Research Article
Volume 13:10, 2022

Journal of Cytology & Histology

ISSN: 2157-7099 Open Access

Histomorphometric Density Measurement of Bone Marrow Cellularity in Broiler Chickens

Floyd D. Wilson[1]*, Lanny Pace[2] and Frederic J. Hoerr[3]

[1]Poultry Research and Diagnostic Laboratory, Mississippi State University, Mississippi State, MS 39762, USA
[2]Mississippi Veterinary Research and Diagnostic Laboratory, Mississippi State University, Mississippi State, MS 39762, USA
[3]Veterinary Diagnostic Pathology, 638 South Fort Valley Road Fort Valley, Virginia, 22652, USA

Abstract

Routine 5 µm H&E-stained histologic bone sections were utilized to investigate the capability of histomorphometry for determining bone marrow cellularity in commercial broiler chickens. Bone marrow mean pixel density was measured using the NIH ImageJ software program and the results for binary digital images were compared to corresponding results for actual bone marrow cell counts and cellular density scoring. A total of 220 bone marrow samples from femur and tibia obtained from broiler chickens, aged from hatch to 42 days were investigated. The average and standard deviation for all density measurements was 117 ± 26 pixels, for all cell counts 312 ± 124 cells per grid field and for all density scores 3.4 ± 0.8. Bone marrow density measurements from binary digital images and cellularity counts were highly correlated (correlation coefficient +0.83). Although more subjective semi quantitative density scoring procedures produced similar and significant age-associated profiles, the scoring results had a lower correlation with density (+0.59) and with cell counts (correlation +0.64). The density method provides a rapid approach for the evaluation of bone marrow cellularity using routine bone marrow histologic sections, standard microscopy, free software, and archived specimens. New bone marrow data are reported on age-associated changes occurring in the bone marrow of broiler chickens raised in a commercial environment.

Keywords: Histology • Bone marrow density • Morphometrics • Severity scoring • Chicken

Introduction

Quantitation of bone marrow for clinical or experimental studies is labor intensive and may require the use of expensive imaging systems and proprietary software programs. Several diseases affect the hematopoietic system in poultry which can be reflected by quantitative changes in the bone marrow [1-4]. However, there are few reports on the enumeration of bone marrow cells for normal birds or those having poultry diseases [5-9].

We previously described results for bone marrow quantitation in poultry obtained by counting bone marrow cells present in routine histology sections [8,9]. In this communication we describe a simple and more rapid method for the quantitative evaluation of bone marrow cellularity by measuring tissue density of histologic bone marrow sections. The results with the density method are compared to those obtained by semiquantitative density scoring and actual cell counting. Results for age-associated changes in the bone marrow are also presented. The method can also be used for cellularity studies with human bone marrow.

Methods and Materials

Samples

A total of 220 bone marrow sections of femur and tibia were included in the study. Decalcified femur or tibia bone sections were prepared as routine 5µm sections stained with hematoxylin and eosin for bone marrow evaluation. The samples were retrieved from our histology slide archives using samples from previous bone studies in commercial broiler chickens from day of hatch to 42 days of age (Table 1).

The bone marrow samples were mostly from clinically normal birds; however, birds having various gross abnormal conditions (small birds cull and/or lame birds) were also present. As statistical analysis did not demonstrate difference between normal and the abnormal birds (data not shown), all values were included in the age-associated evaluations.

Bone marrow quantification

All microscopic evaluations were performed using an Olympus CX31 microscope (Olympus Corporation, Breinigsville, PA). Photomicrographs were taken at 400-x magnification with an AmScope MU1403 camera (AmScope, Irvine, CA) using the associated software-based program. The microscope lamp setting was 5. The software settings were: 400-x at high density (4096 x 3286 pixels taken at @ 25%); color temp 3945; tint 1032; "bit depth" 8 bit; and with the enhance rate and frame rate set at medium.

Density scoring

The number of bone marrow cells were estimated using a semiquantitative cellular density scoring method where 0=no hematopoietic cells observed; 1=minimal cells; 2=small numbers or mild cellularity; 3=moderate cellularity; 4=marked cellularity; and 5=uniformly dense cellularity.

Bone marrow cell counting

The concentration of bone marrow cells was determined by counting the number of nucleated marrow cells within a 1-mm square grid when viewed at 400-x magnification using an ocular reticle. The average for 3 grid fields was determined for each bone marrow sample.

