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Construction of an Automatic Quantification Method for Bone Marrow Cellularity Using Image Analysis Software

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ABSTRACT

Although rapid, the evaluation of bone marrow (BM) cellularity is semi-quantitative and largely dependent upon visual estimates. We aimed to construct an automatic quantification method using image analysis software. We used hematoxylin and eosin (HE)-stained specimens of BM biopsies and clots from patients who underwent BM examination at Tottori University Hospital from 2020 to 2022. We compared image analysis (Methods A, B, and C) with visual estimates in pathology reports of 91 HE specimens in 54 cases (29 males, 25 females), including 38 biopsy and 53 clot specimens. Cellularity was visually scored as hypocellular (n = 17), normocellular (n = 44), or hypercellular (n = 30). Compared with the visual estimates, intraclass correlation coefficients for Methods A, B, and C were 0.80, 0.85, and 0.88, respectively. The most appropriate values were obtained with Method C which detected both non-fatty and cell nuclear areas.

Key words automated image analysis; bone marrow cellularity; visual estimates

Bone marrow (BM) trephine biopsies and aspirations are routinely used in hematology to investigate blood diseases. During visual examination of a biopsy, various aspects of the tissue are evaluated, such as the marrow architecture and cellularity of hematopoietic and stromal components.[1] BM aspiration produces smears and clot sections. The targets of histopathological diagnosis are clot and biopsy sections obtained by solidifying BM specimens. The ratio of hematopoietic cells can be calculated as a percentage, which is the first step for evaluating BM. Categorizing BM as hypo-, normo-, or hypercellular relies on the subjective internal reference frame of the pathologist and knowledge of the normal variations of BM cellularity with age. There is a general consensus that cellularity decreases with age,[1] but there is little consensus in the published literature on the mean values and variation of cellularity per age. Several studies published in the last 60 years disagree with the rate of decrease and the age at which this decrease occurs.[2–5] In histology, cellularity is typically expressed as a visual estimate (VE) of the percentage of an area in the marrow cavity occupied by active hematopoietic marrow. This VE is rapid but semiquantitative in nature. By contrast, point counting (histomorphometry) using a microscope eyepiece with a graticule is a more accurate method of quantifying cellularity, but it is slow and labor-intensive. Quantifying BM cellularity for routine diagnostics using digital image analysis (DIA) offers advantages over manual microscopy techniques. Areas are easily measured and compared, and cells can be counted quickly and exhaustively, giving an objective quantification instead of an estimation of proportions. Several recent studies reported cellularity measurement using digital image analysis techniques on digitized slides that show good concordance with references of VE or point counting.[2, 6, 7]

The aim of this study was to construct an automatic quantification method for BM cellularity using DIA software.

MATERIALS AND METHODS

BM reports from a pathology database were identified between 2020 and 2022. Biopsies with extensive artifactual changes including a predominance of cortical bone, crush artifact, extensive hemorrhage, and fragmentation were excluded. Biopsies exhibiting cell dropout or fibrosis were also excluded, along with clot specimens with poor particles. After applying these exclusion criteria, 54 patients and 91 slides were included in this study. The BM biopsies and clots were independently reviewed by hematopathologists, using VE. Cellularity was estimated to the nearest 10%, and the median value was recorded as the overall cellularity. Hematoxylin

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Abbreviations: AUC, area under the curve; BM, bone marrow; DIA, digital image analysis; HE, hematoxylin and eosin; ICC, intraclass correlation; ROC, receiver operating characteristic; VE, visual estimate

