Segmented Neutrophil Size and Platelet Morphology in HIV/AIDS Patients

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OBJECTIVE: A study was conducted to determine if HIV/AIDS patients have smaller than normal size neutrophils and increased prevalence of abnormal platelet morphology.

DESIGN: Wright's-stained peripheral blood smears from 100 HIV/AIDS patients were evaluated for size of segmented neutrophils and degree of abnormal platelet morphology.

SETTING: East Carolina University, Greenville, North Carolina.

PARTICIPANTS: The study subjects consisted of 100 HIV/AIDS patients seen in an outpatient clinic in a teaching hospital in an academic health center. Peripheral blood smears were made from EDTA tubes drawn as a part of a routine immunology panel.

MAIN OUTCOME MEASURES: Segmented neutrophils from each of ten oil immersion fields were measured for diameter with a micrometer and the average diameter calculated. In addition, any platelet morphology abnormality, which was noted in at least five oil immersion fields, was recorded. One researcher evaluated one slide on each patient, and the second researcher randomly selected 20% of the subjects and performed the same procedure on a second slide for quality assurance of results.

RESULTS: A segmented neutrophil mean diameter of 15.1 microns was found. Though this mean is a mere 0.1 microns above the upper limit of the normal range of 10 to 15 microns, 53% of the patients had an observed average diameter above 15 microns. The HIV/AIDS patients' mean diameter was statistically different when compared to a normal population mean of 12.0 microns (T-test = 16.15, $p < .0001$), thus, showing a tendency for HIV/AIDS patients' segmented neutrophils to be large. Over half of the study subjects demonstrated abnormal platelet morphology including agranularity, small size, and giant size.

CONCLUSION: Neutrophil size as based on cell diameter, was found to be significantly larger for a sample of HIV/AIDS patients than the normal mean size. There was also a tendency for platelet morphology to be abnormal.

ABBREVIATIONS: AIDS = acquired immunodeficiency syndrome; AO = American Optical Corporation; ARC = AIDS related complex; CD = cluster of differentiation; HIV = human immunodeficiency virus.

INDEX TERMS: HIV/AIDS, neutrophil size, platelet morphology.

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The clinical laboratory has played a vital role in monitoring the immunological status of patients with human immunodeficiency virus (HIV) as well as in diagnosing opportunistic infections in those with clinically-defined acquired immunodeficiency syndrome (AIDS). Laboratory procedures have become more advanced in providing detailed and sophisticated information to health care providers who treat and monitor HIV/AIDS patients. Flow cytometry and newer, less costly technologies have enabled clinicians to obtain CD4 and CD8 counts and ratio results along with viral loads and specific genome information on a more frequent basis. Such tests have improved over the past decade in both sensitivity and specificity.[1-5]

The hematological manifestations of HIV/AIDS as monitored by the complete blood count (CBC) and differential, are well documented. These effects include predominantly cytopenia in the leukocytes, erythrocytes and thrombocytes, i.e., platelets. Lymphocytes, specifically the CD4 cells (T-helper lymphocytes), are depleted through direct attack by the virus. Anemia and neutropenia usually are due to inadequate production from bone marrow

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suppression in HIV/AIDS patients, though immune-mediated destruction may also play a role. The thrombocytopenia can be due to inadequate production, splenic sequestration, and/or immune-mediated destruction of platelets in these patients. The cytopenias generally become progressively worse as the clinical condition of the HIV/AIDS patient deteriorates.[6-9]

Neutrophils serve as one important element of cellular immunity. As an HIV/AIDS patient's CD4 lymphocytes decline, it is important to maintain functional neutrophils as a line of immune defense. It has been shown that HIV/AIDS patients can have neutropenia as well as a significant impairment in neutrophil functions including chemotaxis, phagocytosis, bactericidal activity, superoxide production, and oxidative burst. This decrease in neutrophil number and function, along with low CD4 counts, may contribute to the increased infection rate among HIV/AIDS patients.[10-14]

Morphologic abnormalities of neutrophils associated with dysfunction have been described in several disorders including Alder-Reily, Chediak-Higashi, and May-Hegglin anomalies and in congenital neutropenias such as Kostmann's syndrome, though the morphologic changes do not include abnormal size.[15,16] Dysmyelopoiesis as found in myelodysplastic disorders and, particularly, megaloblastic anemias do have large sized neutrophils as a morphologic characteristic.[17-19] Morphologic abnormalities, when demonstrated, may be linked to dysfunction of cellular elements in HIV/AIDS patients as well.

