Fibrosis & Structural Decline of Liver Allografts: what and how to measure & potential underlying causes

Carla Venturi Monteagudo MD, PhD
THE NORMAL LIVER ARCHITECTURE

Parenchymal Cells
- Hepatocytes
- Cholangiocytes

Non-parenchymal Cells
- Endothelial Cells
- Kupffer Cells
- Hepatic Stellate Cells
- Myofibroblasts
- Natural Killer Cells
- B Lymphocytes

Extra Cellular Matrix
- Collagen
- Laminin
- Proteoglycans
- Fibronectin

INTRODUCTION
the zone 3 receives less oxygen and nutrients than zone 1, where the blood flow of the hepatic artery branch and portal vein is poured to conform the sinusoids.
LIVER INJURY AND REGENERATION

INTRODUCTION

PERSISTENT INFLAMMATORY CONDITIONS
Infections-Rejection- biliary / vascular complications- steatohepatitis

ACTIVATE IMMUNE RESPONSE

Hepatic Stellate Cells ACTIVATION

FIBROGENESIS
Production & Degradation of Extra Cellular Matrix

PERPETUATION OF PRO-FIBROGENIC STATUS
EXTRA CELLULAR MATRIX ACCUMULATION

TRANSPLANTED LIVER

ALLOGRAFT FIBROSIS & CIRRHOSIS

Activated HSCs
Myofibroblast phenotype
Stromal Stiffness

INJURY
Proliferation
Oxidative Stress, et al

Fibrofogenesis

PDEG, MCP-1
Matrix Degradation

INTRODUCTION
FIBROSIS IN PEDIATRIC LIVER TRANSPLANTATION

INTRODUCTION

PATHOGENESIS & EVOLUTION

ASSESSMENT & MONITORING
Liver Biopsy
Non-invasive Methods

PREVENTION & REVERSION

LIVER ALLOGRAFT FIBROSIS
FIBROSIS IN PEDIATRIC LIVER TRANSPLANTATION

High proportion of fibrosis described in the long-term, mainly associated to inflammation, chronic hepatitis & chronic rejection

<table>
<thead>
<tr>
<th>Reference</th>
<th>Center</th>
<th>Number of Biopsies</th>
<th>Time After LT</th>
<th>Abnormal Histology</th>
<th>Main Histological Diagnoses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fouquet et al. (2005)</td>
<td>Paris</td>
<td>67</td>
<td>&gt;10 years</td>
<td>73%</td>
<td>Chronic rejection (42%), centrilocular fibrosis (22%), biliary cirrhosis (4%), other (4%)</td>
</tr>
<tr>
<td>Evans et al. (2006)</td>
<td>Birmingham</td>
<td>113, 135, 164</td>
<td>1, 5, 10 years</td>
<td>32% at 1 year, 55% at 5 years, 69% at 10 years</td>
<td>Chronic hepatitis ± fibrosis (64%), biliary fibrosis (2%), recurrent PSC (2%), other (2%)—at 10 years</td>
</tr>
<tr>
<td>Ekong et al. (2008)</td>
<td>Chicago Groningen</td>
<td>63</td>
<td>&gt;3 years</td>
<td>97%</td>
<td>Fibrosis (97%), inflammation (70%)</td>
</tr>
<tr>
<td>Scheenstra et al. (2009)</td>
<td>Groningen</td>
<td>77, 64, 66, 55</td>
<td>1, 3, 5, 10 years</td>
<td>34% at 1 year, 48% at 3 years, 65% at 5 years, 69% at 10 years</td>
<td>Fibrosis (69%)—at 10 years</td>
</tr>
<tr>
<td>Ueno et al. (2011)</td>
<td>Osaka</td>
<td>24</td>
<td>&gt;1 year</td>
<td>&gt;71%</td>
<td>Fibrosis (71%), inflammation (58%)</td>
</tr>
<tr>
<td>Miyagawa-Hayashino et al. (2012)</td>
<td>Kyoto</td>
<td>67</td>
<td>&gt;5 years</td>
<td>&gt;84%</td>
<td>Fibrosis (84%), inflammation (58%)</td>
</tr>
<tr>
<td>Venturi et al. (2012)</td>
<td>Brussels</td>
<td>38</td>
<td>7 years</td>
<td>94%</td>
<td>Fibrosis (94%), inflammation (74%), ductal proliferation (26%), steatosis (26%)</td>
</tr>
<tr>
<td>Tomita et al. (2013)</td>
<td>Tokyo</td>
<td>59</td>
<td>0.2-15 years (median, 6 years)</td>
<td>&gt;86%</td>
<td>Fibrosis (86%), inflammation (39%), steatosis (10%)</td>
</tr>
<tr>
<td>Kosola et al. (2013)</td>
<td>Helsinki</td>
<td>54</td>
<td>&gt;3 years</td>
<td>&gt;43%</td>
<td>Steatosis (43%), ductular reaction (43%), fibrosis (39%), inflammation (22%)</td>
</tr>
<tr>
<td>Briem-Richter et al. (2013)</td>
<td>Hamburg</td>
<td>60</td>
<td>&gt;1 year</td>
<td>40%</td>
<td>Fibrosis (33%), mild acute rejection (20%), steatosis (17%), early chronic rejection (3%)</td>
</tr>
<tr>
<td>Daffai et al. (2014)</td>
<td>King’s College Hospital, London</td>
<td>56</td>
<td>&gt;1 year</td>
<td>84%</td>
<td>Hepatitis (41%), bridging fibrosis/cirrhosis (27%), NRH (16%), biliary problem (12.5%), rejection (4%), other (11%)*</td>
</tr>
<tr>
<td>Sanada et al. (2014)</td>
<td>Tochigi</td>
<td>89, 55</td>
<td>2 and 5 years</td>
<td>&gt;42%</td>
<td>Inflammation (42%), fibrosis (34.5%)—at 5 years</td>
</tr>
</tbody>
</table>

