Evaluation of a novel histology-based fibrosis phenotypic composite score and its correlation with NASH-CRN Fibrosis scores in patients with NASH

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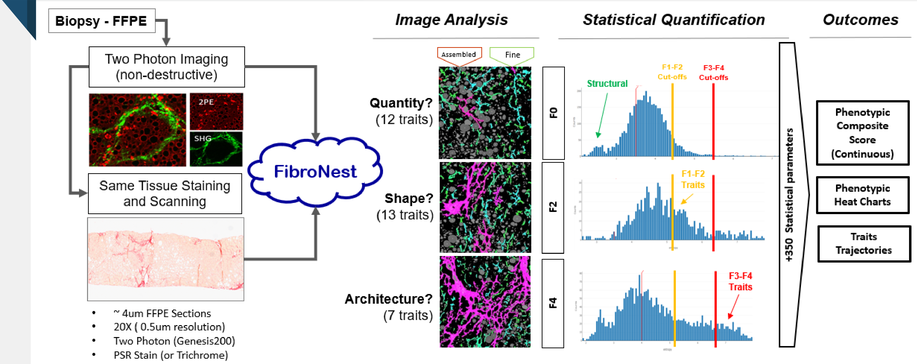
EASL European Association for the Study of the Liver

PharmaNest™

BACKGROUND

We have previously shown that the Phenotypic Fibrosis Composite Score (Ph-FCS) calculated by the FibroNest image analysis platform from second harmonic generation (SHG) images correlates with the NASH-CRN fibrosis scores. Ph-FCS is a novel continuous phenotypic scoring and quantifier of fibrosis. In this study, the same slides imaged non-destructively by SHG have been stained, digitized and then quantified with FibroNest™. This study evaluates the performance of the Ph-FCS obtained from stained slides and its relationship with NASH-CRN fibrosis scores.

METHOD



This retrospective study comprised a cohort of patients (n=77) with NASH diagnosed by histologic assessment of liver biopsy according to NASH CRN criteria by pathologists. Fibrosis stages 0-4

Fibrosis 0	Fibrosis 1	Fibrosis 2	Fibrosis 3	Fibrosis 4
N= 21	N=17	N=20	N=15	N=4

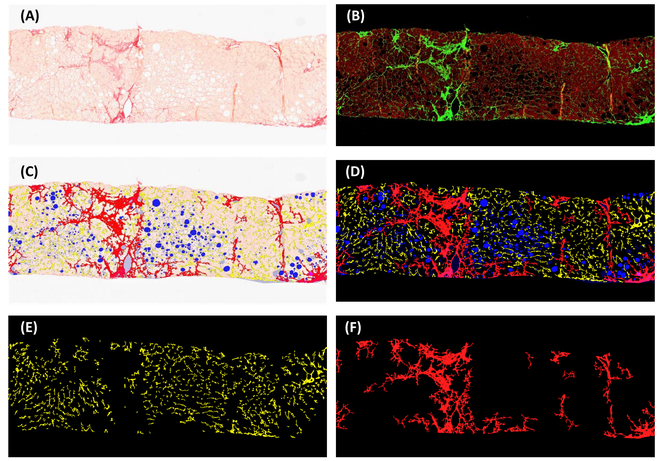
- FFPE sections (3-4 microns) of biopsies were deparaffinized and imaged using the Two Photon microscope Genesis200® at 20X, a non-destructive method (Two Photon Image)
- The same tissues where subsequently stained for collagen with Picro Sirius Red (PSR, Abcam Kit ab150681, no hematoxylin bath) and imaged at 20X in white light with an Aperio AT2 Digital Pathology system at 20X (WSI Image)
- Using FibroNest© for image analysis of both the Two Photon and WSI images, the fibrosis phenotype is described for its collagen content and structure (12 traits), the morphometric traits of the collagen fibers (13 traits), and fibrosis architecture / texture traits (7).
- In each biopsy, each morphometric and texture trait is represented by a histogram distribution (e.g, the distribution of fiber thickness of every single fiber – about 3000 in each biopsy)which is quantified with 7 statistical parameters (qFPs) to account for severity, progression, distortion and variance.
- Because fibrosis progresses from fine collagen structures to complex and assembled bundles, FibroNest also performs this phenotypic analysis on the Fine and Assembled collagens classes.
- Using variance analysis of the qFP vs the F scores, the resulting ~350 qFPs are reduced into a limited set of ~70 Meaningful qFPs, which are combined to calculate the normalized Ph-CFS Phenotypic Fibrosis Composite Score.
- The sensitivity of the Ph-CFS to the PSR process is also evaluated on a separate group of 5 biopsies (thickness: 2-5 um; bath time: 10-60 min).

CONCLUSIONS

- The Ph-CFS calculated with FibroNest from WSI of stained NASH biopsies **significantly correlated with fibrosis stages** and statistically differentiates between F0-1-2-3 stages (p<0.01). Ph-FCS vs F4 statistical performance is limited by N.
- The **very high signal-to-noise** generated by the FibroNest method is the result of recruitment of a large number (+70) quantifiable phenotypic statistical parameters from histogram analysis.
- As a result of this high signal-to-noise and the phenotypic approach of the method, the **Ph-FCS is immune to Tissue processing and staining variation** (Less than 5%)
- The Ph-FCS calculated by FibroNest from digital images of stained tissues **compare to the ones calculated from Two-Photon / SHG images** (reported before by the same team, AASLD2020, weblink)
- **FibroNest™ method provides an objective and reliable evaluation of fibrosis severity and progression in NASH directly from digital images of stained tissues**. This can potentially be used to assess fibrosis regression with pharmacological agents.

