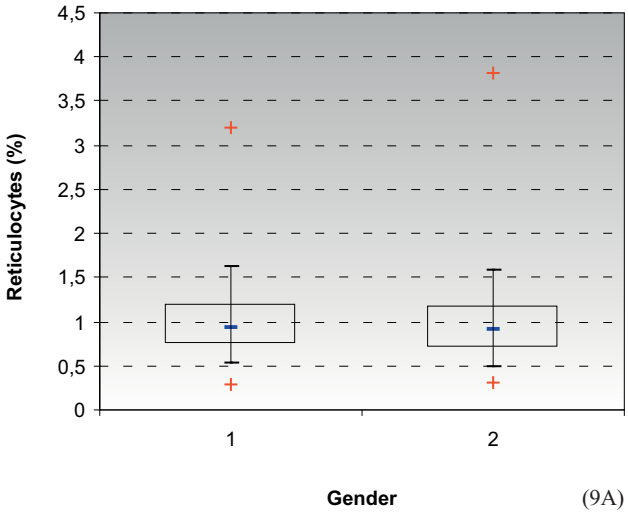
Figure 8 (online only) Red cell distribution width (RDW), comparison of analyzers

Table 7 Mean corpuscular hemoglobin concentration (MCHC).

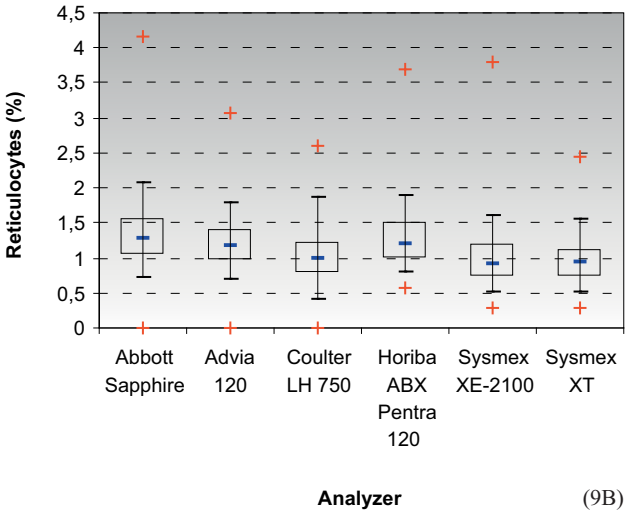
Gender	Analyzer	n	Mean	SD	P 2.5%	P 97.5%
Male	Abbott CD 3200	58	346.037	10.092	328.046	369.731
Male	Abbott CD 3500/3700	109	345.848	6.084	334.409	357.655
Male	Abbott Sapphire/CD 4000	415	341.502	11.352	321.000	363.439
Male	Advia 120	258	351.247	10.156	330.000	372.000
Male	Coulter LH 750	101	348.549	8.221	332.000	365.470
Male	Horiba ABX Pentra 12	126	335.437	14.818	316.000	351.000
Male	Sysmex XE-2100	486	346.314	9.826	328.000	366.000
Male	Sysmex XT	203	343.729	8.621	326.000	360.000
Female	Abbott CD 3200	100	340.575	9.839	321.201	359.712
Female	Abbott CD 3500/3700	198	343.615	6.583	331.057	357.578
Female	Abbott Sapphire/CD 4000	495	339.062	19.970	319.000	365.470
Female	Advia 120	299	344.898	10.153	326.000	365.000
Female	Coulter LH 750	159	340.094	8.167	320.390	354.000
Female	Horiba ABX Pentra 12	161	335.186	9.923	315.000	350.000
Female	Sysmex XE-2100	671	338.009	9.193	319.000	355.000
Female	Sysmex XT	220	336.245	7.852	321.000	351.000

Table 8 Red blood cell distribution width (RDW).

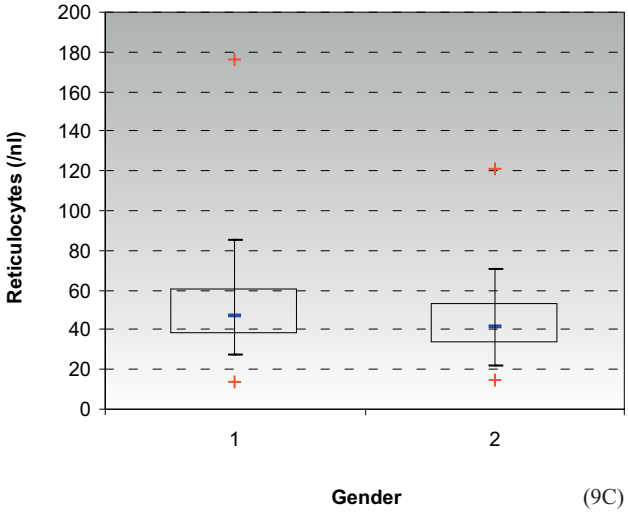
Analyzer	n	Mean	SD	P 2.5%	P 97.5%
Abbott CD 3200	179	11.960	0.705	10.862	13.639
Abbott CD 3500	328	14.146	0.933	12.393	16.100
Abbott Sapphire	765	11.660	0.647	10.800	13.100
Advia 120	558	12.754	0.893	11.500	14.700
Coulter LH 750	95	13.294	0.929	12.200	15.700
Horiba ABX Pentra 12	287	12.722	1.096	10.900	14.900
Sysmex XE-2100	992	13.056	0.686	12.100	14.800
Sysmex XT	423	13.187	0.772	12.100	15.100



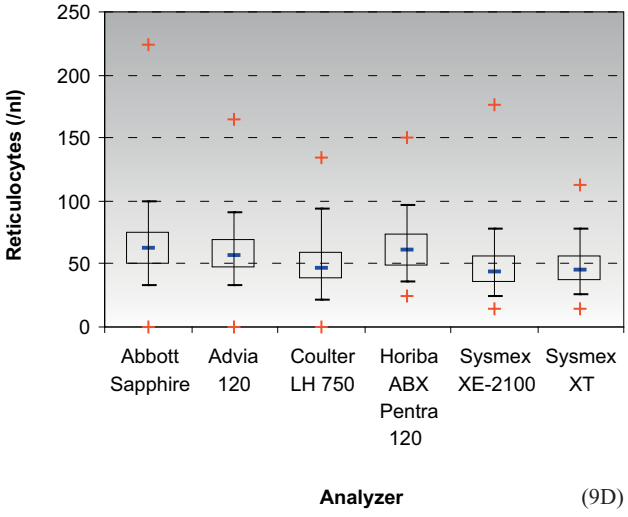
(9A)



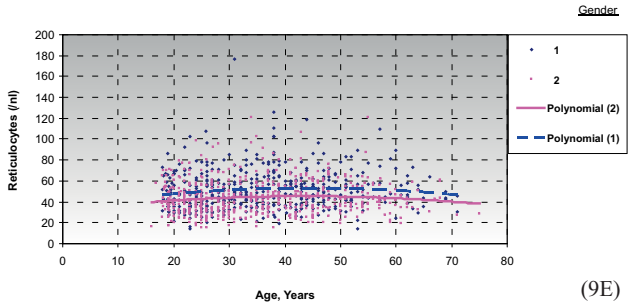
(9B)



(9C)



(9D)



(9E)

Figure 9 (online only) Reticulocytes.
(A) Comparison of percent values of males (1) and females (2) based on measurements at the XE-2100. (B) Comparison of analyzers by percent values. (C) Comparison of males (1) and females (2) by absolute values based on XE-2100 values. (D) Comparison of analyzers by concentration values. (E) Investigation of age dependence

Table 9 Reticulocyte concentration.

