UCDAVIS HEALTH | Department of Pathology and Laboratory Medicine

Comparison between manual trichrome vs Gomori's automated trichrome in Bone Marrow Fibrosis Grading in patients with primary myelofibrosis

Shafagh Parsazad, M.D., Farnoush M Moen, M.D., Denis M Dwyre, M.D., John P Graff, D.O., Zarir E Karanjawala, M.D., Elham Vali Betts, M.D., Ananya Datta Mitra, M.D.

Introduction

Background:
Primary Myelofibrosis (PMF) is a clonal stem cell disorder within the myeloproliferative neoplasms (MPNs), characterized by bone marrow fibrosis due to excessive extracellular matrix (ECM) deposition, mainly type I and III collagens by reactive stromal cells. This process is driven by abnormal cytokine production from atypical megakaryocytes, disrupting hematopoiesis and driving disease progression.

Fibrosis Progression in PMF:

Early (MF-1): Sparse, loosely connected type III collagen (reticulin) fibers.

Intermediate (MF-2): Denser reticulin, emerging type I collagen bundles.

Advanced (MF-3): Extensive collagen deposition, reticulin thickening, and osteosclerosis. Once collagenous fibrosis develops in the marrow it lowers the risk of overall survival for these patients.

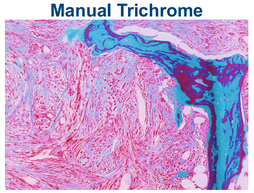
In the era of potentially disease-modifying agents such as Janus kinase inhibitors, accurate grading and differentiation of bone marrow (BM) fibrosis has become more relevant to assess staging of disease and therapeutic effects. However, different fibrosis grading models have been used in the past without uniformity, hence, accurate assessment of collagen and it's grading appear to be essential to discriminate all components of the complex BM fibrous matrix. In clinical practice, trichrome staining is the most employed method for visualizing fibrosis.

The major objectives of the present study were to: (i) comment on the technical pitfalls that may impact on the quality and result of staining; (ii) assess BM fibrosis grading on two different methods of collagen staining and (iii) assess reproducibility of BM fibrosis grading independent from technical influences between the two methods.

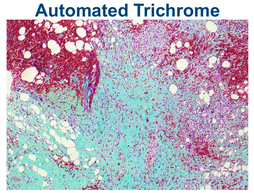
Staining Characteristics

Tissue Component	Manual Trichrome	Automated Gomori's Trichrome
Collagen	Green	Green
Muscle fibers	Red	Red
Cytoplasm	Red	Red
Nuclei	Brown to black	Dark brown to black
Overall Contrast	May vary (operator-dependent)	Consistent, standardized contrast