-Evolutive process? Patient predisposing condition? Could be related to post-transplant persistent injuries?
FIBROSIS IN PEDIATRIC LIVER TRANSPLANTATION

**ASSESSMENT & MONITORING**

**INTRODUCTION**

**Invasive Approach - Liver Biopsy - “GOLD STANDARD”**

**QUANTITATIVE MORPHOMETRIC ANALYSIS**
Quantify the fibrosis area found in the liver biopsy specimen stained by PicroSirius-Red.

**SEMIQUANTITATIVE HISTOLOGIC SCORING SYSTEMS**
Pathologists review the liver biopsy classifying fibrosis in mild, moderate or severe according the scores.

**PicroSirious-Red**
- Collagen I-II-III
- Counterstained

**Masson’s Trichrome**
- Collagen
- Nuclei
- Cytoplasm
**SCHEUER system (1991)**
Combines Necroinflammation and Fibrosis grade 0-4

<table>
<thead>
<tr>
<th>Portal inflammation &amp; necrosis</th>
<th>Lobular inflammation &amp; necrosis</th>
<th>Portal Fibrosis</th>
</tr>
</thead>
</table>

**META VIR system (1994)**
Combines piecemeal and lobular necrosis with inflammation and fibrosis

<table>
<thead>
<tr>
<th>Activity &amp; Necroinflammation A 0-3</th>
<th>Portal Fibrosis 0-4</th>
</tr>
</thead>
</table>

**ISHAK system (1995)**

<table>
<thead>
<tr>
<th>Periportal or periseptal interface hepatitis 0-4</th>
<th>Confluent necrosis 0-6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Focal (spotty) lytic necrosis, apoptosis and focal inflammation 0-4</td>
<td>Portal inflammation 0-4</td>
</tr>
<tr>
<td><strong>Portal</strong> and <strong>Bridging Fibrosis</strong> 0-6</td>
<td><strong>Portal</strong> Fibrosis 0-4</td>
</tr>
</tbody>
</table>
LIVER BIOPSY
Fibrosis at the Three Main Areas of the Liver Parenchyma

Portal Fibrosis  Sinusoidal fibrosis  Centrilobular fibrosis

Conventional systems used to stage fibrosis in the native liver fail to recognize these patterns of graft fibrosis.
Multiparametric MRI No Ped TX
Transient Elastography (AUROC 0.8-0.9)
Acoustic radiation force impulse (AFRI) (AUROC 0.8)
Magnetic Resonance Elastography (MRE) (AUROC 0.92) No Ped TX

**Transient Elastography** Equipment expensive, range of probes are needed, influenced by obesity & inflammation. Reproducible measurements are not possible in 20% of patients. More difficult in split or reduced grafts. Less accurate in middle fibrosis.
INTRODUCTION

FIBROSIS IN PEDIATRIC LIVER TRANSPLANTATION

ASSESSMENT & MONITORING
Non-Invasive Approach
Serum markers of fibrosis

Hyaluronic Acid (HA)
Animo-terminal propeptide of type III collagen (PIIINP)
Tissue inhibitor of matrix metalloproteinase 1 (TIMP1)
APRI: AST/platelet ratio index
Type 4 collagen S, Fibronectin & Laminin

HA: appeared to be a fair predictor of liver allograft fibrosis (Hartley JL, et al. JPGN 2006;43 217-21)
ELF panel*: accurate in pediatric NAFLD (AUROC 0.92); no correlation with the degree of pediatric allograft fibrosis. (Goldschmidt I, et al Ped Transpl. 2013; 17:525-34)

MicroRNAs: Intrahepatic microRNAs predictive of inflammation, rejection, proliferation. (need LB)
Markers of cell Death: CK18, sensitive marker of fibrosis in NAFLD.

Could the serum markers replace Liver Biopsy?

Fibrotest
Alpha 2 macroglobulin
Haptoglobin
Apolipoprotein 1
Total bilirubin
GGT
ALT

0 - 0.10 Probability of fibrosis < 10%
0.10 - 0.60 Liver biopsy recommended
0.60 - 1.00 Probability of fibrosis > 90%

Imbert-Bismuth, Lancet 2001
Autoantibody positivity (SMA-ANA), reflect cause of graft injury; related to chronic hepatitis & fibrosis

Class II donor-specific human leukocyte antigen antibodies (DSAs), mostly DQ, has been associated with graft inflammation, fibrosis, De novo AIH

Donor-specific T cells have been shown to predict the risk of acute rejection following pediatric TX
FIBROSIS IN PEDIATRIC LIVER TRANSPLANTATION

PATHOGENESIS & EVOLUTION

-How is the evolution of activated HSCs in pediatric liver allograft along the time?