Gender	Analyzer	N	Mean	SD	P 2.5%	P 97.5%
Male	Abbott CD 3200	0	.	.	.	.
Male	Abbott CD 3500/3700	0	.	.	.	.
Male	Abbott Sapphire/CD 4000	324	67.756	23.283	31.000	115.000
Male	Advia 120	257	63.865	18.059	36.000	101.100
Male	Coulter LH 750	56	56.654	22.443	21.700	114.500
Male	Horiba ABX Pentra 12	125	67.888	19.962	39.000	113.000
Male	Sysmex XE-2100	431	51.218	18.836	24.800	96.200
Male	Sysmex XT	203	51.050	16.283	26.600	91.900
Female	Abbott CD 3200	0	.	.	.	.
Female	Abbott CD 3500/3700	0	.	.	.	.
Female	Abbott Sapphire/CD 4000	396	60.261	20.861	30.000	117.000
Female	Advia 120	294	54.459	17.638	25.900	97.500
Female	Coulter LH 750	120	47.133	19.903	13.900	98.300
Female	Horiba ABX Pentra 12	158	57.184	17.623	27.000	91.000
Female	Sysmex XE-2100	578	43.766	16.171	19.800	80.700
Female	Sysmex XT	220	44.066	15.880	19.500	82.700

Table 10 Platelet concentration.

Gender	Analyzer	n	Mean	SD	P 2.5%	P 97.5%
Male	Abbott CD 3200	58	254.062	50.439	168.418	400.159
Male	Abbott CD 3500/3700	109	227.823	48.927	148.084	341.000
Male	Abbott Sapphire/CD 4000	415	234.478	50.374	149.583	346.000
Male	Advia 120	258	267.183	54.112	166.000	389.000
Male	Coulter LH 750	101	230.188	53.349	137.000	327.000
Male	Horiba ABX Pentra 12	126	255.619	50.255	168.000	355.000
Male	Sysmex XE-2100	486	229.953	45.868	146.000	328.000
Male	Sysmex XT-	203	242.734	49.053	157.000	355.000
Female	Abbott CD 3200	100	288.016	57.601	190.503	426.290
Female	Abbott CD 3500/3700	198	260.935	51.815	178.000	399.003
Female	Abbott Sapphire/CD 4000	494	267.610	57.279	168.000	405.000
Female	Advia 120	299	302.196	67.215	203.000	445.000
Female	Coulter LH 750	159	267.478	59.331	166.000	387.000
Female	Horiba ABX Pentra 12	161	293.311	69.174	179.000	443.000
Female	Sysmex XE-2100	671	268.048	55.707	176.000	391.000
Female	Sysmex XT	220	273.514	58.986	178.000	412.000

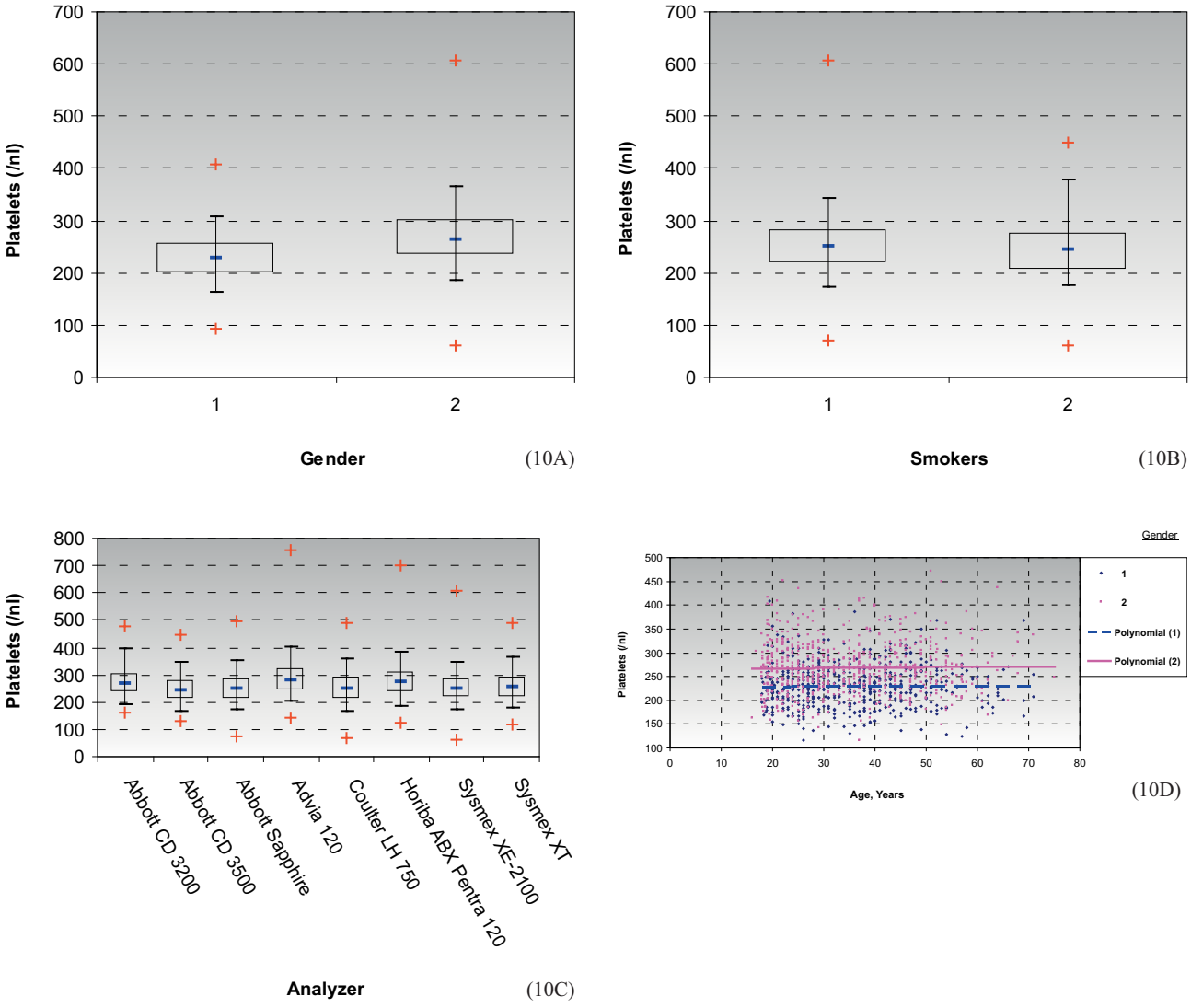


Figure 10 (online only) Platelet concentration.
(A) Comparison of males (1) and females (2). (B) Comparison of non-smokers (1) and smokers (2). (C) Comparison by analyzers. (D) Investigation of age dependence

Table 11 Mean Platelet Volume (MPV).