Determination of Bone Marrow Density

Density measurements (mean pixels/area) were made from the photomicrographs using the ImageJ software program provided by NIH (http://

***Address for Correspondence:** Floyd D. Wilson, Poultry Research and Diagnostic Laboratory, Mississippi State University, Mississippi State, MS 39762, USA, Tel- +1-6017077499 E-mail: floydw8504.fw@gmail.com*

***Copyright:** © 2022 Wilson FD, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.*

Date of Submission: 13 Oct, 2022, Manuscript No. jch-22-77178; **Editor Assigned:** 15 Oct, 2022, PreQC No. P-77178; **Reviewed:** 17 Oct, 2022, QC No. Q-77178; **Revised:** 21 Oct, 2022, Manuscript No. R-77178; **Published:** 28 Oct, 2022, DOI: 10.37421/2157-7099.2022.13.656

imagej.nih.gov/ij/download.html). The evaluation consisted of first converting the color image to a binary format. The density was then measured by the software. Results included measurements of the mean pixel density for the entire 400-x photographed field, the measured area size, and the integrated pixel density. Minimal and maximal gray scale values were also determined.

The density measurement procedure was to first set measurements on the ImageJ menu by

- selecting “Analyze”
- “Set Measurements”
- Select the displayed boxes for “Area”, “Mean gray scale” and “Integrated density”.

The sequence for the measurement of density using the ImageJ software menu is to

- Select “Process”
- Select “Binary”
- Select “Make Binary”
- Select “Analyze”
- Select “Measurement”.

The effect of camera intensity settings on density results accomplished using a single bone marrow section. While keeping all other camera settings constant, the selected section was photographed using the three software camera density settings (low setting 1024 x 822, medium setting 2048 x 1644 and high setting 4096 x 3286). Using the ImageJ software, the varied density-colored photomicrographs and corresponding converted binary images were than measured.

Statistical Analysis

Statistical evaluations were performed using the GraphPad Prism 9.40 software program (Graphpad software, San Diego, CA). One way ANOVA (Kruskal-Wallis test) was applied to study the effects of age on bone marrow severity scores, cellularity and density. Correlation coefficients comparing the results for the 3 methods were determined by the Pearson r test. A p-value of <0.05 between comparisons was considered significant.

Results

The results are shown in Table 1 and Figure 1-5. Histologic examples of low and high bone marrow cellularity are shown in Figure 1a. Total of 209 bone marrow sections of femur and tibia were evaluated using semiquantitative cellular density scoring, cell counting and cellular density image analysis methods (Table 1). The inclusion of normal and abnormal (small, cull, lame) chickens provided a wide spectrum of bone marrow reactivity (Figure 1). Ageing patterns for density determined by ImageJ analysis, by cell counts or by severity scoring were similar (Figure 2).

While performing the density evaluations of the sections, an occasional “reversed image” was displayed by ImageJ showing the intercellular rather than the cellular components after conversion to a binary image (Figure 3a). This was changed to show cellular components by using the editing reverse image tool in ImageJ (Figure 3b). The results for statistical evaluations of density, counts and scores for the age results are compared in Table 2.

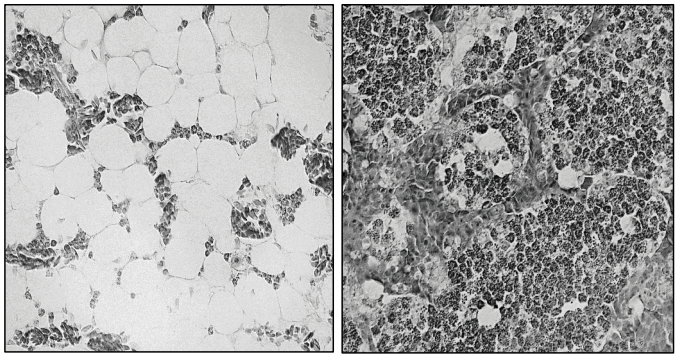
Discussion and Analysis

No significant differences in scores, counts or density between the clinical subgroups were apparent in this study nor were significant differences in these parameters present between the femur and tibia bone marrow samples at the various ages (Figure 4). Also, no differences were present between the proximal metaphysis and distal metaphysis (or diaphysis) sites (Figure 5). While significant differences were present between the various age-groups using binary images, there were no differences nor did positive correlations detect with color or grayscale images (Table 3).