ASSESSMENT & MONITORING

Activated HSCs are identified by ASMA-immunoreactivity in the liver biopsy

PREVENTION & REVERSION

-Could the activated HSCs predict high fibrosis development in the long term?
Major Aims

To Analyze The History Of Pediatric Liver Allograft Fibrosis Over Time

To Evaluate The Influence Of Clinical Variables & Immunosuppression In Fibrosis Development

- DESIGN & VALIDATION OF A NEW ALLOGRAFT FIBROSIS SCORING SYSTEM
- CORRELATION OF NON-INVASIVE METHODS WITH LIVER BIOPSY
- TO STUDY THE DYNAMICS OF PEDIATRIC LIVER ALLOGRAFT FIBROSIS
- EVOLUTION OF ACTIVATED HEPATIC STELLATE CELLS IN THE LIVER ALLOGRAFTS
Patients & Methods
Retrospective analysis 1999-2005 of 170 Pediatric LT recipients

Exclusion Criteria: Re-transplantation; inadequate LB; incomplete follow-up(< 3 LB) = 31

Clinical -Biochemical & Serologic Assessment

Pre-LT factors:
- Donor Age
- Donor type
- Ischemia Time
- Recipient age-gender-weight
- height- blood pressure
- Liver Transplant indication
- CMV - EBV status

Post-LT factors:
- Vascular and biliary complications
- Infections (0-6 months)
- Autoantibodies & gammaglobulins %
- History of Post-transplant lymphoproliferative disease

Available data of Doppler ultrasound- TE
Adequate and available protocol liver biopsy
Patients & Methods- Histologic Assessment

170 Pediatric LT recipients - 31 Excluded

139 patients

595 Liver Biopsies =

Available & adequate protocol liver biopsy

Normal Histology: absent or minimal non-specific portal infiltrate.

Acute & Chronic rejection*

Portal inflammation

Centrilobular dropout

Steatosis

Ductal proliferation

Cholestasis

De Novo autoimmune hepatitis**

Necroinflammatory activity

Fibrosis staging

*AR Episodes: increased liver enzymes ([AST] [ALT] [GGT]:NR 5–50 IU/L, histological features (Banff) and treatment with i.v Steroid.

**De novo AIH: progressive graft dysfunction, increased autoantibodies and serum gamma-globulin levels, with histologic features of chronic active hepatitis (portal inflammation with limiting plate disruption, and lobular hepatitis with or without plasma cell infiltration)
Design of a new histological fibrosis scoring
Liver Allograft Fibrosis Score (LAFSc)
Histologic Features and Staging definitions of the Liver Allograft Fibrosis Score= 0-9 (LAFLSc)

<table>
<thead>
<tr>
<th>Structure</th>
<th>0</th>
<th>I</th>
<th>II</th>
<th>III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Portal Tract</td>
<td>No Fibrosis</td>
<td>Non-expanding fibrosis in less than 50% of portal tracts.</td>
<td>Fibrosis in more than 50% of portal tracts and/or expansion into short fibrous septa into the periportal parenchyma.</td>
<td>Marked expansion of most or all portal tracts with bridging fibrosis expanding to other portal tracts or central areas with or without occasional nodules.</td>
</tr>
<tr>
<td>Sinusoids (zones 1, 2)</td>
<td>No Fibrosis</td>
<td>Little fibrosis with thin focal collagen deposits involving less than 50% of sinusoids.</td>
<td>Little fibrosis with thin diffuse collagen deposits involving more than 50% of sinusoids, or thicker but focal fibrosis in less than 50% of sinusoids.</td>
<td>Thick, marked, diffuse sinusoidal fibrosis.</td>
</tr>
<tr>
<td>Centrolobular Vein (zone 3)</td>
<td>No Fibrosis</td>
<td>Circular perivenular fibrosis involving less than 50% of central veins without invasion into the perivenular parenchyma.</td>
<td>Circular perivenular fibrosis in more than 50% of central areas and/or expansion into short fibrous septa into the perivenular parenchyma.</td>
<td>Marked centrolobular fibrosis with bridging to other central areas and/or portal tracts.</td>
</tr>
</tbody>
</table>

Design of a new histologic fibrosis scoring
Patients & Methods

Clinical, biochemical and serological data
Available & Adequate LB at 6 months and 7 years
Data of Non-invasive methods (TE & APRI index) at 7 years

POPULATION INCLUDED

38 patients/ 76 LB

Liver Transplant indication
- Biliary Atresia: 21 (55%)
- Metabolic Diseases: 8 (21%)
- Cholestasis: 8 (21%)
- Tumors: 1 (3%)

Donor Type
- Living Related Donor (n): 23 (60%)
- Deceased Donor (n): 15 (40%)

Immunosupression received at LT
- TAC + Steroides: 18 (47%)
- TAC + Basiliximab: 14 (37%)
- TAC monotherapy: 6 (16%)

Validation of the new semi-quantitative scoring system
Patients & Methods- Histologic Assessment

**Protocol liver biopsies**

- **1 year**
- **2 years**
- **3 years**
- **5 years**
- **10 years**

**1.** 76 New tissue sections cut & stained for Hematoxilin & Eosin (inflammation-activity) Masson’s Trichrome (fibrosis scored by the New Score, METAVIR- Ishak)

**2.** COMPUTER-ASSISTED MORPHOMETRIC ANALYSIS used as reference PATTERN for the new score validation (PicroSirius-Red stain), that measure the proportion of collagen found at the digitalized image of each liver biopsy.