Analyzer	n	Mean	SD	P 2.5%	P 97.5%
Abbott CD 3200	179	8.804	1.568	6.380	12.420
Abbott CD 3500/3700	327	8.551	1.129	6.816	11.028
Abbott Sapphire/CD 4000	766	8.232	1.068	6.594	10.600
Advia 120	558	7.711	1.026	5.900	9.900
Coulter LH 750	95	9.180	0.995	7.600	11.700
Horiba ABX Pentra 12	287	8.990	0.839	7.600	10.800
Sysmex XE-2100	964	10.671	0.859	9.200	12.500
Sysmex XT	422	10.586	0.824	9.200	12.200

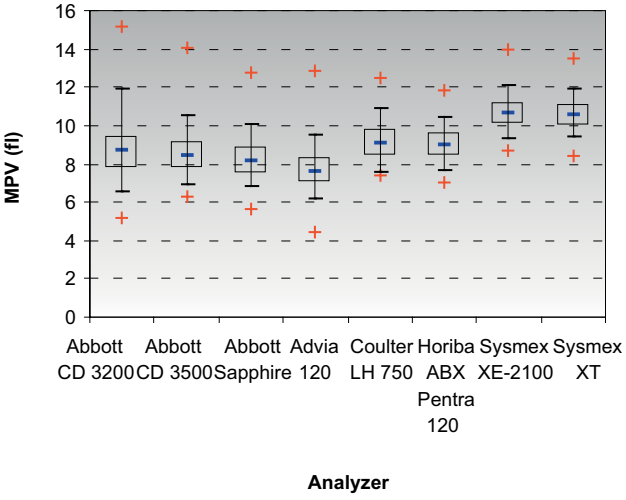


Figure 11 (online only) Mean Platelet Volume (MPV), analyzer comparison.

provoke reflex-type reactions in the sending physician. For example, the use of different reference ranges between different laboratories can mean that a test of one and the same blood sample fulfills the defining criteria of anemia in one laboratory, but not in the other. At this point it should again be emphasized that an intra-individual comparison with preceeding values is superior to an inter-individual comparison, especially in follow-up observations [29]. Up to now gender-specific standard ranges were focused on erythrocytes, Hb and HCT. In the future and not least within the scope of accreditation procedures, the source of reference ranges for the mean corpuscular hemoglobin concentration (MCHC), the red blood cell distribution width (RDW), the reticulocyte concentration, the platelet concentration, the mean platelet volume (MPV) or the white blood cell concentration (WBC) will be scrutinized. The percentages of the individual leukocyte subgroups in the differential blood count represent only auxiliary values for calculating their absolute concentrations as primary evaluation criteria. Hence only these were discussed and graphically represented in this study. The more detailed differentiations offered in part by some of the analyzers are company-specific (large unstained cells [LUC] or immature granulocytes [IG]) and were not included in the evaluation of the study, since they cannot or can only be partially verified with microscopic and immunologic reference procedures [30]. As demonstrated by the example of a female smoker, even in healthy persons outside the 95% percentiles reticulocytes disperse upward up to 3.8% with normal blood count values, so that an accusation of doping with

Table 12: Leukocyte concentration.

Gender	Analyzer	n	Mean	SD	P 2.5%	P 97.5%
Male	Abbott CD 3200	58	6519.679	1448.720	4539.500	9586.400
Male	Abbott CD 3500/3700	109	6140.061	1350.253	4100.000	9253.500
Male	Abbott Sapphire/CD 4000	415	6341.346	1562.213	3930.000	10100.000
Male	Advia 120	258	6429.240	1646.177	3790.000	10330.000
Male	Coulter LH 750	101	6195.842	1363.234	4410.000	9340.000
Male	Horiba ABX Pentra 12	126	6493.651	1599.637	3900.000	10300.000
Male	Sysmex XE-2000	486	6196.523	1479.203	3920.000	9810.000
Male	Sysmex XT-2100	203	6193.350	1610.873	3740.000	9890.000
Female	Abbott CD 3200	100	6838.586	1522.200	4240.200	10118.400
Female	Abbott CD 3500/3700	198	6623.308	1530.045	3950.000	9950.000
Female	Abbott Sapphire/CD 4000	494	6756.031	1834.663	4030.000	11200.000
Female	Advia 120	299	6882.382	1899.954	4050.000	11840.000
Female	Coulter LH 750	159	6836.604	1685.822	3940.000	10900.000
Female	Horiba ABX Pentra 12	161	7124.224	1814.310	4000.000	11500.000
Female	Sysmex XE-2100	671	6682.219	1720.087	3960.000	10410.000
Female	Sysmex XT-2000	220	6594.273	1947.729	3950.000	11570.000