A high correlation was present between cell count and density (r +0.83), but lower correlations occurred for density or counts with the scores (Table 2). Highly statistically significant differences occurred between the various age-groups using all three methods (Figure 2).

Table 1. Cell counting and cellular density image analysis methods.

Clinical group	Age (Days)	Number	Age group total
Normal	0	40	40
Normal	5	9	
Lame	5	6	15
Normal	11	8	
Small Normal	11	6	
Lame	11	7	
Cull Lame	11	5	26
Normal	22	6	
Small Normal	22	17	
Cull Lame	22	15	38
Normal	27-29	24	
Small Normal	27-29	32	
Cull Lame	27-29	24	80
Normal	42	21	21
	Total all		220



(a) (b)

Figure 1. Examples of low and high bone narrow cellularity. **(a)** Femur low cellularity @ 400-x. Score=1.5, total cell count=73 and density=42 and **(b)** Femur with high cellularity @ 400-x. Severity Score=5.0, total cell count=594 and density=154.

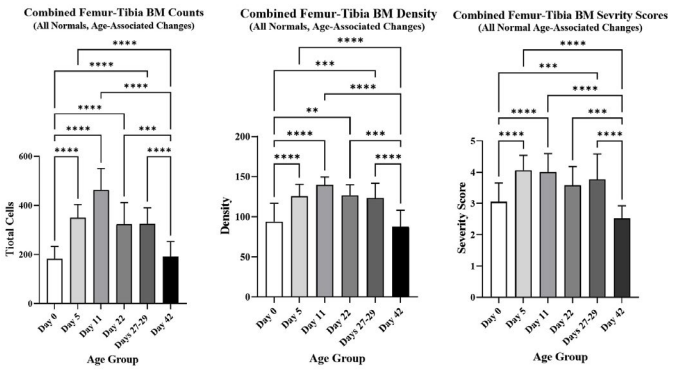
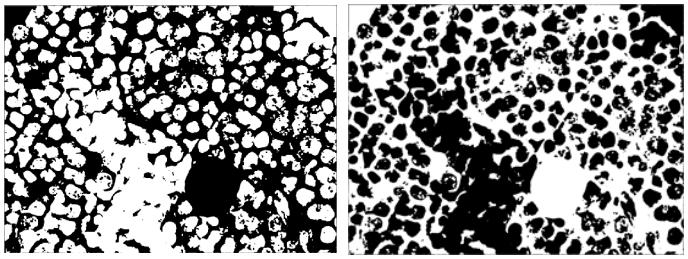
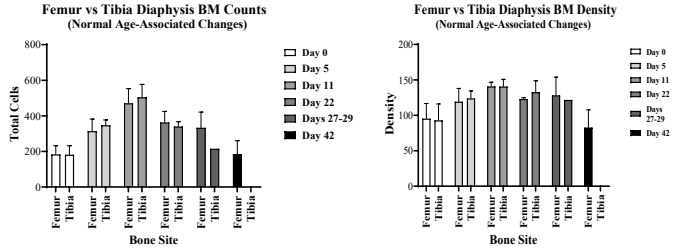


Figure 2. Ageing patterns for density determined by ImageJ analysis, by cell counts or by severity scoring.



(a) (b)

Figure 3. Example of "reversed" Image. **(a)** Initial binary image with selection of apparent interstitial regions (density=138.8) and **(b)** "Inverted" binary image with selection of apparent bone marrow cells (density=131.4).



(a) (b)

Figure 4. (a) The total counts and density results obtained for the femur and tibia at the various ages are compared and **(b)** the results for the age-groups are very similar for the two sites using both methods with no significant difference appreciated.

