Automated grading of reticulin stain of bone marrow trephine using AUTORETIC algorithm

H Cualing[1] MD, E Zhong MS

Background: Determining the grade of reticulin fibrosis in the bone marrow is currently performed subjectively using manual and visual grade assignment by hematopathologists with unavoidable intra- and interobserver bias. Because of this inconsistency, a rapid, accurate, reproducible computerized algorithm is needed. Here, we describe a novel AUTORETIC algorithmic results and compared them with pathologist's manual grading.
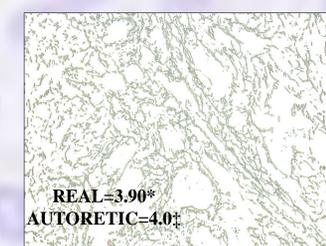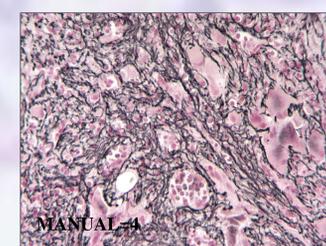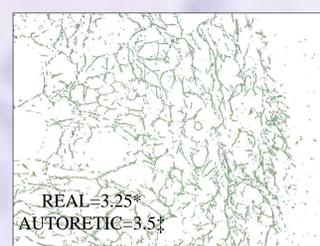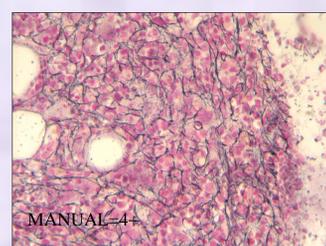
Materials and Methods: A test and a control group of images are compared. Test group has 65 patients, 23-80 years of age, M:F ratio 1:1.6 with various hematological diseases, including MDS, CML, AML, ALL, Hodgkin and non-Hodgkin lymphomas, and plasma cell myeloma. . The trephine core biopsy reticulin staining was performed using VENTANA Nexus automated system. A 20x digital color image was captured: average of 3 frames (1-7) per case. The results of the manual grading by two pathologists are compared with the automated grading by AUTORETIC software that was developed and provided by IHCflow/GreenGreat corp. using proprietary algorithm. A control group of 200 images from patients with similar diseases, with marrow biopsy stained with a non-automated reticulin technique, using a different microscope CCD setup, is graded by a different pathologist, but with images likewise run in AUTORETIC to compare reproducibility. Grading was performed based on Bain's criteria. (BJ, Bain, Jul 2001, Bone Marrow Pathology textbook) by 3 trained pathologists/fellows. Algorithm was modeled after the Bain's images and 6 images scored by pathologists with intermediate results in an ordinal real number scale rounded to 0, 1, 2, 3, 4.
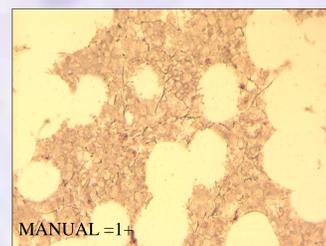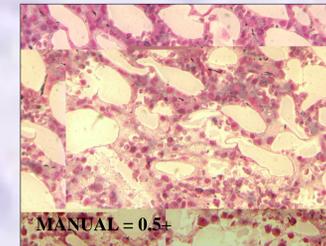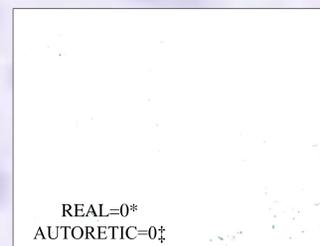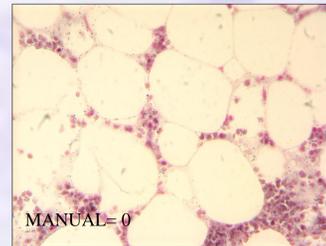
Irrelevant objects and cells are discarded and size, length, pixel numbers and entrapment are used as parameters for grading.

Criteria for reticulin staining :

- **0** No reticulin fibers (normal)
- **1** Occasional fine individual fibers and foci of a fine fiber network(normal)
- **2** Fine fiber network throughout most of the sections, no coarse fibers(normal)
- **3** Diffuse fiber network with scattered thick coarse fibers, occasional megakaryocytes encircled (Reticulin Fibrosis)
- **4** Diffuse often coarse fiber network with back to back fibers and many cells including megakaryocytes encircled (Myelofibrosis)

REF: Adopted modified from BJ, Bain, July 2001, Bone Marrow Pathology Textbook.