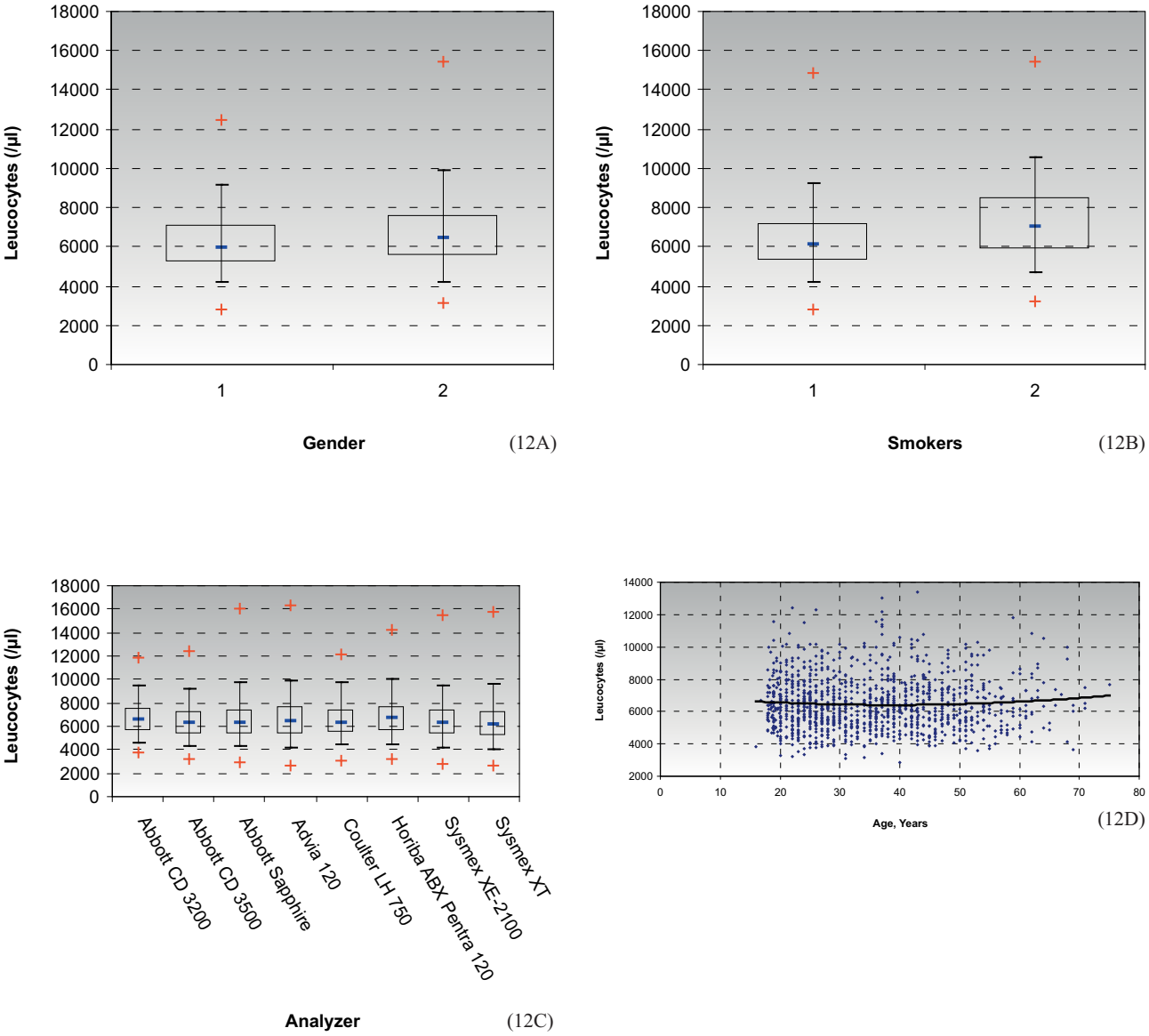


Figure 12 (online only) Leukocyte concentration.
(A) Comparison of males (1) and females (2). (B) Comparison of non-smokers (1) and smokers (2). (C) Comparison by analyzers. (D) Investigation of age dependence.

Table 13 Concentration of neutrophilic granulocytes.

Gender	Analyzer	n	Mean	SD	P 2.5%	P 97.5%
Male	Abbott CD 3200	58	3739.924	1129.824	2151.800	6199.700
Male	Abbott CD 3500/3700	109	3456.878	1046.509	2010.000	6098.400
Male	Abbott Sapphire/CD 4000	411	3593.960	1253.389	1650.000	6990.000
Male	Advia 120	253	3725.106	1318.481	1780.000	7000.000
Male	Coulter LH 750	57	3894.912	1330.834	2010.000	6700.000
Male	Horiba ABX Pentra 12	126	3680.952	1278.290	1760.000	6700.000
Male	Sysmex XE-2100	481	3488.942	1167.837	1781.450	6230.000
Male	Sysmex XT	203	3505.714	1261.501	1750.000	6950.000
Female	Abbott CD 3200	100	4176.974	1358.146	2094.800	7461.600
Female	Abbott CD 3500/3700	198	3906.458	1387.967	1850.000	7313.900
Female	Abbott Sapphire/CD 4000	493	3981.571	1529.205	2000.000	7680.000
Female	Advia 120	284	4113.375	1581.720	2070.000	7730.000
Female	Coulter LH 750	120	4195.833	1421.009	2200.000	7510.000
Female	Horiba ABX Pentra 12	161	4158.075	1419.960	2210.000	7880.000
Female	Sysmex XE-2100	668	3883.850	1417.587	1910.000	7337.270
Female	Sysmex XT	220	3856.318	1584.036	1710.000	7870.000

Table 14 Lymphocyte concentration.

Gender	Analyzer	n	Mean	SD	P 2.5%	P 97.5%
Male	Abbott CD 3200	58	2006.852	579.449	1126.300	3422.300
Male	Abbott CD 3500/3700	109	1954.473	527.472	1040.000	3241.400
Male	Abbott Sapphire/CD 4000	411	1996.195	549.463	1100.000	3160.000
Male	Advia 120	253	1929.255	561.566	1070.000	3120.000
Male	Coulter LH 750	57	1868.246	493.324	1170.000	3000.000
Male	Horiba ABX Pentra 12	126	2013.016	541.849	1060.000	3090.000
Male	Sysmex XE-2100	481	1983.858	547.267	1051.380	3240.000
Male	Sysmex XT	203	1888.473	549.296	1080.000	3040.000
Female	Abbott CD 3200	100	2012.232	487.184	1270.300	3018.200
Female	Abbott CD 3500/3700	198	2067.197	567.911	1230.000	3350.000
Female	Abbott Sapphire/CD 4000	493	2118.751	592.598	1240.000	3630.000
Female	Advia 120	284	2069.349	593.287	1170.000	3450.000
Female	Coulter LH 750	120	2148.583	521.963	1100.000	3450.000
Female	Horiba ABX Pentra 12	161	2206.149	612.409	1060.000	3490.000
Female	Sysmex XE-2100	668	2138.255	591.889	1220.000	3560.000
Female	Sysmex XT	220	2017.045	585.168	1140.000	3540.000