Thrombocytopenia as noted by a platelet count of less than 50,000/μL has been noted in approximately 5% to 10% of patients infected with HIV and becomes a chronic problem in about a third of patients with clinically-defined AIDS.[20-22] Increased platelet turnover as evidenced by plasma glycocalicin levels has also been demonstrated in HIV-positive adults and children even with platelet counts of greater than 100,000/μL.[23] As abnormal platelet morphology can be associated with decreased platelet function in a variety of disorders, such morphologic variations in HIV/AIDS patients could also be clinically noteworthy.[24,25]

PURPOSE OF THE STUDY

During work as a bench technologist in hematology, the senior author anecdotally observed that a higher proportion of HIV/AIDS patients as compared to other patient populations, appeared to exhibit small size neutrophils and bizarre platelet morphology on peripheral blood smears. As no current studies of leukocyte size or platelet visual morphology were found in the literature, the authors decided to undertake an assessment of neutrophil size and platelet morphology in a convenience sample of HIV/AIDS patients to determine if observations at the bench were verified by a formal study. The authors chose to limit the assessment to neutrophil size and platelet morphology due to 1) the possible relationship between these two variables with decreased function of neutrophils and platelets as described in several non-HIV disorders which may also hold true with HIV/AIDS and 2) the ease of assessment and minimal costs involved in such a study.

The study described in this article was designed to answer the following research questions: 1) Does the average size of segmented neutrophils in HIV/AIDS patients differ from a normal value? 2) Does the morphology of platelets in HIV/AIDS patients differ from the normal morphology of platelets?

METHODS AND MATERIALS

Sample

The patients in the study sample included 100 persons who came through an HIV clinic during a one-month period (August 1999). The clinic is operated by a tertiary-care, teaching hospital that is part of the School of Medicine at East Carolina University in Greenville, North Carolina. The convenience sample was heterogeneous as it included both adult and adolescent males and adult and adolescent females. Persons with asymptomatic HIV, AIDS related complex (ARC), and those with clinically defined AIDS were included in the study sample. Patient identity, demographics, and clinical condition were withheld from the researchers to prevent bias in the smear evaluations and data analysis.

Procedures

Peripheral blood smears were made from an EDTA tube collected as part of a routine immune panel on each of the 100 patients. All smears were made within eight hours of collection, though the majority was made within three to four hours. The smears were made using the Mini-prep automated slide maker that utilizes a push-slide technique. Two smears were made on each patient. All smears were Wright's stained using an Ames automated slide stainer.

One smear from each patient was evaluated for the diameter of segmented neutrophils. For each slide, one segmented neutrophil in each of ten fields was measured for diameter with a micrometer using the oil immersion objective of an American Optical Corporation (AO) binocular microscope. The ten diameters were averaged for each patient. If the ten diameter values had a range of more ten microns, then the second slide on that patient was evaluated for neutrophil diameter. Using standard technique for microscope calibration of a micrometer, a correction factor of 0.93 was utilized in measuring neutrophil diameter with the oil immersion objective. The micrometer used was a 20-micron micrometer reticle manufactured by AO and designed as a removable instrument for a microscope eyepiece.

The same smear for each patient was also evaluated for platelet morphology, though this was a more subjective assessment. A total of ten oil immersion fields were observed for platelet morphology. Any morphology that deviated from 'normal' was noted. If a morphology variant was noted in at least half of the fields viewed, it was recorded as an abnormal platelet morphology for that patient. Abnormal platelet morphologies included in the study were

small (less than two microns diameter), large (five to eight microns diameter), and giant (greater than eight microns diameter) size as well as elongated, vacuolated, hypogranular, and agranular.

The second author evaluated the slides on all 100 patients in the sample. The first author validated these results. This validation was done by evaluating the second slide on 20 patients randomly selected from the sample for both neutrophil diameter and platelet morphology using the same techniques and criteria as used in the first slide evaluation. These neutrophil diameter results were compared with the original results and were found to be within five percent of the first-smear results in all 20 patients. For platelet morphology on the 20 patients used for result validation, the same abnormal morphologies as originally recorded were observed in each case.

For purposes of the neutrophil size, the authors did not conduct the same procedures on a sample of healthy subjects due to the difficulty of recruiting subjects for such an assessment. A search of the literature finds that a reference range for neutrophil diameter which is commonly given in hematology textbooks and instructional materials is 10 to 15 microns, though no reference to the original study from which this reference range was derived could be found.[26-30] A reference mean for neutrophil diameter has been cited as 12 microns and was used in this study for comparison to the HIV/AIDS sample mean.[31]

Data Analysis

All patient results were entered into an Excel database. Analyses of frequencies, descriptive statistics, and statistical analysis were performed using this software package.