**3.** Morphometric analysis results were correlated with the New Score, METAVIR, Ishak & TE – APRI index

**4.** Correlation between Pathologists (intra/inter observers agreement)

H&E and Masson’s trichrome-stained samples evaluated by external pathologist
Fibrosis staged by LAFSc- METAVIR & Ishak systems

<table>
<thead>
<tr>
<th></th>
<th>6 MONTHS</th>
<th>7 YEARS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>METAVIR F0–F4</strong></td>
<td>F0: 10 (26.3%); F1–F2: 28 (73.6%) F3: 0</td>
<td>F0: 4 (10.5%); F1–F2: 31 (81.5%) F3:3 (7.9%)</td>
</tr>
<tr>
<td><strong>Ishak F0–F6</strong></td>
<td>F0: 11 (28.9%); F1–F2: 15 (39.4%) F3–F4: 12 (31.5%)</td>
<td>F0: 2 (5.2%); F1–F2: 11 (28.9%) F3–F4: 20 (52.6%); F5: 6 (15.7%)</td>
</tr>
<tr>
<td>LAFSc F0–F9</td>
<td>F0: 4 (10.5%); F1: 3 (7.9%); F2: 8 (21.1%); F3:7 (18.4%); F4: 11 (28.9%); F5:2 (5.3%); F6: 3 (7.9%); F7–F9:0</td>
<td>F0: 1 (2.6%); F1: 2 (5.3%); F2: 8 (21.1%); F3: 5 (13.2%); F4: 4 (10.5%); F5: 6 (15.8%); F6: 9 (23.7%); F7: 2 (5.3%); F8: 1 (2.6%); F9:0</td>
</tr>
</tbody>
</table>

Correlation among morphometric analysis with LAFSc- METAVIR & Ishak

<table>
<thead>
<tr>
<th>Spearman Correlation</th>
<th>LAFSc</th>
<th>METAVIR</th>
<th>Ishak</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morphometry (rho; p value)</td>
<td><strong>0.731</strong> <strong>p &lt; 0.000</strong></td>
<td>0.571** <strong>p &lt; 0.000</strong></td>
<td>0.566** <strong>p &lt; 0.000</strong></td>
</tr>
<tr>
<td>Ishak (rho; p value)</td>
<td><strong>0.759</strong> <strong>p &lt; 0.000</strong></td>
<td><strong>0.940</strong> <strong>p &lt; 0.000</strong></td>
<td></td>
</tr>
<tr>
<td>METAVIR (rho; p value)</td>
<td><strong>0.739</strong> <strong>p &lt; 0.000</strong></td>
<td><strong>0.940</strong> <strong>p &lt; 0.000</strong></td>
<td></td>
</tr>
</tbody>
</table>

LAFSc was the most accurate semi-quantitative score for evaluating fibrosis.

Design of a new histologic fibrosis scoring
Results I

Correlation between collagen deposits (morphometric analysis) & LAFSc

<table>
<thead>
<tr>
<th>Equation</th>
<th>R²</th>
<th>F</th>
<th>gl1</th>
<th>gl2</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linear Regression</td>
<td>0.493</td>
<td>73.78</td>
<td>1</td>
<td>76</td>
<td>0.000</td>
</tr>
<tr>
<td>Quadratic Regression</td>
<td>0.508</td>
<td>38.78</td>
<td>2</td>
<td>75</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Reproducibility of Liver allograft fibrosis score analysed by observers

High intra-observer agreement 0.97, p < 0.0001
Inter-observer agreement: and 0.79, p < 0.0001
Intraclass correlation coefficient

Design of a new histologic fibrosis scoring
**Results I**

Correlation among morphometric analysis and semi-quantitative scoring with non-invasive methods for fibrosis assessment (n=38)

<table>
<thead>
<tr>
<th>Noninvasive methods</th>
<th>Invasive methods</th>
<th>PSR%</th>
<th>LAFSc</th>
<th>METAVIR</th>
<th>Ishak</th>
</tr>
</thead>
<tbody>
<tr>
<td>TE (FibroScan®)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rho</td>
<td></td>
<td>-0.126</td>
<td>-0.225</td>
<td>0.132</td>
<td>0.036</td>
</tr>
<tr>
<td>p value</td>
<td></td>
<td>p = 0.47</td>
<td>p = 0.19</td>
<td>p = 0.44</td>
<td>p = 0.83</td>
</tr>
<tr>
<td>APRI</td>
<td></td>
<td>-0.155</td>
<td>-0.245</td>
<td>-0.308</td>
<td>0.168</td>
</tr>
<tr>
<td>rho</td>
<td></td>
<td>p = 0.36</td>
<td>p = 0.14</td>
<td>p = 0.06</td>
<td>p = 0.34</td>
</tr>
<tr>
<td>p values</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

TE = transient elastography; APRI index = (AST × upper normal limit) × 100/platelet count (10⁹/L).