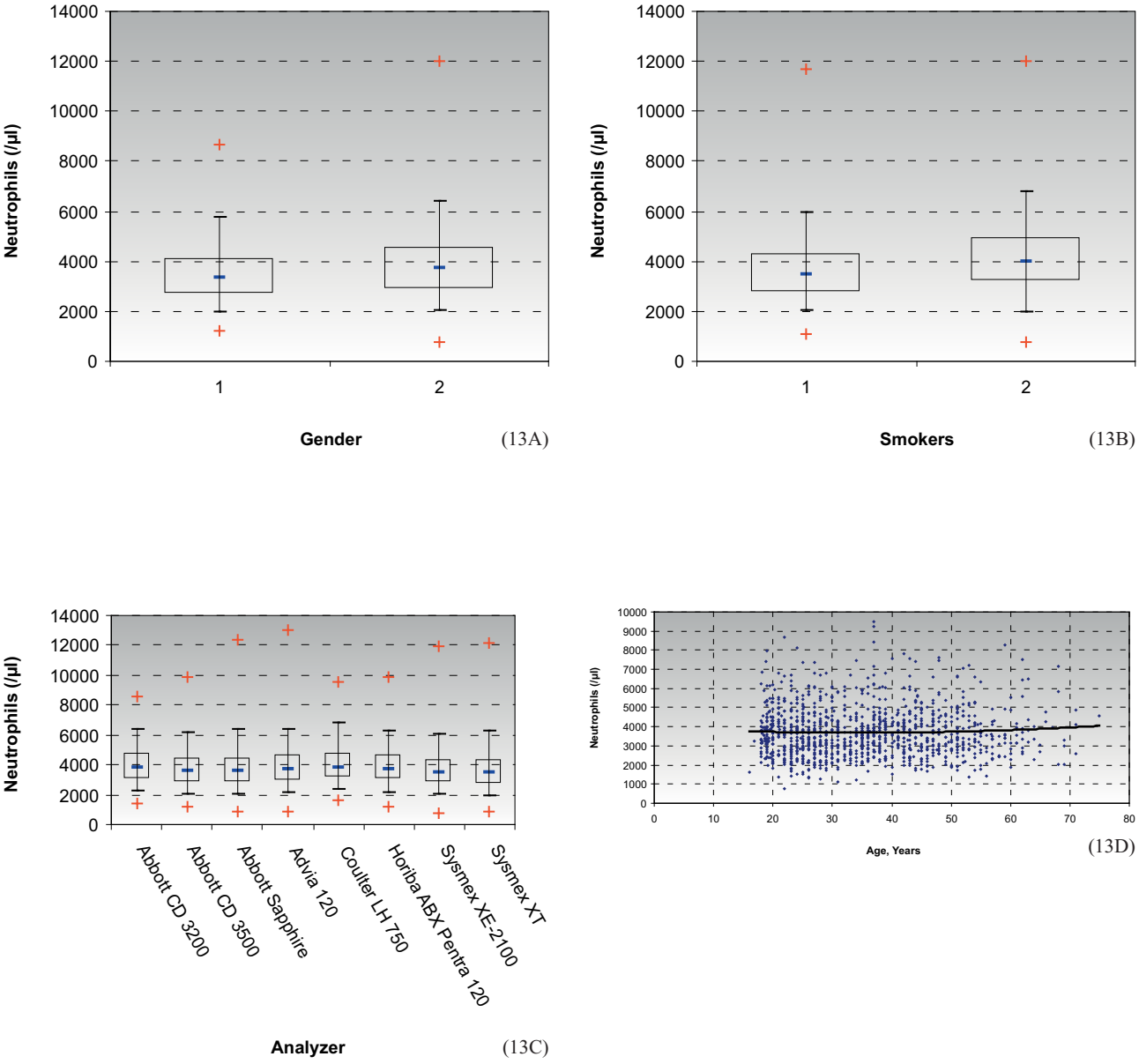
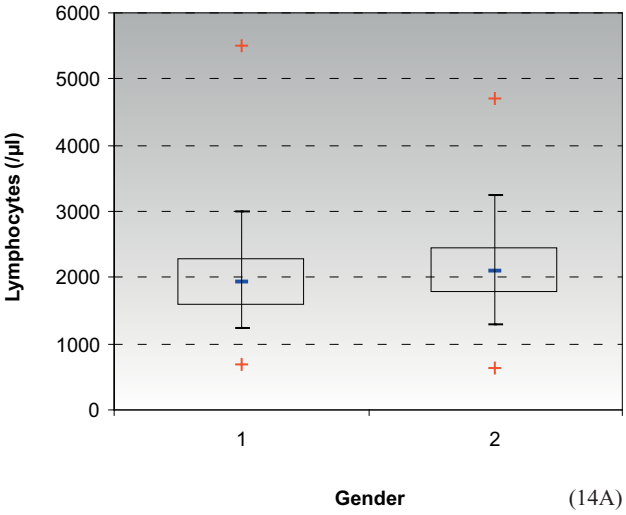
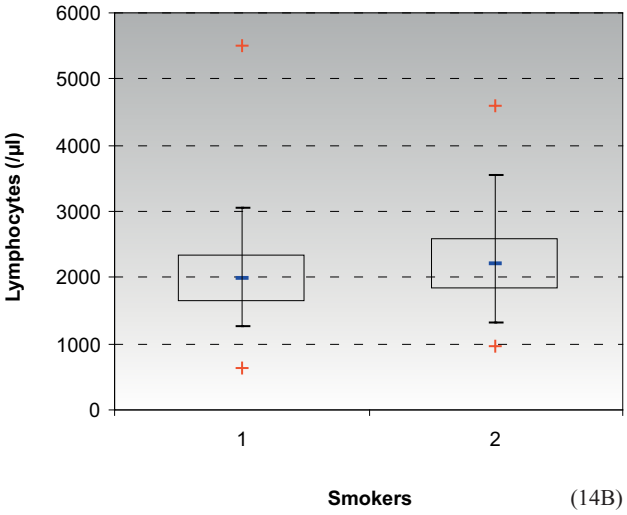


Figure 13 (online only) Concentration of neutrophilic granulocytes.
(A) Comparison of males (1) and females (2). (B) Comparison of non-smokers (1) and smokers (2). (C) Comparison by analyzers. (D) Investigation of age dependence.



(14A)



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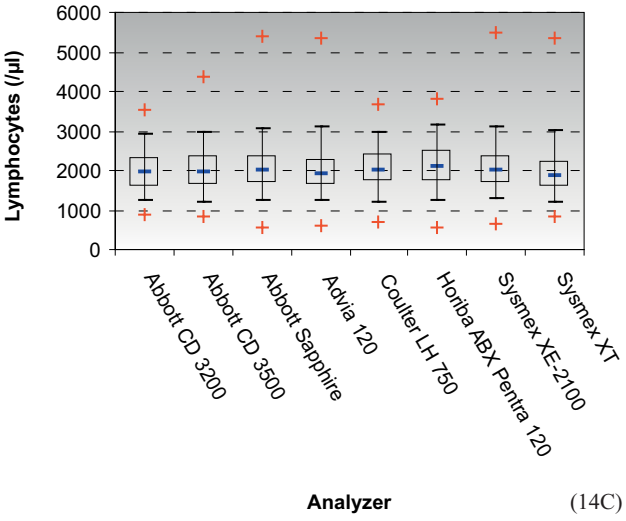
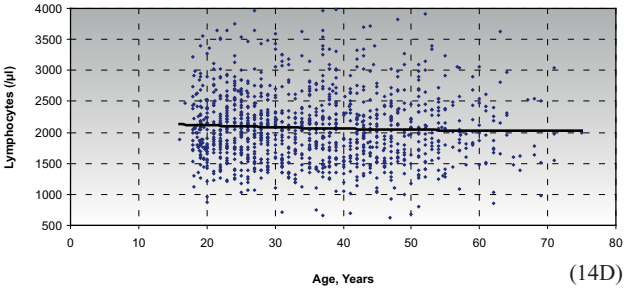


(14C)



(14D)

Figure 14 (online only) Lymphocyte concentration.
(A) Comparison of males (1) and females (2). (B) Comparison of non-smokers (1) and smokers (2). (C) Comparison of analyzers. (D) Investigation of age dependence.

Table 15 Monocyte concentration.

Gender	Analyzer	n	Mean	SD	P 2.5%	P 97.5%
Male	Abbott CD 3200	58	554.031	139.570	339.000	850.500
Male	Abbott CD 3500/3700	109	512.760	145.000	287.000	828.700
Male	Abbott Sapphire/CD 4000	411	536.474	168.834	246.000	941.000
Male	Advia 120	253	432.172	126.930	240.000	730.000
Male	Coulter LH 750	57	557.193	148.730	290.000	860.000
Male	Horiba ABX Pentra 12	126	556.667	162.363	250.000	900.000
Male	Sysmex XE-2100	481	527.295	164.831	260.850	870.000
Male	Sysmex XT	203	601.675	187.889	320.000	1090.000
Female	Abbott CD 3200	100	454.405	138.007	232.500	745.700
Female	Abbott CD 3500/3700	198	447.540	152.126	207.200	797.000
Female	Abbott Sapphire/CD 4000	493	479.566	156.602	230.000	824.000
Female	Advia 120	284	381.957	118.599	200.000	650.000
Female	Coulter LH 750	120	498.917	144.233	260.000	810.000
Female	Horiba ABX Pentra 12	161	505.714	170.542	220.000	930.000
Female	Sysmex XE-2100	668	493.329	156.685	250.000	850.000
Female	Sysmex XT	220	550.182	179.898	280.000	990.000

Table 16 Eosinophilic granulocytes.

