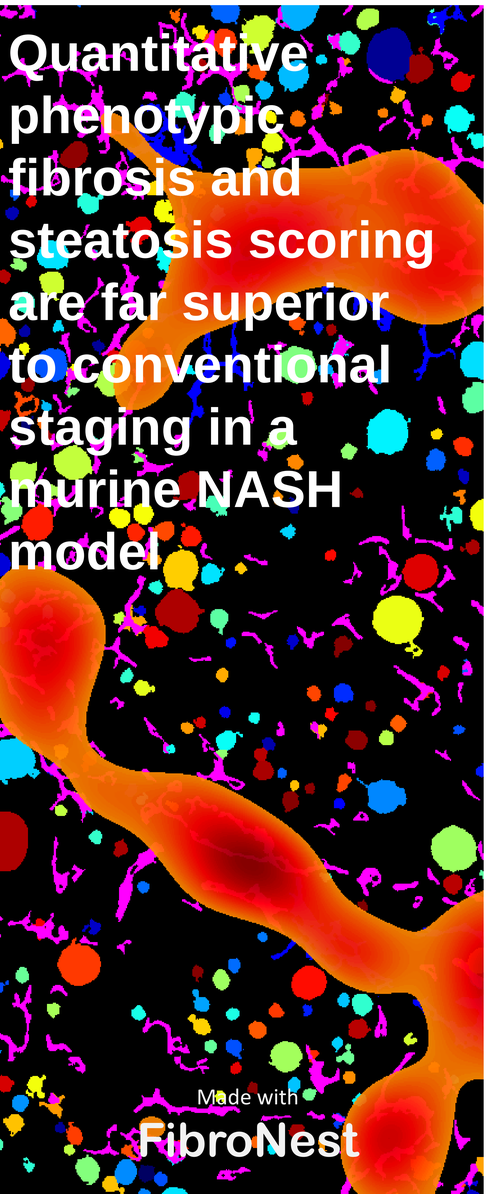

Advanced quantitative phenotypic fibrosis and steatosis scoring is more superior to histology-based conventional staging in NASH animal models

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1 Introduction

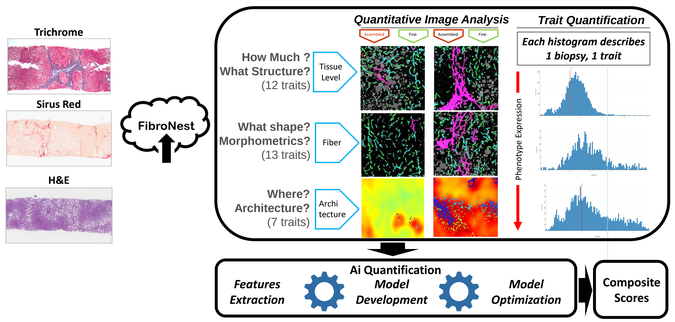
The defining pathologic elements of non-alcoholic steatohepatitis (NASH) are fat accumulation, inflammation, and fibrosis. While conventional histopathology remains the gold standard for diagnosis and staging, this method has its limitations, as it uses a narrow range for scoring, qualitative evaluation, and it is also prone to inter-observer variability.

2 Aim

The Phenotypic Fibrosis Composite Score (Ph-FCS) calculated by the FibroNest image analysis platform is a novel continuous phenotypic scoring and quantifier of fibrosis and steatosis. Here, we compared Ph-FCS to the conventional scoring in the FAT-NASH model, whose features closely align with human NASH (Tsuchida et al, J. Hepatology 2018; doi: 10.1016/j.jhep.2018.03.011).

3 Method

- Mice (n=5 per group) were fed a normal diet or the FAT-NASH regimen (high fat, high fructose, high cholesterol, and very low dose CCl4) for 12 or 24 weeks to induce NASH.
- In other groups, NASH mice (n= 8) were treated with vehicle or with the clinical-stage drug obeticholic acid (OCA, 30 mg/kg, by gavage) for 24 weeks.
- Liver histological sections were stained with picrosirus red for collagens and imaged at 40X in white light with an Aperio Digital Pathology system.
- FibroNest™, a cloud-based quantitative, single fiber, image analysis platform, was used to quantify the fibrosis phenotype including 32 traits for collagen content and structure, fiber morphometry, and architecture (measures the organization of the fibers) (flow chart below).



- Principal quantitative fibrosis traits (up to 315 qFTs) are automatically detected and combined into a normalized Phenotypic Composite Fibrosis Score (Ph-FCS).
- FibroNest outcomes are compared to conventional image analysis methods (panel A) and histologic stages, both o characterize the progression of fibrosis in the model, and the response top OCA

5 Conclusions

FibroNest shows a markedly wider dynamic range of difference between the groups in the FAT-NASH study. This allows greater detection and sensitivity for subtle changes. FibroNest™ provides a more sensitive and reliable evaluation of fibrosis severity and progression in NASH and for the evaluation of therapeutic drug efficacy.