No correlation was found among TE or APRI index with morphometric analysis, METAVIR, Ishak & LAFSc
Dynamics Of Allograft Fibrosis In Pediatric Liver Transplantation
Patients & Methods

POPULATION INCLUDED

- Clinical, biochemical, serological data,
- Immunosuppression
- Doppler Ultrasound
- Available & adequate LB at 6 months, 3 and 7 yrs.

Dynamics of liver allograft fibrosis

Protocol liver biopsies - long-term follow-up

Demographic Data

<table>
<thead>
<tr>
<th>Liver Transplant Indication</th>
<th>Median Age at LT (years, range)</th>
<th>Median Weight at LT (kg, range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biliary Atresia</td>
<td>1.28 (0.2-15.7)</td>
<td>7.66 (3.8-53.7)</td>
</tr>
<tr>
<td>P.I.F. Cholestasis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metabolic diseases</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tumors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alagille Syndrome</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver Transplant Indication</td>
<td>30 (55%)</td>
<td>9 (16%)</td>
</tr>
<tr>
<td>Donor Type</td>
<td>8 (15%)</td>
<td>5 (9%)</td>
</tr>
<tr>
<td>Living Related Donor/ Deceased Donor (n,%)</td>
<td>29 (53%) - 25 (47%)</td>
<td></td>
</tr>
<tr>
<td>Median donor age (years, range)</td>
<td>30.1 (0.4- 50.3)</td>
<td></td>
</tr>
<tr>
<td>Median Ischemia time (minutes, range)</td>
<td>169.5 (68- 892)</td>
<td></td>
</tr>
<tr>
<td>Imunosuppression received at LT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TAC+ Steroids</td>
<td>24 (44%)</td>
<td></td>
</tr>
<tr>
<td>TAC+ Basiliximab</td>
<td>23 (43%)</td>
<td></td>
</tr>
<tr>
<td>TAC monotherapy</td>
<td>7 (13%)</td>
<td></td>
</tr>
</tbody>
</table>
Patients & Methods

**Clinical Considerations**
- Normal vs increased liver enzymes along the time (NV = 5-50 AST, ALT, GGT)
- Patients who did not received Steroids anytime.
- Tacrolimus monotherapy < 4ng/ml with normal liver enzymes (prope-T)
- Two or more immunosuppressors or Tacrolimus monotherapy > 4 ng/ml.

**Histologic Assessment**
- New tissue sections stained for H&E, Masson’s Trichrome, PicroSirious-Red & Activated Hepatic Stellate Cells (ASMA immunostaining)
- Pathologist Review & Fibrosis scoring: METAVIR (F0-F4) & Liver Allograft Fibrosis Score (LAFSc 0-9)
- Fibrosis & ASMA-positive area quantified by morphometric analysis

1-Correlation among fibrosis with clinical variables, IS and histologic features associated
2-Correlation among ASMA-positive area with fibrosis (LAFSc & PSR%) at same period/long-term

**Statistical Methods:** SPSS 18.0 Chicago, IL. Results expressed as percentage, median, mean and SD; statistical significance for p-values < 0.05. Relation among variables evaluated by Pearson correlation. Linear and quadratic regressions were fitted to analyze relationship among variables.
Fibrosis progressed along the time in 40 (74%) patients.

Stable or reduced fibrosis was found in 14 (26%) patients.

Patients with increased liver enzymes show similar amount of fibrosis than those with normal liver function.
Results II- Fibrosis evolution at parenchymal areas

**Portal Fibrosis**

- **6m**
  - F0: 6%
  - F1: 24%
  - F2: 24%
  - F3: 55%
- **3y**
  - F0: 24%
  - F1: 39%
  - F2: 35%
  - F3: 18%
- **7y**
  - F0: 15%
  - F1: 15%
  - F2: 28%
  - F3: 15%

**Centrilobular Fibrosis**

- **6m**
  - F0: 9%
  - F1: 39%
  - F2: 50%
  - F3: 20%
- **3y**
  - F0: 9%
  - F1: 65%
  - F2: 41%
  - F3: 20%
- **7y**
  - F0: 11%
  - F1: 20%
  - F2: 37%
  - F3: 11%

**Sinusoidal Fibrosis**

- **6m**
  - F0: 28%
  - F1: 54%
  - F2: 52%
  - F3: 20%
- **3y**
  - F0: 54%
  - F1: 52%
  - F2: 37%
  - F3: 20%
- **7y**
  - F0: 9%
  - F1: 20%
  - F2: 9%
  - F3: 0%