Analyzer	n	Mean	SD	P 2.5%	P 97.5%
Abbott CD 3200	179	131.91	90.456	19.800	365.90
Abbott CD 3500/3700	328	144.14	106.30	23.700	433.00
Abbott Sapphire/CD 4000	923	158.67	110.54	30.303	448.00
Advia 120	538	155.95	108.66	30.000	470.00
Coulter LH 750	166	160.36	117.55	40.000	400.00
Horiba ABX Pentra 120	287	170.98	93.235	60.000	400.00
Sysmex XE-2100	1149	149.40	106.45	30.000	440.00
Sysmex XT	423	152.74	109.33	30.000	440.00

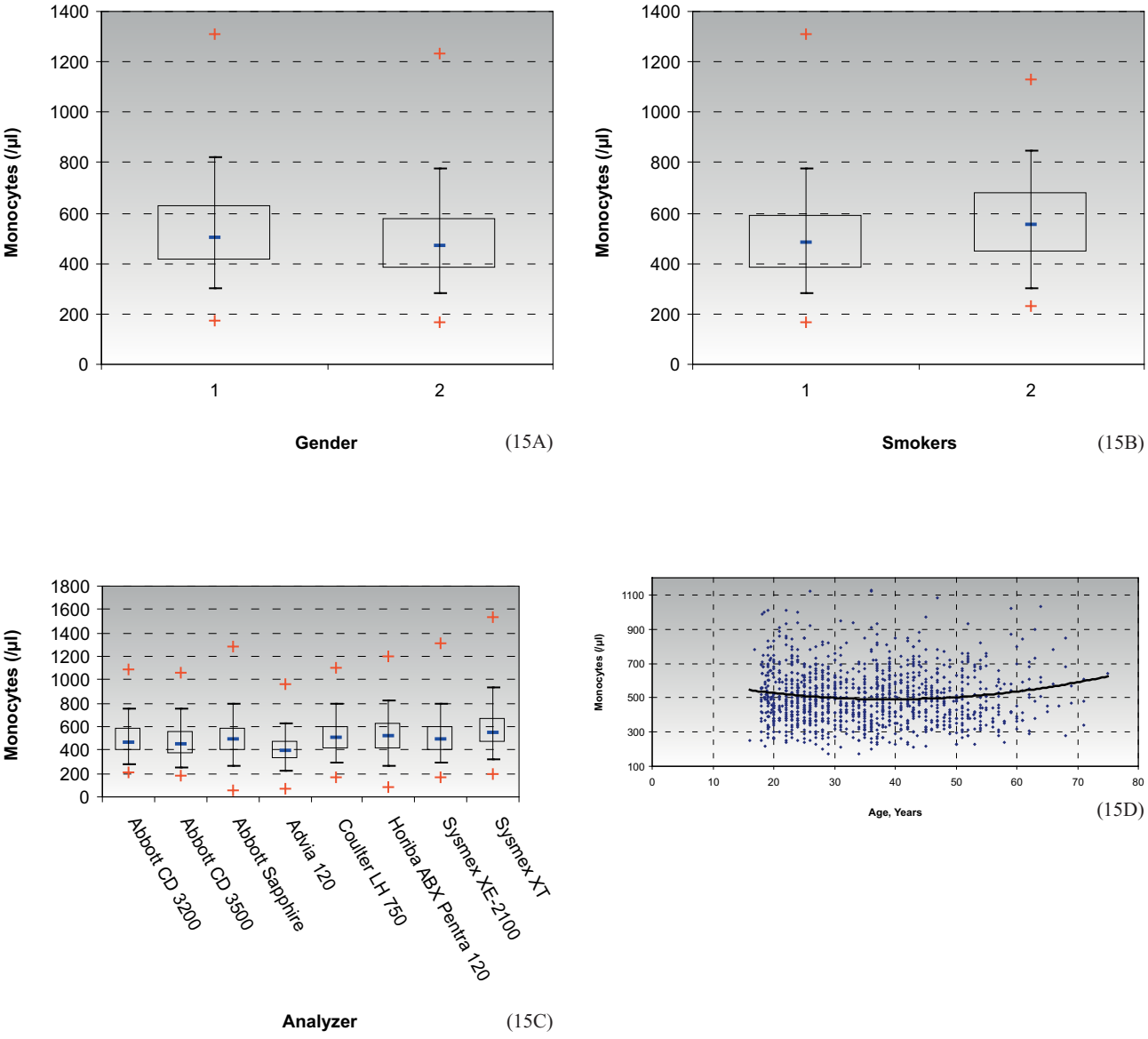


Figure 15 (online only) Concentration of monocytes.
(A) Comparison of males (1) and females (2). (B) Comparison of non-smokers (1) and smokers (2). (C) Comparison of analyzers. (D) Investigation of age dependence