RESULTS

Neutrophil Diameter

For the 100 mean diameters of segmented neutrophils from the subjects in the study, descriptive statistics are displayed in Table 1. The median and mode are in the reference range (10 to 15 microns) and the mean of the patient neutrophil diameter averages is 0.1 microns above the upper limit of the reference range. Comparison of the sample mean of 15.1 to a reference mean for neutrophil diameter of 12.0 using a one-sample T-test demonstrated the HIV/AIDS patients' neutrophil diameter to be statistically larger than a reference population (T-test = 6.15, p <.0001).

Table 1. Statistics for neutrophil diameter means (n = 100)

Statistic	Value in micrometers
Mean	15.1
Median	14.9
Mode	14.0

Standard deviation = 1.92

The distribution of individual patient neutrophil diameter means for the sample is displayed as increments of 0.5 micron in the histogram in Figure 1. The largest percentage, 22%, of neutrophil means was within one micron, i.e., 15.0 to 16.0 microns above the upper limit of reference. The second largest percentage, 10%, were 14.1 to 14.5 microns, while the next largest percentage had means of either 13.1 to 13.5 microns or 14.6 to 15.0 microns, 9% each. Though the mean of the patients' neutrophil diameter averages was only 15.1, slightly over half, 53%, of the individual patients had a neutrophil diameter average greater than 15.0. This display of data shows a relatively normal distribution and supports the observation that the neutrophil size for the HIV/AIDS patients in this study tended toward being large.

Platelet Morphology

The most prevalent platelet morphology variant was agranular platelets that were noted in 80% of the HIV/AIDS patients. Small platelets, defined as less than two microns in diameter, were noted in 76% of the study subjects. Most of these small platelets appeared as fragments. Platelets over eight microns in diameter, termed giant platelets, were found in 31% of the study subjects. Most of the giant platelets were also vacuolated.

DISCUSSION AND CONCLUSIONS

Based on previous observations and experiences of one of the researchers, the question for this study was whether or not smaller than normal segmented neutrophils and abnormal platelet morphology would be found on the peripheral blood smears from HIV/AIDS patients. Contrary to the authors' suspicions, the size of the patients' neutrophils as reflected by mean diameter was actually found to be on the large side. Though the arithmetic mean, 15.1 microns, of the patients' diameter means was a mere 0.1 micron above the upper limit of the reference range (10 to 15 microns), 53% had a mean neutrophil diameter greater than 15.0 with some as high as 19.5 microns. The mean diameter of 15.1 was found to be statistically higher than a normal population mean of 12.0 microns based on a one-sample T-test (T-test = 16.15, p <.0001). This finding is the opposite of the original belief that the neutrophils would be small.

The results did demonstrate platelet morphologic abnormalities in over half of the HIV/AIDS patients with 76% exhibiting platelets of small size, <two microns diameter, and 80% with agranular platelets. Giant platelets of greater than eight microns diameter were found in about a third, 31%, of the study subjects.

Whether the observed variations of large size neutrophils, giant and small platelets, and agranular platelets contribute to the functional abnormalities found in these two cellular elements as manifested in HIV/AIDS patients requires further research of a molecular nature. Additionally, the findings of this study are limited in their application by the convenience nature of the sample. The study limitations include: 1) the heterogeneous nature of the study sample relative to gender, age, and clinical condition; 2) the homogeneous nature of

the study sample relative to geographical residence, i.e., eastern North Carolina; 3) the lack of multiple microscopist verification of results on every study subject which was cost prohibitive; 4) comparison of the neutrophil diameter sample mean to a reference population of unknown standard deviation; and 5) lack of determination of neutrophil diameter for a healthy population of equal sample size and drawn from the same geographical area.

The study results do provide preliminary findings that may verify what microscopists in clinical settings are observing as to hematological variations in manual peripheral blood smear evaluations in their HIV and AIDS patients. Future research should clarify the role of these variations in functionality of the neutrophils and platelets in this patient population.