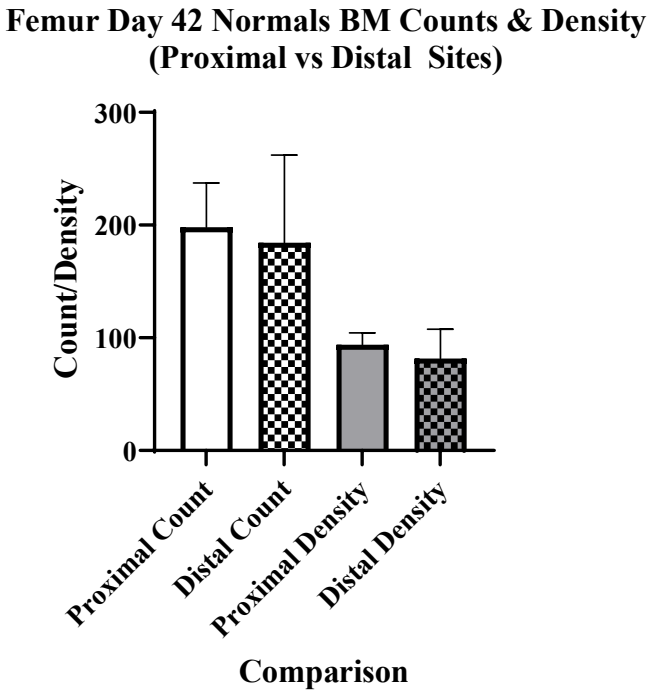


Figure 5. The combined results for cell counts and density comparing the proximal metaphyseal to the diaphyseal sites on day 42 are shown. There were no significant differences between the bone sites using either method.

Conclusion

The general approach of using binary digital image density measurements to quantitate bone marrow cellularity of broiler chickens appears valid. It provides a simple and inexpensive method for quantification of bone marrow cellularity using routine histology sections. Evaluations use routine microscopy and a free software program. The method also provides an approach for evaluating archived specimens. Our approach for measuring bone marrow density is like the method previously reported for quantitation of bone marrow

Table 2. Statistical comparison of age differences (Combined bone data).

Statistical Evaluations (Combined Data)			
	Density	Count	Score
	Dunn's multiple comparisons test		
Comparison	*P Value	*P Value	*P Value
Day 0 *vs.* Day 5	<0.0001	<0.0001	0.0003
Day 0 *vs.* Day 11	<0.0001	<0.0001	0.0003
Day 0 *vs.* Day 22	<0.0001	<0.0001	0.7185
Day 0 *vs.* Days 27-29	<0.0001	<0.0001	0.0011
Day 0 *vs.* Day 42	>0.9999	>0.9999	0.5041
Day 5 *vs.* Day 11	0.0598	0.0119	>0.9999
Day 5 *vs.* Day 22	>0.9999	>0.9999	>0.9999
Day 5 *vs.* Days 27-29	>0.9999	>0.9999	>0.9999
Day 5 *vs.* Day 42	<0.0001	<0.0001	<0.0001
Day 11 *vs.* Day 22	0.007	0.0043	>0.9999
Day 11 *vs.* Days 27-29	0.0036	<0.0001	>0.9999
Day 11 *vs.* Day 42	<0.0001	<0.0001	<0.0001
Day 22 *vs.* Days 27-29	>0.9999	>0.9999	>0.9999
Day 22 *vs.* Day 42	<0.0001	<0.0001	0.0056
Days 27-29 *vs.* Day 42	<0.0001	<0.0001	<0.0001

*Significant difference indicated by shaded values ($p \geq 0.05$)

Table 3. Severity scores, counts, densities and correlation coefficients

Score	Count	Density	Correlation coefficients	
1.5	213.8 ± 0.8 (2)	115.4 ± 6.3 (2)	Score versus Count	0.65
2	194.0 ± 83.0 (17)	87.8 ± 28.5 (17)	Score versus Density	0.58
2.5	217.1 ± 85.3 (32)	96.6 ± 21.5 (32)	Count versus Density	0.87
3	277.8 ± 97.7 (34)	113.4 ± 23.5 (34)		
3.5	333.5 ± 83.9 (36)	125.4 ± 17.6 (36)		
4	384.7 ± 91.4 (65)	132.4 ± 13.6 (65)		
4.5	440.9 ± 110.2 (23)	135.8 ± 17.9 (23)		
5	479.3 ± 98.0 (11)	134.8 ± 11.1 (11)		

density in chickens infected with chicken anemia virus (Quantitative Analytical Technique Applied to Histopathology of Birds Infected Experimentally by the Virus of Chicken Anemia Virus. Luz García et al, Proceedings Diagnostic Pathology 2008, 3(Suppl 1): S21doi). Those authors used a positive pixel count algorithm for quantification of bone marrow cellularity which measured area and staining intensity, Pixel counts with this method measure strong positive (red), positive (orange), weak positive (yellow), and negative (blue) pixels. The positive pixel count algorithm demonstrated the number of strong positive (% positivity) of controls were higher than CAV bone marrows (average 61% and 25% respectively). However, that method requires an image analysis optic system and proprietary software. In contrast, our density approach is performed with a standard microscope using a software program provided free for download from NIH.

