นิพนธ์ต้นฉบับ

Automated hematology 1 : reference values for leucocyte parameters on the flow cytometric system (Technicon H*1).

Pranee Krailadsiri*

Navapun Charuruks*

Krailadsiri P, Charuruks N. Automated hematology I:reference values for leucocyte parameters on the flow cytometric system (Technicon H*1). Chula Med J 1994 Jun; 38(6): 337-342

*The reference values for leucocyte parameters on the flow cytometric system (Technicon H*1) were established based on the theory of reference ranges advocated by the International Committee for Standardization in Haematology (ICSH). The parameters included leucocyte count, relative number (%) of differential leucocyte counts (neutrophil, lymphocyte, monocyte, eosinophil, basophil and large unstained cell), lobularity index, and mean peroxidase index. The samples were obtained from 1005 healthy subjects, aged between 19-60 years, 603 were male and 402 were female. The 95% confidence interval for two-tailed tests of each constituent except lobularity index was calculated to determine the lower and upper limits of reference ranges. For lobularity index, one-tailed test was used.*

Key words : *Automation, Hematology, Reference values, Leucocyte parameter, Flow cytometry.*

Reprint request : Krailadsiri P, Department of Laboratory Medicine, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand.

Received for publication. January 4,1994.

*Department of Laboratory Medicine, Faculty of Medicine, Chulalongkorn University.

ปราณี ไกรลาศศิริ, นวพรรณ จารุรักษ์. การนับเม็ดเลือดด้วยเครื่องอัตโนมัติ 1 : ค่าอ้างอิงของเม็ดเลือดขาวที่วิเคราะห์โดยเครื่อง เทคนิคอน เอช*1. จุฬาลงกรณ์เวชสาร 2537 มิถุนายน;38(6): 337-342

*การนับเม็ดเลือดด้วยเครื่องอัตโนมัติ ให้ผลถูกต้อง แม่นยำ ประหยัดเวลาและแรงงาน อย่างไรก็ตามโดยทั่วไปยังคงต้องอาศัยค่าอ้างอิงที่ได้จากการนับโดยวิธีเดิม เนื่องจากค่าอ้างอิงเป็นค่าเฉพาะสำหรับการวิเคราะห์แต่ละวิธี และแต่ละห้องปฏิบัติการ การศึกษานี้จึงมีวัตถุประสงค์เพื่อหาค่าอ้างอิงของเม็ดเลือดขาว ที่ได้จากการวิเคราะห์ด้วยเครื่องอัตโนมัติระบบ flow cytometry (Technicon H*1) โดยอาศัยตัวอย่างเลือดจากคนปกติ 1005 คน อายุระหว่าง 19-60 ปี เป็นเพศชาย 603 คน และเพศหญิง 402 คน ค่าอ้างอิงของแต่ละพารามิเตอร์ได้จากการคำนวนหาระดับความเชื่อมั่นที่ร้อยละ 95 ของการทดสอบสองทาง ยกเว้นค่าอ้างอิงของ lobularity index ใช้การทดสอบทางเดียว*

Many laboratories have been using automated equipments to perform the complete blood count and the leucocyte differential count. However, for diagnostic discrimination, manually derived reference values found in standard textbooks are still used in most cases. Because the principle and method are different, some of the parameters cannot be compared.

The objective of this study is to establish the reference values for the leucocyte parameters on the flow cytometric system, using Technicon H*1, based on the theory of reference ranges recommended by the International Committee for Standardization in Heamatology (ICSH).[2,3] These parameters are leucocyte count, relative number (%) of differential leucocyte counts (neutrophil), lymphocyte, monocyte, eosinophil, basophil and large unstained cell (LUC), lobularity index (LI) and mean peroxidase index (MPXI).

Materials and Methods

Blood sample was drawn into disodium ethylene diamine tetraacetic acid (Na2 EDTA) from the antecubital vein of sitting subjects attending the annual check up program. Using evacuated tubes (Venoject), 3 ml of blood was collected and the ratio of 1.5 mg Na2 EDTA to 1 ml of blood was maintained. Samples were stored at room temperature (20-25C) and then analysed by a Technicon H*1. The system was calibrated and operated in accordance with the manufacturer's operating instruction (Technicon Instrumentes Corporation).[4] Storage times in the anticoagulant before analysis varied from one to eight hours. All experiments were performed at the same room temperature.

The age, gender, history of illness, and medication of each subject were noted at the time of sample collection. Physical examinations were performed by physicians. All subjects had fasted at least twelve hours before venipuncture as the samples for lipid profiles were also collected at the same time.