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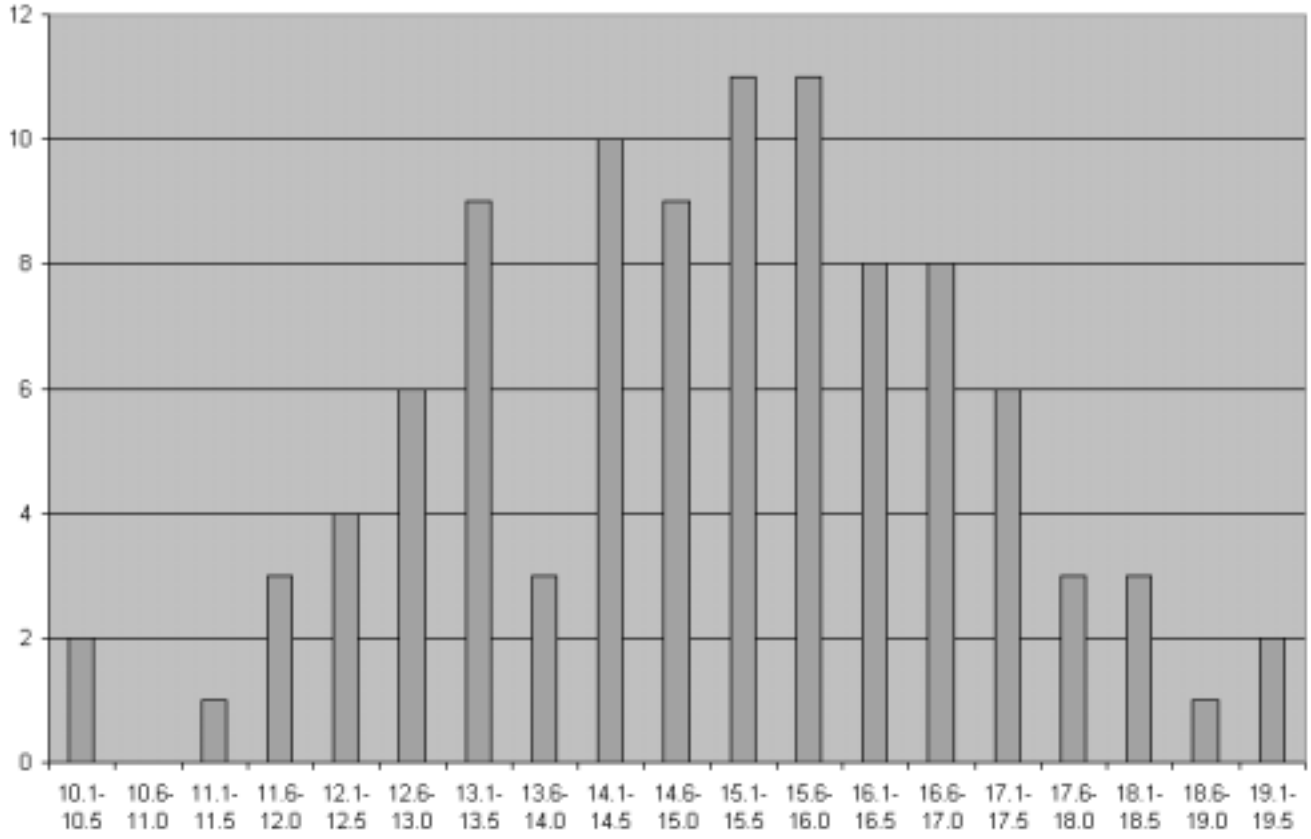
REFERENCES

1. Anderson K. Advances on the AIDS horizon: 1994. 7th ed. Sacramento CA: Anderson Continuing Education; 1994. p 1, 9-12.
2. Bartlett JG, Finkbeiner AK. The guide to living with HIV infection. Baltimore MD: The Johns Hopkins University Press; 1993. p 115-6.
3. McKenzie SB. Textbook of hematology. 2nd ed. Baltimore, MD: Williams and Wilkins; 1996. p 303-4.
4. Louie J, Parker JW. Flow cytometry. In: Steine-Martin EA, Lotspeich-Steininger CA, Koepke JA, editors. Clinical hematology: principles, procedures, correlations. 2nd ed. Philadelphia: Lippincott-Raven; 1998. p 556-7.
5. Wilson, KA. HIV alters cellular genetic expression within days of infection. Lab Med 2000;31:128.
6. Coutton C, Chermann JC. In vitro study of seraspenide on HIV-induced inhibition of granulopoiesis. Eur J Haematol 1997;59:184-9.
7. Coyle TE. Hematologic complications of human immunodeficiency virus infection and the acquired immunodeficiency syndrome. Med Clin of N Am 1997;81:449-70.
8. Murphy MF, Metcalfe P, Waters AH, and others. Incidence and mechanism of neutropenia and thrombocytopenia in patients with human immunodeficiency virus infection. Br J Haematol 1987;66:337-40.
9. Costello C. The haematological manifestations of HIV disease. In Hoffbrand AV, Lewis SM, Tuddenham EGD, editors. Postgraduate haematology. 4th ed. Oxford, England: Butterworth-Heinemann; 1999. p 311-5.
10. Ellis M, Sudhir G, Galant S, and others. Impaired neutrophil function in patients with AIDS or AIDS-related complex: a comprehensive evaluation. J Infect Dis 1988;158(6):1268-75.