Statistically significant differences between bird age-groups were present in the bone marrow density when using the binary images. There was a high correlation (r +83) between cell counts and binary density, but a lower correlation was present between counts and cellularity scores (r +59). However, no significant differences were present between density measurements made for the femur compared to the tibia, or between the metaphyseal and diaphyseal sites.

One cautionary note is that conversion of color images to binary format with ImageJ can occasionally produce "negative" images showing the intercellular rather than cellular components. This is readily apparent when viewing the image and can be reversed to view the marrow cellular components using the ImageJ software editing feature.

The study also provides reference data on age-associated changes in the bone marrow in clinically normal, cull, and lame commercial broiler chickens. The cellular density scoring in this study was performed by an experienced investigator. However, as differences in semiquantitative scoring can occur

between different observers, binary digital density measurements results can also allow for the calibration of scoring results with actual bone marrow density ranges.

Recommendations for Future Studies

The method provides a new rapid method for estimating bone marrow cellularity that can provide a useful approach for quantitation of marrow for clinical and experimental studies using human and experimental animals. The approach does not require use of expensive digital analysis systems nor proprietary software. It also provides a method for quantitative validation of semi quantitative cellular scoring results. Refinement of the method by employment of various filters or special staining is also possible.

Acknowledgments

The cases described herein comprised archival specimens of client-submitted diagnostic cases and pathology surveys of broiler chickens from commercial environments.

Care and use of animals

Bird treatment from which the histology slides used in the study were obtained were treated in accordance with the principles and specific guidelines presented in Guide for the Care and Use of Agricultural Animals in Research and Teaching, 4th edition, 2020

Conflicts of Interest

There are no conflicting interests in the manuscript.

References

1. Barnes, H. John., and Oscar J. Fletcher. Hemic System: Avian Histopathology; Omni Press, USA, 2016.
2. Wilson, Floyd. D., Shuping Zhang, Sue. A. Hubbard and Danny Magee, et al. “Unusual Pathology Manifested with an Outbreak of Ornithobacterium rhinotracheale Associated Meningoencephalitis in Commercial Broiler Chickens.” *Am Assoc Avian Pathol* (2009).
3. Wilson, Floyd D., William R. Maslin, Robert W. Wills and Phil A. Stayer, et al. “High Incidence of Glomerulonephritis Associated with Inclusion Body Hepatitis in Meat-type Chickens: Routine Histopathology and Histomorphometric Studies.” *Avian Dis* 54(2010):975-980.
4. Zhang Z, Floyd Wilson, Robert Read, et al. “Detection and Characterization of Naturally Acquired West Nile Virus Infection in a Female Wild Turkey.” *J Vet Diagn Invest* 18 (2006): 204-208.
5. Glick, Bruce, and Cornelius Rosse. "Cellular composition of the bone marrow in the chicken. I. Identification of cells." The Anat Rec 185 (1976): 235-245.
6. Glick, Bruce, and Cornelius Rosse. "Cellular composition of the bone marrow in the chicken: II. The effect of age and the influence of the bursa of Fabricius on the size of cellular compartments." *The Anat Rec* 200 (1981): 471-479.
7. García, Luz, Victor Bermudez, Mariela Brett and Luzmila Peroza, et al. "Quantitative analytical technique applied to histopathology of birds infected experimentally by the virus of chicken anemia virus." In "Diagn Patho, 3 (2008): 1-3.
8. \Wilson, Floyd. D., Timothy Cumming, Sue A. Hubbard and Danny Magee et al. “Morphometric Studies on Inflammatory Diseases in Poultry.” Presented at the AAVLD Annual Meeting, Greensboro, NC, 2004.
9. Wilson, Floyd D. "Histomorphometry of Bone Marrow and Other Tissues in Diseases of Broiler Chickens." American College of Veterinary Pathologists, American Society for Veterinary Clinical Pathology (2011).

How to cite this article: Wilson, Floyd D., Lanny Pace and Frederic J. Hoerr. “Histomorphometric Density Measurement of Bone Marrow Cellularity in Broiler Chickens.” *J Cytol Histol* 13 (2022): 656.