The inclusion criteria were that the subjects should not receive medication or have history of illness likely to influence the complete blood cell count. All of them had normal results of physical examinations, urinalysis, stool examinations for parasites and chest X-ray. No anemia was found using the WHO criteria.[5]

The exclusion criteria were those subjects receiving drug therapy, having chronic medical conditions requiring treatment, having abnormal results of physical examinations, urinalysis, parasites in stool, anemia or flags on leucocyte parameters reported by the Technicon H*1. Pregnant women were also excluded.

The reference ranges for leucocyte count, relative number (%) of differential leucocyte counts and mean peroxidase index were estimated by the 95% confidence interval for two-tailed tests. The lower and upper limits of each constituent were calculated by $\bar{x} \pm z.05$ (standard error of sample mean). The z.05 for two-tailed tests equals 1.96.

The reference values for lobularity index were calculated by the 95% confidence interval for one-tailed tests. The $\bar{x}.05$ for one-tailed tests was 1.645.

Results

A total of 1005 healthy subjects were analysed, 603 (60%) males and 402 (40%) females, aged between 20 and 60 years, arithmatic mean (x) 38.9 years, standard error 8.7 years. Figure 1 illustrates the age distribution with sex partition.

The reference range of each parameter from 1005 subjects is shown in Table 1. The reference ranges with sex partition are shown in Table 2.

Table 1. Reference ranges from 1005 healthy subjects.

Parameters	Reference ranges
WBC ($x10^9$/L)	3.6-9.8
Neutrophil (%)	36.4-68.9
Lymphocyte (%)	20.4-49.0
Monocyte (%)	2.5-8.1
Eosinophil (%)	0-8.4
Basophil (%)	0-2.5
Large unstained cell (%)	0-9.5
Lobularity index	>1.1
Mean peroxidase index	(-11)-(2)

Table 2. Reference ranges with sex partition.

Parameters	Male (n=603)	Female (n=402)
WBC ($x10^9$/L)	3.7-9.9	3.4-9.5
Neutrophil (%)	35.4-67.6	38.4-70.2
Lymphocyte (%)	20.8-49.7	20.0-47.8
Monocyte (%)	2.8-8.2	2.2-8.0
Eosinophil (%)	0-9.0	0-7.5
Basophil (%)	0.7-1.3	0.2-1.5
Large unstained cell (%)	1.6-4.9	1.3-5.0
lobularity index	> 1.6	> 0.2
Mean peroxidase index	(-11)-(2)	(-10)-(3)

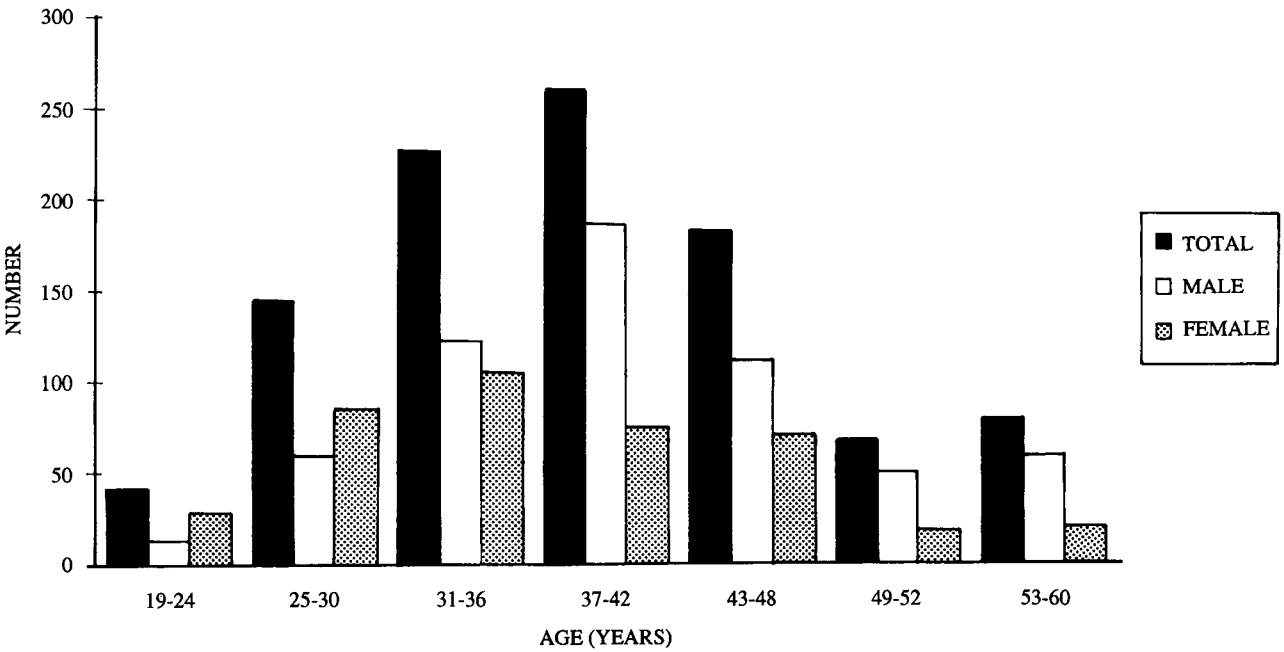

Figure 1. Histogram of age distribution.