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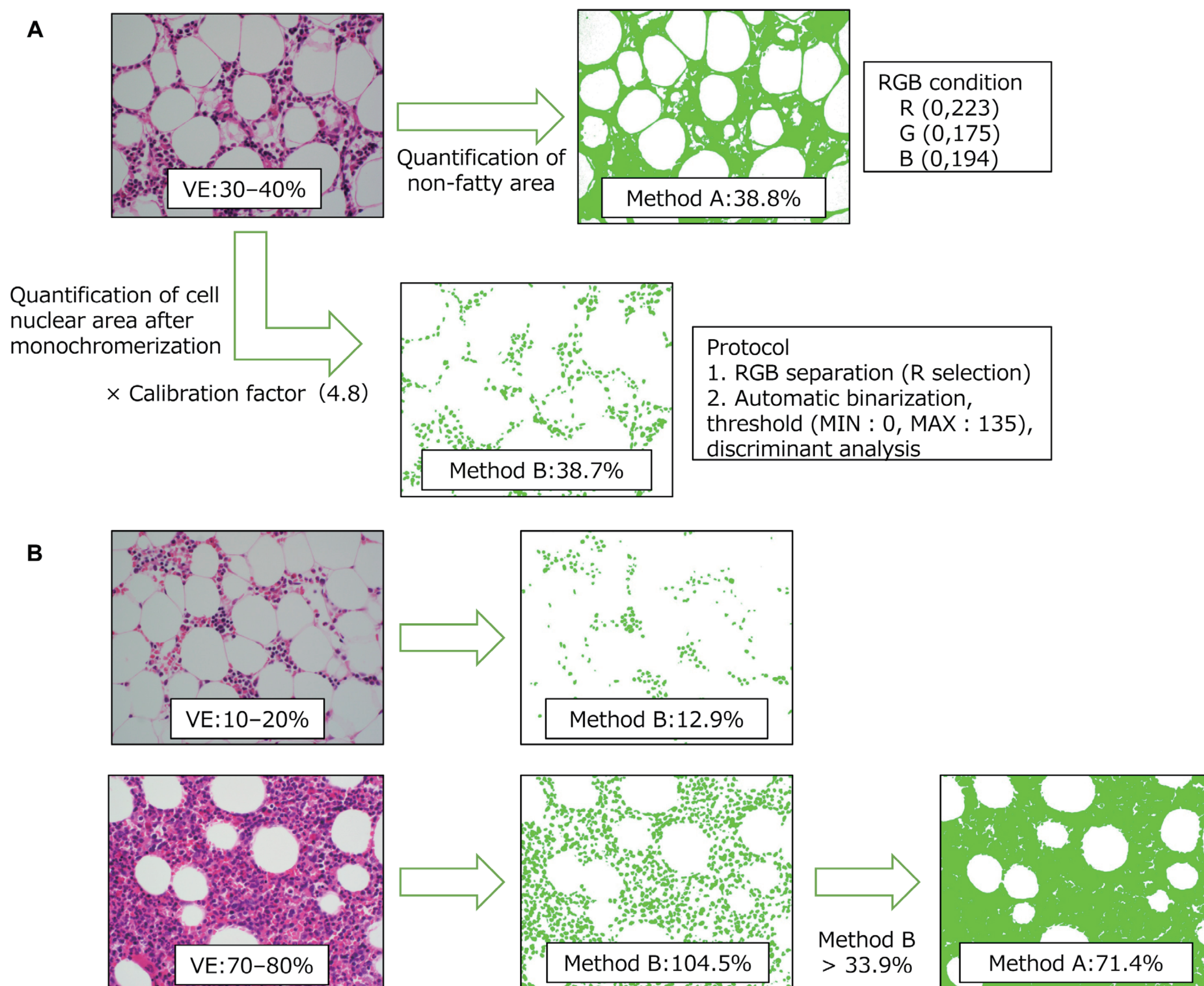

Fig. 1. (**A**) Overview of Methods A and B to quantify BM cellularity and the analysis conditions. Method A was developed to detect the non-fat area as a quantification value for cellularity. Method B was developed to detect the nuclear area after monochromatizing the images, and the cellularity was calculated by multiplication with the calibration factor. (**B**) Overview of Method C. The program of Method C was constructed to adopt the quantification value by Method B if it was < 33.9% and Method A if it was > 33.9%. VE, visual estimate.

and eosin (HE)-stained sections were digitally scanned using cellSens (Olympus, Tokyo, Japan). WinROOF 2015 imaging software (Mitani Corporation, Osaka, Japan) was used to analyze digital slides with adequate tissue histology. We developed a method in which the non-fat area is used as a quantification value for cellularity (Method A), and a method in which the nuclear area of cells is quantified by discriminant analysis after monochromatizing the images and cellularity calculated (Method B) (Fig 1A):

$$Method\ A: Cellularity\ (\ ) = non\text{-}fat\ area\ (\ )$$

$$Method\ B: Cellularity\ (\%) = nuclear\ area\ of\ cells\ (\%) \times 4.8$$

The calibration factor of 4.8 in Method B was obtained from a training set using five normocellular specimens. Next, we developed a third Method C, by combining Methods A and B. Method C was constructed to adopt the quantification values by Method B if it was less than 33.9% and by Method A if it was more than 33.9% (Fig 1B). This cut-off value was determined from the median cellularity of normal cellularity slides in Method B.

$$Method\ C: Cellularity\ (\%) = IF(Method\ B < 33.9, Method\ B, Method\ A)$$

The cases were analyzed using three protocols and were stratified into those with low (<30%), normal

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Table 1. Comparison of pathology reports with the three methods developed in this study

	Method A	Method B	Method C
ICC* (95% CI)	0.80 (0.72–0.87)	0.85 (0.78–0.90)	0.88 (0.82–0.92)
AUC for hypocellular (95% CI)	0.86 (0.76–0.95)	0.94 (0.89–0.99)	0.94 (0.88–0.99)
P value†	–	< 0.001	< 0.001
AUC for hypercellular (95% CI)	0.96 (0.93–0.99)	0.93 (0.89–0.98)	0.97 (0.93–1.00)
P value‡	0.18	–	0.058

AUC, area under the curve; CI, confidence interval; ICC, intraclass correlation. *ICCs calculated with Spearman's correction. †*P* values of Methods B and C were calculated with DeLong's test compared to Method A. ‡*P* values of Methods A and C were calculated with DeLong's test compared to Method B.