Figure 1. Mean neutrophil diameters



11. Roilides E, Mertins S, Eddy J, and others. Impairment of neutrophil chemotactic and bactericidal function in children infected with human immunodeficiency virus type 1 and partial reversal after in-vitro exposure to granulocyte macrophage colony stimulating factor. J of Pediatr 1990;117:531-40.
12. Shalekoff S, Tiemessen CT, Gray CM, Martin DJ. Depressed phagocytosis and oxidative burst in polymorphonuclear leukocytes from individuals with pulmonary tuberculosis with or without human immunodeficiency virus type 1 infection. Clin and Diag Lab Immunol 1998;5:41-4.
13. Farber BF, Lesser M, Kaplan MH, and others. Clinical significance of neutropenia in patients with human immunodeficiency virus infection. Infect Control Hosp Epidemiol 1991;12:429-34.
14. Battaglione VJ, Fischer F, Michiels J, and others. Ultrastructure of the polymorphonuclear leukocytes in human immunodeficiency virus infection. Pathology 2000;32:119-25.
15. Finnegan K. Leukocyte disorders. In: Lehmann CA, editor. Saunders manual of clinical laboratory science. Philadelphia: WB Saunders; 1998. p 908-9.
16. Mills EL, Noya FJD. Congenital neutrophil deficiencies. In: Abramson JS, Wheeler JG, editors. The natural immune system: the neutrophil. Oxford, England: Oxford University Press; 1993. p 188-91.
17. Roper D, Stein S, Payne M, Coleman M. Anemias caused by impaired production of erythrocytes. In: Rodak BF, editor. Diagnostic hematology. Philadelphia: WB Saunders; 1995. p 186-91.
18. Rodak BF, Carr J. Myelodysplastic syndromes. In: Rodak BF, editor. Diagnostic hematology. Philadelphia: WB Saunders; 1995. p 391-9.
19. Pierre RV. The dysmyelopoietic disorders. In: Stiene-Martin EA, Lotspeich-Steininger CA, Koepke JA, editors. Clinical hematology: principles, procedures, correlations. 2nd ed. Philadelphia: Lippincott-Raven; 1998. p 430-9.
20. Borgia G, Reynaud L, Cicciarello S, and others. Thrombocytopenia and AIDS: possible direct role of HIV. AIDS 1996;10:1606-7.
21. Cole JL, Marzec UM, Gunthel CJ, and others. Ineffective platelet production in thrombocytopenic human immunodeficiency virus-infected patients. Blood 1998;9:3239-46.
22. Sullivan PS, Hanson DL, Chu SY, and others, Adult/adolescent spectrum of disease group. Surveillance for thrombocytopenia in persons infected with HIV: results from the multistate audit and adolescent spectrum of disease project. J Acquired Immune Defic Syndromes Hum Retrovirol 1997;14:374-9.
23. Williams SB, Sano M, Smith N, and others. Glycocalicin levels in the plasma of HIV+ patients: an indicator of platelet turnover. J Lab Clin Med 1998;132:303-7.
24. Castellone DD. Evaluation of bleeding disorders. In: Lehmann CA, editor. Saunders manual of clinical laboratory science. Philadelphia: WB Saunders; 1998. p 976-84.
25. Davis GL. Quantitative and qualitative disorders of platelets. In: Steine-Martin EA, Lotspeich-Steininger CA, Koepke JA, editors. Clinical hematology: principles, procedures, correlations. 2nd ed. Philadelphia: Lippincott-Raven; 1998. p 717-30.
26. Carr JH, Rodak BF. Clinical hematology atlas. Philadelphia: WB Saunders; 1999. p 51.
27. Finnegan K. Hematopoiesis. In: Lehmann CA, editor. Saunders manual of clinical laboratory science. Philadelphia: WB Saunders; 1998. p 854.
28. Lawrence LL. The Leukocytes. In: Steine-Martin EA, Lotspeich-Steininger CA, Koepke JA, editors. Clinical hematology: principles, procedures, correlations. 2nd ed. Philadelphia: Lippincott-Raven; 1998. p 305.
29. Miale JB. Laboratory medicine: hematology. 6th ed. St Louis: CV Mosby; 1982. p 131.
30. Wood BL, Curtis JD, Murray C, and others. Peripheral Blood Tutor™ CD-ROM. From: Department of Laboratory Medicine, University of Washington. Philadelphia: Lippincott-Raven; 1995.
31. Erslev AJ, Gabuzda TG. Pathophysiology of blood. 3rd ed. Philadelphia: WB Saunders; 1985. p 135.