**Linear progression p<0.01**

- **Portal Fibrosis**
- **Centrilobular Fibrosis**
- **Sinusoidal Fibrosis**

**No linear p=0.2**

**Dynamics of liver allograft fibrosis**
Results III- Evolution of Fibrosis & Activated-HSCs (ASMA)

Fibrosis progressed along the time $p<0.001$

Activated HSCs decreased along the time $p<0.01$

LAFSc: mild = 1-3; moderate = 4-6; severe = 7-9

Increment by areas in the long-term:
- Sinusoidal: 33%
- Centrilobular: 45%
- Portal: 57%

Activated-HSCs showed inverse evolution respect to Fibrosis in the long-term

Liver Transplantation 2016 Jun;22(6):822-9
Results III- Evolution of Fibrosis according to Activated HSCs at 6m

**Activated-HSCs at 6 months = ≥ 8% = 20 patients**

\[= \leq 8\% = 34 \text{ patients}\]

---

**Table:**

<table>
<thead>
<tr>
<th></th>
<th>Fibrosis 6m</th>
<th>Fibrosis 3 y</th>
<th>Fibrosis 7 y</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ASMA ≥ 8</strong></td>
<td>20</td>
<td>16.7 ± 8</td>
<td>11.9 ± 7</td>
<td>24.6 ± 8</td>
</tr>
<tr>
<td><strong>ASMA ≤ 8</strong></td>
<td>34</td>
<td>12.3 ± 7</td>
<td>11.4 ± 6</td>
<td>17.5 ± 7</td>
</tr>
<tr>
<td><strong>p-value</strong></td>
<td>=0.03</td>
<td>=0.8</td>
<td>&lt; 0.01</td>
<td></td>
</tr>
</tbody>
</table>

*Note: p-values represent the significance between means*

---

**Graph:**

- **PSR%**: 0% to 30% over 6 months, 3 years, and 7 years.
- **r²**: 0.48 for PSR%, p<0.01.
- **r²**: 0.30 for LAFSc, p=0.03.

**Note:** Statistical method: Mixed regression.

**Activated-HSCs ≥ 8 at 6 months a risk factor for fibrosis development at 7 years**

*Liver Transplantation 2016 Jun;22(6):822-9*
## Results IV Demographic data of the 139 LT recipients

<table>
<thead>
<tr>
<th>Description</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients / Liver biopsies (n)</td>
<td>139 (69 boys) /595</td>
</tr>
<tr>
<td>Median age at LT (years, range, range)</td>
<td>1.4 (0.2- 16.8)</td>
</tr>
<tr>
<td>Median Weight at LT (kg, range)</td>
<td>8.4 (3.7- 63.2)</td>
</tr>
<tr>
<td>LT Indication: (n, %)</td>
<td></td>
</tr>
<tr>
<td>Biliary Atresia</td>
<td>75 (54%)</td>
</tr>
<tr>
<td>Metabolic diseases</td>
<td>21 (15%)</td>
</tr>
<tr>
<td>Progressive Intrahepatic Familial Cholestasis</td>
<td>17 (12%)</td>
</tr>
<tr>
<td>Tumors</td>
<td>11 (8%)</td>
</tr>
<tr>
<td>Alagille Syndrome</td>
<td>11 (8%)</td>
</tr>
<tr>
<td>Others</td>
<td>4 (4%)</td>
</tr>
<tr>
<td>Living Related Donor/ Deceased Donor (n, %)</td>
<td>66 (47 %) / 66 (47%)</td>
</tr>
<tr>
<td>Split Liver/ Reduced Deceased Donor</td>
<td>4 (3%) /  3 ( 2.5%)</td>
</tr>
<tr>
<td>Median donor age (years, range)</td>
<td>29 (0.4- 56.6)</td>
</tr>
<tr>
<td>Median Ischemia time (minutes, range)</td>
<td>232.0 (66- 892)</td>
</tr>
<tr>
<td>Immunosuppression at LT (n, %): TAC+ Basiliximab</td>
<td></td>
</tr>
<tr>
<td>TAC+ Steroids</td>
<td>33 (24%)</td>
</tr>
<tr>
<td>TAC monotherapy</td>
<td>28 (20%)</td>
</tr>
<tr>
<td>TAC+MMf+Steroids</td>
<td>13 (9%)</td>
</tr>
<tr>
<td>TAC+MMF+Daclizumab</td>
<td>8 (6%)</td>
</tr>
<tr>
<td>TAC+Basiliximab+MMf</td>
<td>6 (4%)</td>
</tr>
<tr>
<td>TAC+MMf</td>
<td>6 (4%)</td>
</tr>
<tr>
<td>TAC+Steroids+Daclizumab</td>
<td>3 (2%)</td>
</tr>
</tbody>
</table>
# Results IV Evolution of clinical variables studied

<table>
<thead>
<tr>
<th>Clinical Variables</th>
<th>Time of follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-6m.</td>
</tr>
<tr>
<td>LB:595/ Patients 139</td>
<td>115</td>
</tr>
<tr>
<td>Vascular complications</td>
<td>12 (10%)</td>
</tr>
<tr>
<td>Biliary complications</td>
<td>19 (16%)</td>
</tr>
<tr>
<td>Post-LT AA</td>
<td>26 (23%)</td>
</tr>
<tr>
<td>AR Steroids treated</td>
<td>64 (56%)</td>
</tr>
<tr>
<td>PTLD (EBER +) n=28</td>
<td>8 (7%)</td>
</tr>
<tr>
<td>Gammaglobulins&gt; 15%</td>
<td>40 (35%)</td>
</tr>
<tr>
<td>Gammaglobulins (X)</td>
<td>13.6</td>
</tr>
</tbody>
</table>