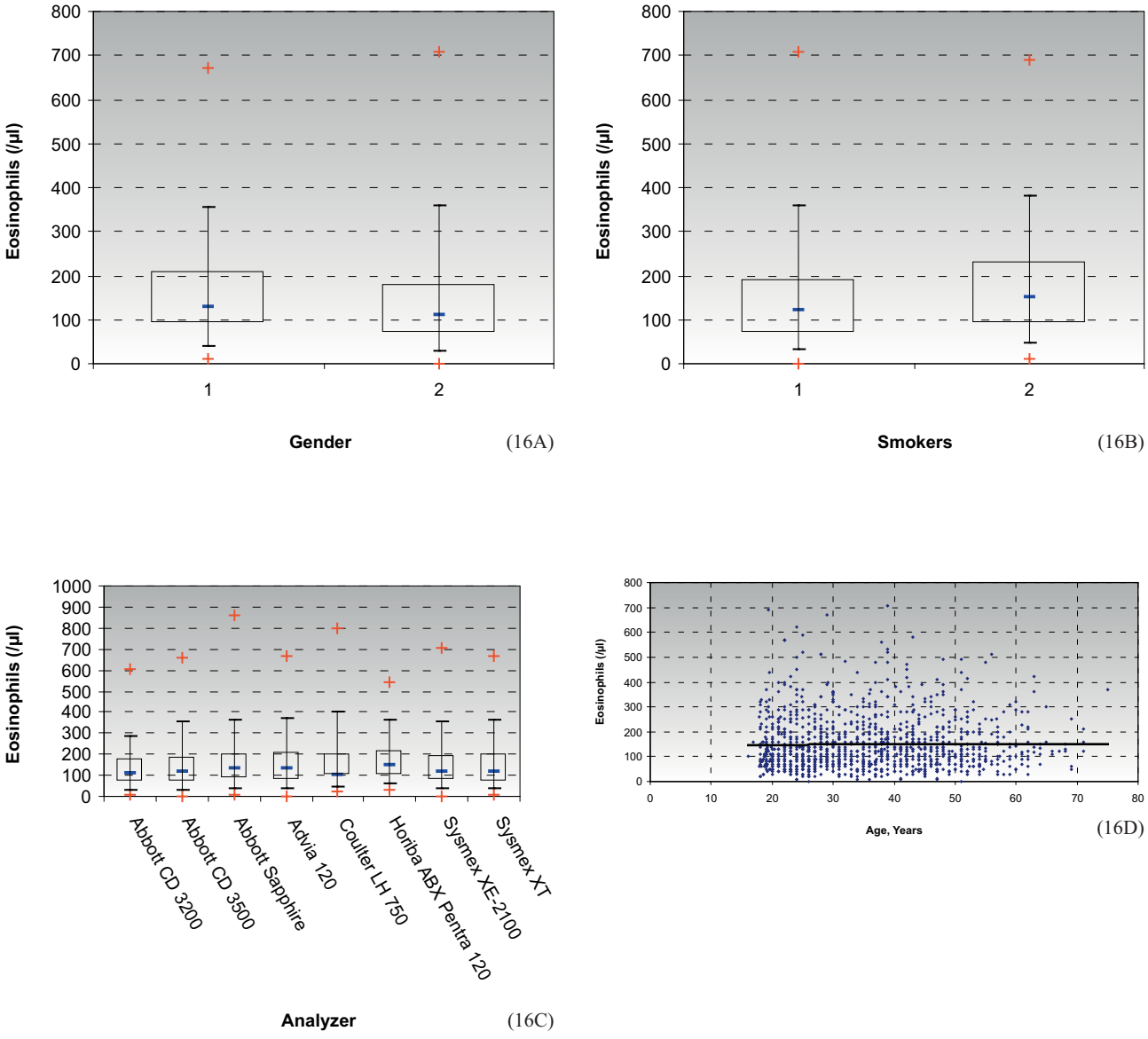


Figure 16 (online only) Concentration of eosinophilic granulocytes.
(A) Comparison of males (1) and females (2). (B) Comparison of non-smokers (1) and smokers (2). (C) Comparison of analyzers. (D) Investigation of age dependence

erythropoietin based solely on a reticulocyte value of 3.3% is not legally tenable, as occurred recently in a prominent case.

The equipment- and location-dependent differences found in this study should serve as a reminder of the laboratory's obligation to equalize the hematology analyzers within a laboratory or a hospital. This requires the use of normal and pathologic blood samples and prohibits stabilized or artificial control material, which is analyzer-dependent and in part is even treated differently by the software.

One must be aware of that the described analyzer-related differences as described in this study with healthy persons are smaller than those that can be achieved in the practice with diseased persons, e.g. with the latter the equipment has considerable problems delimiting anisocytosis as well as poikilocytosis of the platelets and erythrocytes and also with activated lymphocytes and variant monocytes, because all instruments were developed with the aid of blood samples from healthy persons. The study did not look at erythroblasts that can be quantified by certain devices, since they occur in only very low concentrations in healthy persons and present statistical problems. In summary, the results of this study offer medical laboratories the possibility to examine their reference ranges when providing care to healthy Central Europeans while illustrating to the colleagues requesting blood counts the need to qualify the sharp limits stated in the laboratory reports.

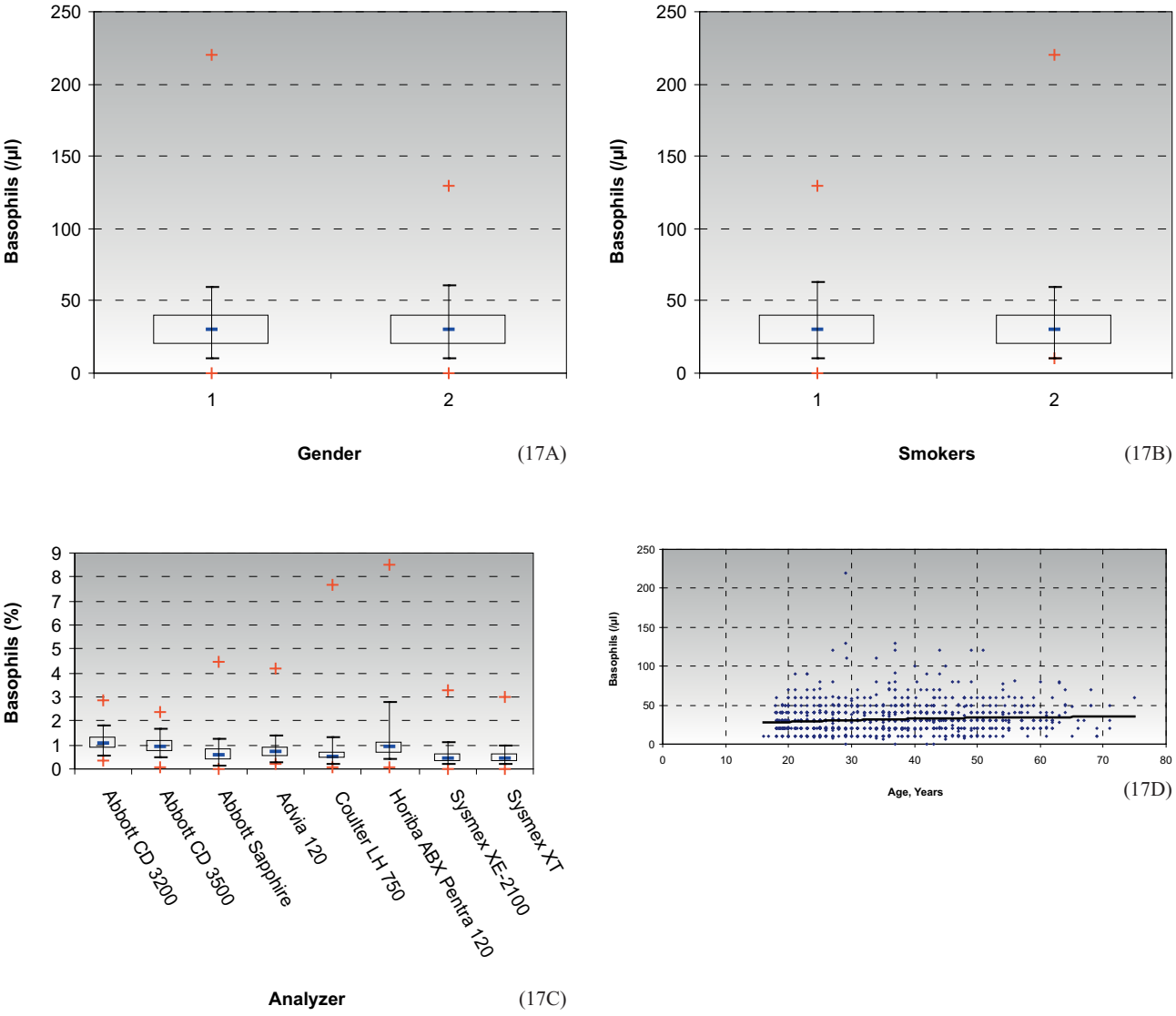


Figure 17 (online only) Concentration of basophilic granulocytes.
(A) Comparison of males (1) and females (2). (B) Comparison of non-smokers (1) and smokers (2). (C) Comparison of analyzers. (D) Investigation of age dependence.