RESULTS

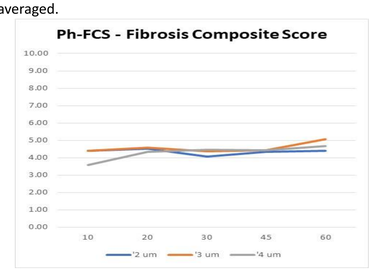
IMAGE ANALYSIS RESULTS AND VISUALIZATION



(A) 20x Biopsy (Sirius Red) (B) FibroNest collagen deconvolution (not dependent of the staining color) Green Channel: collagens, Red Channel: tissue. A third channel (Blue) is used to eliminate scanning artifacts and white balance differences between WSI scanners – not shown here. This step is skipped for the Two Photon Images which generate a native 2-channel image with SHG(Collagens 1&3) in the Green Channel and Two Photon fluorescence (Tissue) in the Red Channel. (C) Augmented visualization of the Digital Image (aid to adjudication) (D) FibroNest quantification Red: assembled collagen, Yellow : interstitial collagen, Blue: steatosis (E) Yellow : interstitial collagen alone (D) Red: assembled collagen. The coalescence of interstitial collagen (yellow) into assembled collagen (Red) is a marker of fibrosis progression.

SENSITIVITY OF COMPOSITE SCORES VS STAINING PROCEDURE

In order to simulate common tissue preparation and staining variability, A unique adequate NASH biopsy FFPE Bloc is processed using consecutive sections at 2, 3 and 4 microns. The PSR Stain bath duration is varied from 10 to 60 minutes. The Ph-FCS is calculated on each tissue segment, then averaged.

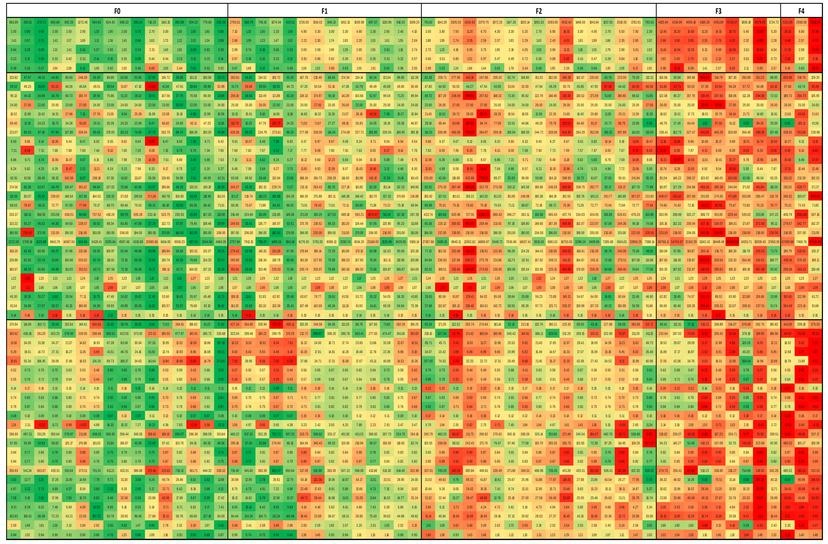


Ph-FCS mean from all biopsy segments

um / Mins	10	20	30	45	60	Δ/2σ
2	3.84	3.87	3.68	4.03	3.98	4.5%
3	3.93	4.30	4.02	4.28	4.34	5.0%
4	3.36	4.00	3.88	4.05	4.05	9.0%
Δ/2σ	7.7%	5.2%	4.4%	3.1%	4.4%	

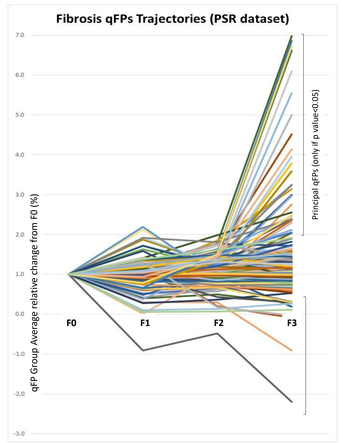
Ph-CFS varies less than 5% when tissue thickness varies from 3 to 4 um and PSR bath time from 20 to 30 mins)

FIBROSIS PHENOTYPIC MAPS (PSR Dataset)

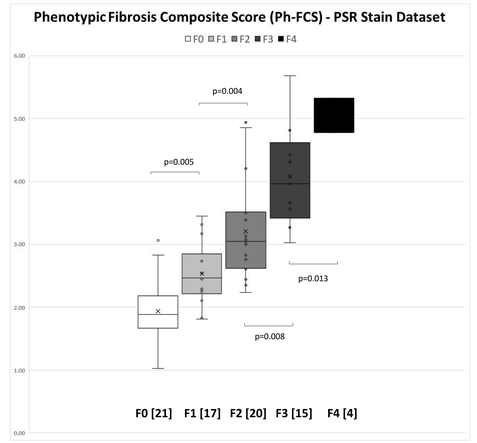


Principal qFPs (rows) are normalized and their progression is illustrated on form of a color severity scale. The relative variation of the F-group averages of each qFP are displayed in the qFP trajectories (below). The Fibrosis Phenotypic signature of each patient (column) is used to assist in the evaluation of the fibrosis phenotype. The normalized values of the qFPs are combined to calculate the Ph-FCS

qFP TRAJECTORIES



Ph-FCS PERFORMANCE



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