Manual Trichrome

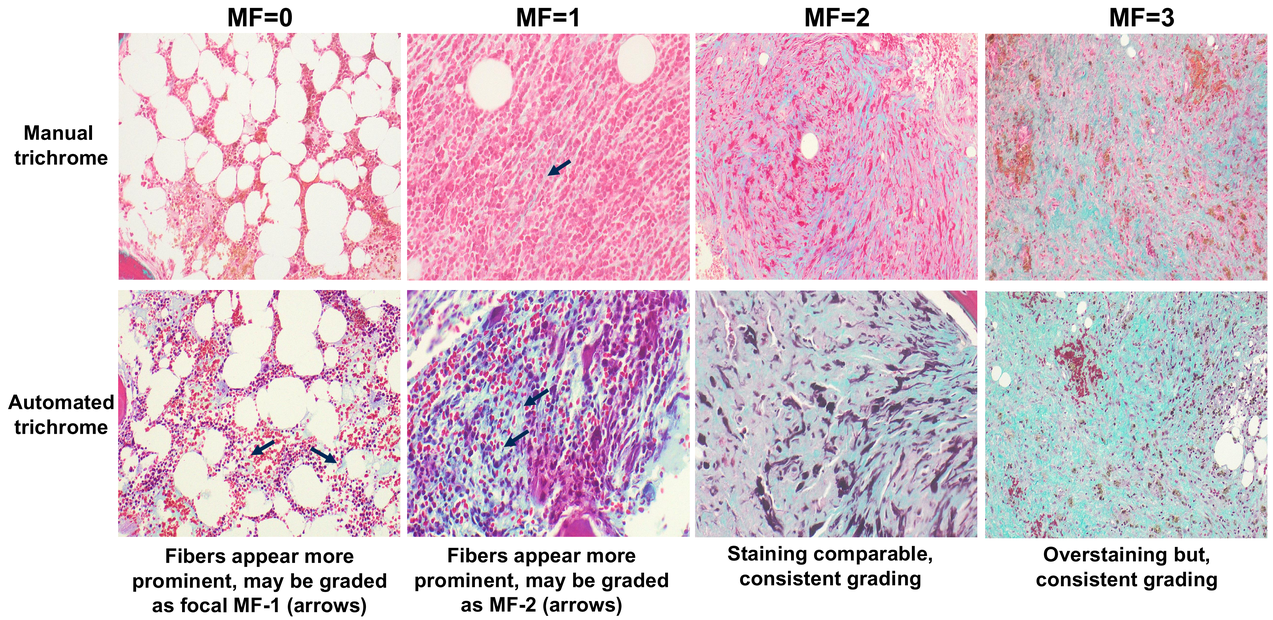


Automated Trichrome



Design

Case selection: Retrospective cohorts were retrieved from archived samples of the Department of Pathology, UCDH. The cases were reviewed and confirmed of the diagnosis based on morphology, reticulin and manual Trichrome stain between two board-certified hematopathologists. Cases not having a Trichrome stain were stained and reevaluated. 21 cases of primary myelofibrosis were identified grading MF-0 to MF-3. All patient identifiers were removed, and the samples complied with the guidelines of our institutional review board.



Results

WHO Fibrosis Grade	Manual Trichrome (Reference Method)	Gomori's Green Trichrome (Test Method)	Interpretation / Diagnostic Concordance
MF-0 (No fibrosis)	No increase collagen fibers; preserved marrow architecture.	Increased staining intensity may highlight background fibers; occasional apparent fiber prominence.	Risk of overestimation of minimal fibrosis with Gomori's method.
MF-1 (Loose fibrosis)	Loose, delicate collagen fibers around marrow spaces.	Delicate fibers appear more prominent due to stronger staining intensity.	Tendency toward overcalling MF-1 from MF-0 due to accentuated fibers.
MF-2 (Moderate fibrosis)	Moderate increase in collagen fibers with focal intersections.	Comparable fiber distribution and density; staining similar to manual method.	Good concordance between methods; minimal grading discrepancy.
MF-3 (Severe fibrosis)	Diffuse, dense collagen fibrosis with coarse bundles.	Dense coarse fibrosis well demonstrated; no significant difference from manual method.	High concordance; reliable detection of advanced fibrosis.

Trichrome Staining Methods

Feature	Manual Trichrome	Automated Gomori's Trichrome
Platform	Manual (bench-top staining)	Automated (Artisan staining system)
Sample Type	Paraffin-embedded sections (3–5 µm)	Paraffin-embedded sections (3–5 µm)
Fixative Preference	Any fixative (Bouin's enhances staining)	Neutral buffered formalin preferred
Pre-treatment	Bouin's solution (oven or microwave)	Not required
Reagent Preparation	Requires preparation of solutions	Ready-to-use reagents
Staining steps	Operator-dependent, manual	Instrument-controlled
Procedure Complexity	Multi-step, labor-intensive	Simplified, automated
Timing	~1.5–2 hours (variable)	Shorter, standardized
Reproducibility	Variable	High
Quality Control	Manual control slides required	Standardized + controls recommended
Risk of Error	Higher	Lower
Flexibility	High	Limited
Cost	Lower reagents, higher labor	Higher cost (instrument + kit)

Summary and Conclusions

This comparative evaluation of fibrosis grading demonstrates overall good diagnostic agreement between the manual trichrome and automated trichrome, with variability primarily observed in low-grade fibrosis assessment. Both methods produce similar staining patterns, but the automated method ensures more consistent and reproducible color intensity, however it demonstrates increased staining intensity in low-grade fibrosis, which may lead to overestimation of early fibrotic change and may lead to inappropriate risk stratification and potentially impact patient management and treatment planning. In contrast, MF-2 and MF-3 cases show strong concordance between the two methods. The manual staining method provides flexibility in execution but is highly dependent on operator skill, leading to variability in results. In contrast, the automated method ensures standardized processing, improved reproducibility, and greater efficiency.

Overall, while Gomori's Trichrome is a useful and reproducible method for identifying established marrow fibrosis, caution is warranted when interpreting minimal or early fibrotic changes, where manual trichrome may provide more conservative and accurate grading.