Table 17 Basophilic granulocytes.

Analyzer	n	Mean	SD	P 2.5%	P 97.5%
Abbott CD 3200	179	72.101	27.763	37.000	137.50
Abbott CD 3500/3700	328	61.797	24.899	23.600	121.00
Abbott Sapphire/CD 4000	923	39.645	29.207	6.870	90.800
Advia 120	538	50.714	24.697	20.000	110.00
Coulter LH 750	177	31.243	43.779	0.000	100.00
Horiba ABX Pentra 12	287	69.547	58.759	20.000	270.00
Sysmex XE-2100	1149	31.431	18.899	10.000	79.500
Sysmex XT	423	30.827	19.178	10.000	80.000

References

1. Thomas L. Labor und Diagnose, 5. Auflage. Frankfurt:TH-Books Verlagsgeselischaft, 1998.
2. Greiling H, Gressner AM. Lehrbuch der Klinischen Chemie und Pathobiochemie, 3. Auflage. Stuttgart, New York: Schattauer, 1995
3. Keller H. Klinisch-chemische Labordiagnostik für die Praxis: Analyse, Befund, Interpretation, 2. Auflage. Stuttgart, New York: Thieme, 1991.
4. Greer JP, Foerster J, Lukens JN, et al. Wintrobes clinical hematology, 12th ed., New York: Lea&Fibiger, 1993
5. Herklotz R, Lüthi U, Ottiger C, Huber AR. Metaanalysis of reference values in hematology. Ther Umsch 2006;63:5–24.
6. Van den Bossche J, Devreese K, Malfait R, Van de Vyvere M, Wauters A, Neeis H, et al. Reference intervals for a complete blood count determined on different automated haematology analysers: Abx Pentra 120 Retic, Coulter Gen-S, Sysmex SE 9500, Abbott Cell Dyn 4000 and Bayer Advia 120. Clin Chem Lab Med 2002;40:69–73.
7. Takubo T, Tatsumi N, Satoh N, Matsuno K, Fujimoto K, Soga M, et al. Evaluation of hematological values obtained with reference automated hematology analyzers of six manufacturers. Southeast Asian J Trop Med Public Health. 2002;33(Suppl 2):62–7.
8. Marwaha N, Marwaha RK, Narang A, Thusu K, Garewal G, Bhakoo ON. Routine hematological values in term newborns. Indian Pediatr 1992;29:1095–9.
9. Bao W, Dalferes ER Jr, Srinivasan SR, Webber LS, Berenson GS Normative distribution of complete blood count from early childhood through adolescence: the Bogalusa Heart Study. Prev Med 1993;22:825–37.
10. Viprakasit V, Suwanthol L, Sangpraypan T, Glomglao W, Utto W, Veerakul G. Hematological parameters and red blood cell indices in healthy Thai children: a revision for 2005. J Med Assoc Thai 2005;88(Suppl 8):S188–96.
11. Taylor MR, Holland CV, Spencer R, Jackson JF, O'Connor GI, O'Donnell JR. Haematological reference ranges for schoolchildren. Clin Lab Haematol 1997;19:1–15.
12. Takubo T, Tatsumi N. Reference values for hematologic laboratory tests and hematologic disorders in the aged. Rinsho Byori. 2000;48:207–16.
13. Chomón B, Vázquez L, Castro D. Reference intervals of hematologic parameters in the elderly. Sangre (Barc) 1989;34:229–33.
14. Shiga S, Koyanagi I, Kannagi R. Clinical reference values for laboratory hematology tests calculated using the iterative truncation method with correction: Part 1. Reference values for erythrocyte count, hemoglobin quantity, hematocrit and other erythrocyte parameters including MCV, MCH, MCHC and RDW. Rinsho Byori 1990;38:93–103.
15. Shiga S, Koyanagi I, Ohsaga J, Ichiyama S, Kannagi R. Clinical reference values for laboratory hematology tests calculated using the iterative truncation method with correction: Part 2, Reference values for white blood cell (WBC) count, WBC differential including segmented neutrophil, band neutrophil, lymphocyte, monocyte, eosinophil, basophil, platelet count and mean platelet volume] Rinsho Byori 1999;47:281–8.
16. Lim EM, Cembrowski G, Cembrowski M, Clarke G Race-specific WBC and neutrophil count reference intervals. Int J Lab Hematol 2010 Mar 3.
17. Cheng CK, Chan J, Cembrowski GS, van Assendelft OW. Complete blood count reference interval diagrams derived from NHANES III: stratification by age, sex, and race. Lab Hematol 2004;10:42–53.
18. Bain BJ. Ethnic and sex differences in the total and differential white cell count and platelet count. J Clin Pathol 1996;49:664–6.
19. Giorno R, Clifford JH, Beverly S, Rossing RG. Hematology reference values. Analysis by different statistical technics and variations with age and sex. Am J Clin Pathol 1980;74:765–70.
20. Lurie S, Rahamim E, Piper I, Golan A, Sadan O. Total and differential leukocyte counts percentiles in normal pregnancy. Eur J Obstet Gynecol Reprod Biol 2008;136:16–9.
21. Cembrowski GS, Fairbanks VF. Can hematology reference intervals be derived from hospitalized patients' data?. Clin Chem 1995;41:1048–51.
22. Trowbridge EA, Reardon DM, Bradey L, Hutchinson D, Warren CW. Automated haematology: construction of univariate reference ranges for blood cell count and size. Med Lab Sci 1989;46:23–32.
23. Balloch AJ, Cauchi MN. Reference ranges for haematology parameters in pregnancy derived from patient populations. Clin Lab Haematol 1993;15:7–14
24. Chin-Yee I, Keeney M, Lohmann RC. Flow cytometric reticulocyte analysis using thiazole orange; clinical experience and technical limitations. Clin Lab Haematol 1991;13:177–88.
25. Charuruks N, Limpanasithikul W, Voravud N, Virochpoka T, Nuchprayoon C. Reference ranges of reticulocytes in adults. J Med Assoc Thai 1998;81:357–64.
26. d'Onofrio G, Kim YR, Schulze S, Lorentz T, Dörner K, Goossens W, et al. Evaluation of the Abbott Cell Dyn 4000 automated fluorescent reticulocyte measurements: comparison with manual, FACScan and Sysmex R1000 methods. Clin Lab Haematol 1997;19:253–60.
27. Nebe T, Diem H, Heimpel H. Aktuelle Aspekte zur Bestimmung der Retikulozytenzahl. J Lab Med 2010;34:295–304.
28. 28. Williams GW, Burns TL, Schork MA. The distribution of selected hematology measurements in the CAP surveyAm J Clin Pathol 1980;74(4 Suppl):595–9.
29. Ross DW, Ayscue LH, Watson J, Bentley SA. Stability of hematologic parameters in healthy subjects. Intraindividual versus interindividual variation. Am J Clin Pathol 1988;90:262–7.
30. Bentley SA, Johnson TS, Sohier CH, Bishop CA. Flow-cytochemical differential leukocyte analysis with quantitation of neutrophil left shift. An evaluation of the Cobas-Helios analyzer. Am J Clin Pathol 1994;102:223–30.