MANUAL= 0

REAL=0*
AUTORETIC=0‡

MANUAL = 0.5+

REAL=0.67*
AUTORETIC=0.5

MANUAL =1+

REAL= 1.1*
AUTORETIC=1.0‡

MANUAL=1+

REAL=1.37*
AUTORETIC= 1.5‡

MANUAL=2+

REAL=2+*
AUTORETIC=2.4‡

MANUAL=3+

REAL=2.5*
AUTORETIC=2.5‡

MANUAL=2+

REAL=2.8*
AUTORETIC=3.0‡

MANUAL=3.5

REAL=2.76*
AUTORETIC=3.0‡

MANUAL=4+

REAL=3.25*
AUTORETIC=3.5‡

MANUAL=4

REAL=3.90*
AUTORETIC=4.0‡
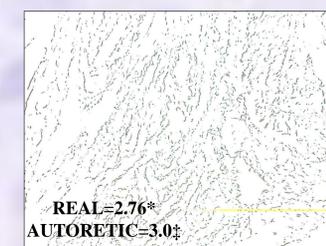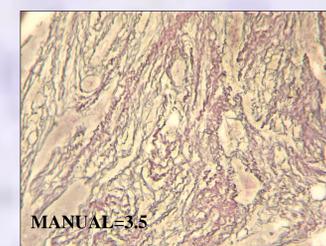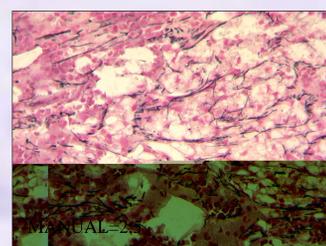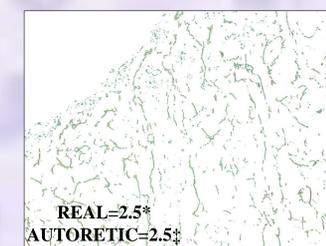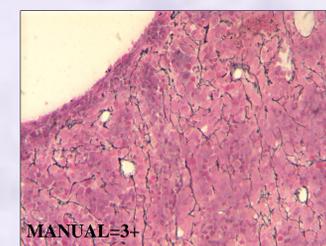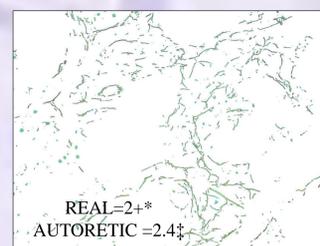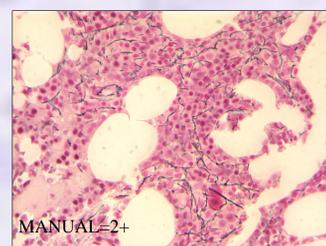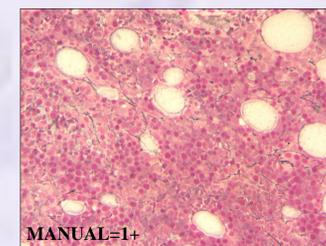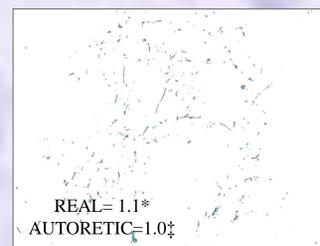
Results: The AUTORETIC grading finished in 2-3 seconds with output continuous rounded in 0.5 units from 0+, 1+ 2+ 3+, 4+. Agreement between the pathologist and computer was judged true if the difference between grades is 0.5 or less. Results demonstrate positive strong correlation between the manual grading and computer grading using our test and control groups. In the test group, the mean reticulin manual grading is 1.8 (95% CI 1.58 - 2.07) vs AUTORETIC mean of 1.734 with 95% CI(1.47 - 1.99) with no significant difference between the mean(SD). The correlation was high with r 0.8699 (nonparametric Spearman p<0.0001). In the control group, the correlation was likewise high with r 0.7687(Spearman, 95% CI 0.71-0.82).

In conclusion, the mean and SD for manual and AUTORETIC program are similar and they highly correlate with each other, indicating the software is a rapid, accurate and reproducible computerized technique that will be objective and useful in clinical bone marrow analysis. The AUTORETIC program could be a surrogate to subjective reporting. In addition, the program is robust and adaptable to variability of conditions and is applicable to bone marrow stained manually or automatically, digitized in a different microscope, or scored by a different user.

References:

1)BJ, Bain's, July 2001, Bone Marrow Pathology.

*REAL= Autoretic continuous score from 0-4.
‡ AUTORETIC = Rounded score to the nearest 0.5 unit.