(30–60%), and high cellularity (>60%). The concordance between VE and DIA in each subcondition was assessed.

All statistical analyses were performed using EZR software (Saitama Medical Center, Jichi Medical University, Saitama, Japan), a Japanese user interface for R (The R Foundation for Statistical Computing, Vienna, Austria).[8]

Ethical clearance

This study was approved by the Ethics Committee at Tottori University, Faculty of Medicine (approval number: 22A032). Informed consent was obtained through an opt-out approach.

RESULTS

We enrolled 54 patients with lymphoma (n = 13), myeloproliferative neoplasms (n = 9), abnormal cellular blood count (n = 8), myelodysplastic syndromes (n = 8), acute myelogenous leukemia (n = 6), reactive amyloidosis (n = 5), chronic myelogenous leukemia (n = 2), malignant melanoma (n = 2), or acute lymphoblastic leukemia (n = 1). The majority of patients had a history of lymphoma, with negative staging biopsies. A total of 91 HE slides were used. The median patient age was 71 years (range, 32–92 years), and the male to female ratio was 29:25, and 38 biopsy and 53 clot specimens were included. Cellularity was visually scored as hypocellular (n = 17), normocellular (n = 44), or hypercellular (n = 30). In comparison with the VE in the pathology reports, the intraclass correlation coefficients (ICC) of Methods A and B were 0.80 and 0.85, respectively. In receiver operating characteristic (ROC) analysis with hypocellularity as the objective variable, the value of area under the curve (AUC) for Method B was significantly high (0.95 vs 0.88, $P < 0.01$). In ROC analysis with hypercellularity as the objective variable, the AUC value of Method A was higher than that of Method B (0.96 vs 0.93, $P = 0.18$). Hypocellular cases were overestimated in Method A and hypercellular cases were overestimated in Method B. Next, we developed Method C by combining Methods A and B. When comparing Method C with the VE in the pathology reports, the ICC was 0.88, and AUC values for hypocellularity and hypercellularity were 0.94 and 0.97, respectively (Table 1).

DISCUSSION

Although rapid, evaluation of BM cellularity is roughly semi-quantitative and largely dependent upon VE. This study demonstrates that BM cellularity measured using DIA shows good agreement with cellularity assessed by VE, confirming that DIA can provide concordant estimations of cellularity. Method A tended to overestimate hypocellular values owing to the detection of non-fat areas, which did not include cells. Usually, HE-stained pathological specimens are used, but the staining time and composition of the staining solution are different for each facility, and differences in the density of pathological tissue images can be seen. Specifically, the staining of the nuclear area varies depending on cell density, and the nuclear area could not be quantified under the same detection conditions. Using R selective images after RGB separation for analysis after conversion into monochrome images, we were able to detect the nuclear area clearly. According to this protocol, it was possible to quantitate hypocellular specimens. However, Method B tended to overestimate hypercellular values because the calibration factor was calculated, using normocellular specimens, as a ratio of the cell nuclear and non-fat areas, and that of hypercellular specimens was lower than that of normocellular specimens. Method C combined the advantages of Methods A and B. Several recent studies reported good agreement between cellularity measurement using DIA techniques on digitized

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slides and references using VE or point counting,[2, 6, 7] our method is consistent with the methods used in these studies. The Image software used in this study was easy to analyze, facilitating the completion of all the analysis steps within a few seconds. This study had several limitations. First, correlation reference methods of pathological reports among histopathologists were not standardized. We did not use the gold standard of point counting to measure cellularity. Second, it was impossible to measure cases of nonconformity as well as VE. Comparisons of BM with significant artifacts, fibrosis, absence of particle, or interstitial depletion (cell dropout) could not be made because such cases were excluded from this study.

In conclusion, this study found that cellularity measured by DIA shows good concordance with cellularity observed by VE. It may be possible to estimate cellularity using either DIA or VE in appropriate clinical settings. The most favorable values were obtained with Method C, which detected non-fatty and cell nuclear areas.

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The authors declare no conflict of interest.

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