Abbreviations: LB, liver biopsy; LT, liver transplantation; AA, autoantibodies; AR, acute rejection; PTLD, post-transplant lymphoproliferative disease; EBER, Epstein Barr virus RNA +.
Results IV  Fibrosis evolution over time

N=139pts.  595LB

Dynamics of liver allograft fibrosis

<table>
<thead>
<tr>
<th>Time</th>
<th>Sinusoidal</th>
<th>Centrilobular</th>
<th>Portal</th>
<th>LAFSc</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 m</td>
<td>0.72 (0.1)</td>
<td>1.29 (0.6)</td>
<td>1.30 (0.5)</td>
<td>3.30 (0.6)</td>
<td>115</td>
</tr>
<tr>
<td>1 y</td>
<td>0.72 (0.1)</td>
<td>1.14 (0.6)</td>
<td>1.24 (0.5)</td>
<td>3.14 (1.6)</td>
<td>112</td>
</tr>
<tr>
<td>2 y</td>
<td>0.80 (0.0)</td>
<td>1.45 (0.7)</td>
<td>1.47 (0.8)</td>
<td>3.73 (1.7)</td>
<td>110</td>
</tr>
<tr>
<td>3 y</td>
<td>0.89 (0.4)</td>
<td>1.41 (0.6)</td>
<td>1.60 (0.9)</td>
<td>3.85 (1.8)</td>
<td>96</td>
</tr>
<tr>
<td>5 y</td>
<td>0.89 (0.4)</td>
<td>1.47 (0.7)</td>
<td>1.64 (0.9)</td>
<td>3.94 (1.9)</td>
<td>81</td>
</tr>
<tr>
<td>7 y</td>
<td>0.89 (0.7)</td>
<td>1.51 (0.6)</td>
<td>1.74 (0.8)</td>
<td>4.14 (1.8)</td>
<td>57</td>
</tr>
<tr>
<td>10 y</td>
<td>1.21 (0.6)</td>
<td>1.46 (0.7)</td>
<td>1.96 (0.7)</td>
<td>4.63 (1.8)</td>
<td>24</td>
</tr>
</tbody>
</table>

Fibrosis Increment by areas over time

<table>
<thead>
<tr>
<th>Sinusoidal</th>
<th>Centrilobular</th>
<th>Portal</th>
</tr>
</thead>
<tbody>
<tr>
<td>68%</td>
<td>13%</td>
<td>50%</td>
</tr>
</tbody>
</table>
LIVER ALLOGRAFT FIBROSIS IS A DYNAMIC PROCESS
### Results IV Association between fibrosis & clinical variables

<table>
<thead>
<tr>
<th>Clinical Variables</th>
<th>Fibrosis</th>
<th>Fibrosis</th>
<th>Fibrosis Location</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N=54</td>
<td>N=139</td>
<td></td>
</tr>
<tr>
<td>Deceased donor grafts</td>
<td>p&lt;0.001</td>
<td>46.3%</td>
<td>Portal p=0.001- p=0.003- p=0.01</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.02</td>
<td>47.5%</td>
<td></td>
</tr>
<tr>
<td>Lymphoproliferative disease</td>
<td>p=0.001</td>
<td>18.5%</td>
<td>Portal p=0.01(7y)</td>
</tr>
<tr>
<td></td>
<td>p=0.01</td>
<td>20%</td>
<td></td>
</tr>
<tr>
<td>Ischemia time &gt; 400 min</td>
<td>p&lt;0.01</td>
<td>11%</td>
<td>Portal p=0.06 (6m), p&lt;0.01(3y)</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.03</td>
<td>10%</td>
<td></td>
</tr>
<tr>
<td>Vascular complications (0-6m)</td>
<td>p=0.04</td>
<td>11%</td>
<td>Centrilobular p=0.04 (7y)</td>
</tr>
<tr>
<td></td>
<td>p=0.04</td>
<td>10%</td>
<td></td>
</tr>
<tr>
<td>Gammaglobulins &gt; 15%</td>
<td>p=0.02</td>
<td>18.5%</td>
<td>Centrilobular p=0.02 (7y)</td>
</tr>
<tr>
<td>Positives AutoAntibodies (&gt;1/40)</td>
<td>p=0.01</td>
<td>19%</td>
<td>Centrilobular p=0.01 (3y)</td>
</tr>
<tr>
<td></td>
<td>p=0.01</td>
<td>10%</td>
<td></td>
</tr>
<tr>
<td>Biliary complications 0-6 m</td>
<td>p=0.01</td>
<td>24%</td>
<td>Sinusoidal p=0.05 (6m), p=0.01 (3y)</td>
</tr>
<tr>
<td></td>
<td>p=0.03</td>
<td>16%</td>
<td></td>
</tr>
<tr>
<td>Male gender</td>
<td>p=0.01</td>
<td>50%</td>
<td>Sinusoidal p=0.001</td>
</tr>
<tr>
<td></td>
<td>p=0.002</td>
<td>50%</td>
<td>Centrilobular p=0.04 (7y)</td>
</tr>
</tbody>
</table>
Results IV  Main histological features found at 595 LB