Discussion

Altomated leucocyte count and differential counts give accurate results since more than 10,000 cells are analysed in each sample. Moreover the turnaronud time is also shorten. Therefore, those abnormal results requiring manual differentials; accuracy can be increased as technicians spend more time at the microscopes.

This study uses the recommendation of the International Committee for Standardization in Haematology (ICSH) to establishe the reference ranges for leucocyte parameters on the flow cytomatric system, using Technicon H*1. However, it is impractial to satisfy two of the stringent conditions of standardization laid down by the ICSH which require technicians to perform venipuncture without using a tourniquet and having subjects in lying or semi-lying position. Indeed, these conditions are generally unobtainable in sampling for routine hematology and are unlikely to be adhered to in general paractice. Nevertheless, since reference values are system-specific, they should be estabished by each laboratory.[6] Furthermore, due to the differences in methodology, some of the leucocyte parameters derive from the flow cytometric system cannot be compared with the values obtained from manual methods. These are LUC, LI, and MPXI. The principles of how these values are derived have already been described.[1,7-10]

The reference ranges for leucocyte parameters from this study are compared to the ranges recommended by the manufacture as shown in Table 3. Most of the parameters have similar ranges. However, the MPXI from this study is lower (-11 to 2 vs -10 to 10).

Finally due to the strict exclusion and inclusion criteria used, the reference values for the leucocyte parameters on the flow cytometric system from this study hopefully will be useful.

Table 3. Comparison of the reference ranges from this study and the manufacturer.

Parameters	The study	The manufacturer
WBC ($x10^9/L$)	3.6-9.8	4.8-10.8
Neutrophil (%)	36.4-68.9	40.0-74.0
Lymphocyte (%)	20.4-49.0	19.0-48.0
Monocyte (%)	2.5-8.1	3.4-9.0
Eosinophil (%)	0-8.4	0-7.0
Basophil (%)	0-2.5	0-1.5
Large unstained cell (%)	0-9.5	0-4.0
lobularity index	>1.1	>1.9
Mean peroxidase index	(-11)-(2)	(-10)-(10)

Acknowledgement

We would like to extend our gratitude to the following people for their help during the study : Assc. Prof. Dr. Sunit Chandaraprasert who organized the check up program : Assc. Prof. Dr. Suebsun Mahasandana who gave invaluable suggestions: the staff of Clinical Epidemiology Unit who gave assistance in data analysis : the staff of Central Laboratory, Department of Laboratory Medicine, and Ms Kanjana Vichittumaros, Technicon specialist from Bayer Thai Co., LTD., who gave assistance and cooperation throughout the program.

References

1. ปราณี ไกรลาศศิริ. Automated blood cell analyzers. จุฬาลงกรณ์เวชสาร 2535 กรกฎาคม; 36(5) : 489-97
2. International Committee for Standardization in Haematology (ICSH). The theory of reference values. Clin Lab Haematol 1981; 13(2) : 369-73
3. International Committee for Standardization in Heamatology (ICSH). Standardization of blood specimen collection procedure for reference values. Clin Lab Haematol 1982; 4 (1):83-6
4. Technicon Instruments, Basingstoke, Hampshire, England.
5. WHO Scientific Group. Nutritional anemia. WHO Tech Rep Ser 1972;503:29
6. Trowbridge EA, Reardon DM, Bradey L, Hutshinson D, warren CW. Automated haematology : construction of univariate reference ranges for blood cell count and size. Med Lab Sci 1989 Jan;46 (1):23-32
7. Nelson DA, Morris MW. Basic examination of blood. In:Henry JB, ed. Clinical Diganosis & Management by Laboratory Methods. Philadelphia : WB Saunders, 1991:553-603
8. Reardon DM, Hutshinson D, Bradey L, Trowbridge EA. Automated Hematology : a comparative study of cell counting and sizing using apertuer inpedance and flow cytomehic systems. Med Lab Sci 1987 Oct;44(4):320-5
9. Bollinger PB, Drewinko B, Brailas CD, Smeeton NA, Trujilo JM. The Technicon H-1 TM. An automated hematology analyzer for today and tomorrow. Am J Clin Pathol 1987 Jan;87(1):71-8
10. D'Onpfrio G. Medical relevance of myeloperoxidase measurement by flow cytometry. Technicon Internation Colloquium on laboratory Hematology. Maison de la Clinic Paris, Fec 19th,1988.