6 Contact information

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4 Results

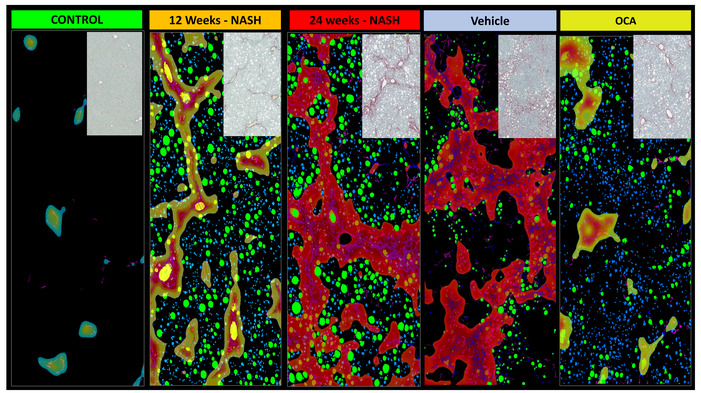
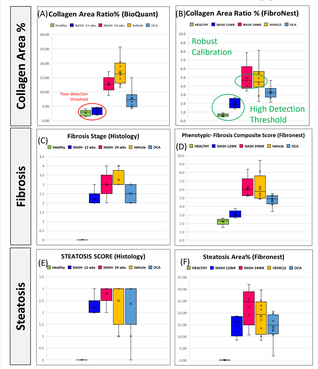


Figure 1. *Liver histology stained with PicroSirus red for Collagens I/III (Inset).*
Image Analysis: Collagen overlapped with architecture (yellow/red cloud). Fat Vacuoles. Large (green), small (blue).

Comparison with Histology



The FibroNest's Fibrosis Phenotypic Score (Ph-FCS) is continuous and incorporates the expression of trees complementary phenotypes: the collagen content levels and related structure, the Single fiber morphometry and the fibrosis Architecture and their and related changes (remodeling).

FibroNest scores outperforms conventional image analysis method ((A) – (B)) and overcomes the limitations of Categorical stages that are not suited to animal models, for the assessment of fibrosis ((C) vs (D)) and Steatosis ((E) vs (F)) severity and response.

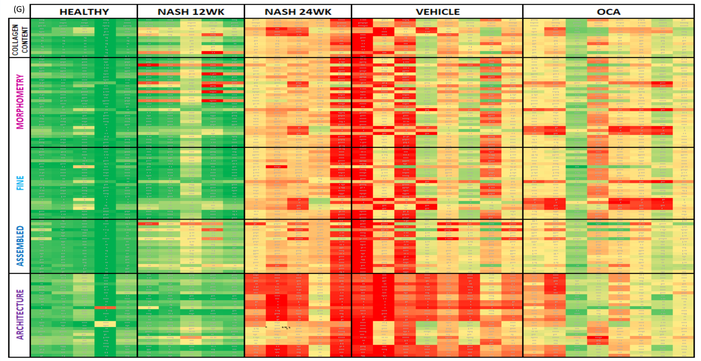
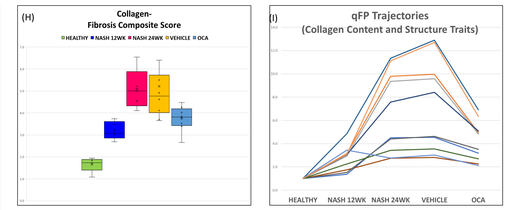


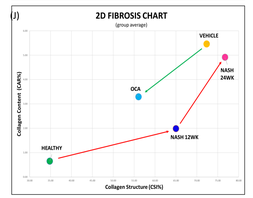
Figure 3. Heatmap of the phenotypic fibrosis quantification, including collagen content, morphometric, and architecture traits: Principal fibrosis parameters (row) are normalized, and their progression is showed in color scale chart. Each column represents an animal. Fibrosis parameters are combined to form the phenotypic Fibrosis Composite Scores (Ph-FCS).

Collagen Content and Reticulation

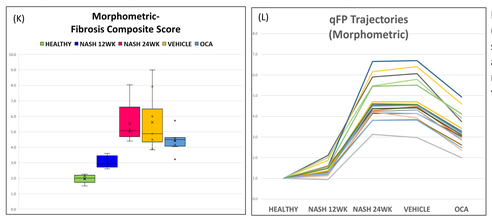


The Collagen Composite Score (H) consist of traits related to collagen content and structures. The progression (and regression under treatment) of each Individual Traits is described in panel (I).

The 2D Fibrosis Charts summarizes the remodeling of collagens into complex structures as the disease progress/ regress, also seen in the architectural phenotypes below (panels M, N)



Single Collagen Fiber Morphometry



Morphometric scores and Trajectories ((K) (L) and inserts) describe the signatures of progression, regression and drug effect on fibers morphometry, as they grow from Fine to Assembled complex fibers.

Similarly, the expression of the architectural phenotype, and specifically the built up (and regression) of septal bridges, is quantified by the FibroNest method ((M), (N) and inserts below)

Fibrosis Architecture