- Normal liver histology  5%, 3% & 1 % of LB at 6 mo. 3 & 5 years.
- Isolated Fibrosis  8- 19% over time.
- Fibrosis + mild unspecific portal inflammatory infiltrate  15-33% over time (70% NLE)

<table>
<thead>
<tr>
<th>Periodos of evaluation</th>
<th>Total</th>
<th>6m</th>
<th>1yr.</th>
<th>2yrs.</th>
<th>3yrs.</th>
<th>5yrs.</th>
<th>7yrs.</th>
<th>10yrs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>LB</td>
<td>595</td>
<td>115</td>
<td>112</td>
<td>110</td>
<td>96</td>
<td>81</td>
<td>57</td>
<td>24</td>
</tr>
<tr>
<td>No fibrosis</td>
<td></td>
<td>2%</td>
<td>6 (5%)</td>
<td>3 (3%)</td>
<td>3 (3%)</td>
<td>-------</td>
<td>1 (1%)</td>
<td>-------</td>
</tr>
<tr>
<td>Isolated Fibrosis</td>
<td>14%</td>
<td>9 (8%)</td>
<td>16 (14%)</td>
<td>16 (14%)</td>
<td>15 (16%)</td>
<td>13 (16%)</td>
<td>11 (19%)</td>
<td>2 (8%)</td>
</tr>
<tr>
<td>Fibrosis + mild portal infiltrate</td>
<td>22%</td>
<td>24 (21%)</td>
<td>29 (26%)</td>
<td>25 (23%)</td>
<td>14 (15%)</td>
<td>18 (22%)</td>
<td>14 (24%)</td>
<td>8 (33%)</td>
</tr>
<tr>
<td>Ductal proliferation</td>
<td>44%</td>
<td>57 (49%)</td>
<td>51 (45%)</td>
<td>47 (43%)</td>
<td>44 (46%)</td>
<td>36 (44%)</td>
<td>20 (35%)</td>
<td>9 (37%)</td>
</tr>
<tr>
<td>Steatosis</td>
<td>21%</td>
<td>29 (25%)</td>
<td>29 (26%)</td>
<td>21 (19%)</td>
<td>17 (18%)</td>
<td>12 (15%)</td>
<td>11 (19%)</td>
<td>5 (21%)</td>
</tr>
<tr>
<td>Inflammatory infiltrate</td>
<td>81%</td>
<td>94 (82%)</td>
<td>100 (89%)</td>
<td>85 (77%)</td>
<td>80 (83%)</td>
<td>61 (75%)</td>
<td>44 (77%)</td>
<td>22 (91%)</td>
</tr>
<tr>
<td>Cholestasis</td>
<td>12%</td>
<td>25 (22%)</td>
<td>15 (13%)</td>
<td>15 (14%)</td>
<td>10 (10%)</td>
<td>6 (7%)</td>
<td>1 (2%)</td>
<td>1 (4%)</td>
</tr>
<tr>
<td>Interface hepatitis</td>
<td>17%</td>
<td>18 (16%)</td>
<td>18 (16%)</td>
<td>19 (17%)</td>
<td>21 (22%)</td>
<td>11 (14%)</td>
<td>11 (19%)</td>
<td>2 (8%)</td>
</tr>
</tbody>
</table>
Results IV- Histological Features associated to fibrosis

PORTAL FIBROSIS
- Unspecific inflammation: 1y p=0.001; 3y p=0.002; 5y p<0.001
- Ductal proliferation: 6mo p<0.001; 1y p=0.002; 5y p=0.003; 7y p=0.02
- Cholestasis: 6mo p=0.007

CENTRILOBULAR FIBROSIS
- Steatosis 5 & 10 y p= 0.04

SINUSOIDAL FIBROSIS
- Steatosis 6 mo;1y & 2 y p<0.001
- Ductal proliferation: 1y p=0.006; 2y p= 0.005; 5y p=0.03

Patients with steatosis did not show waning of it
Cellular drop out & interface hepatitis did not show correlation with fibrosis location
Steroid therapy was not associated with reduced fibrosis in this population.

**Results IV: Immunosuppression-Fibrosis evolution over time**

**Steroids vs Steroids-free patients**

- **Steroids at LT**
  - 21 (87%) kept on ST 1 year
    - x dose: 0.25 ± 0.1 mg/kg
  - 13 (54%) kept on ST 2 years
    - x dose: 0.11 ± 0.1 mg/kg

- 13 (43%) Further ST for AR
  - x dose: 0.3 ± 0.1 mg/kg

**N=54**

**N=139**

- **STEROIDS** 97 (70%)
- **STEROIDS-free** 42 (30%)

**PSR%**

- 37 vs 17
  - [p=0.8]

- [p=0.2]

Dynamics of liver allograft fibrosis.
Results IV  Immunosuppression-Fibrosis according to Prope-Tolerance status

<table>
<thead>
<tr>
<th>Period</th>
<th>6 mo.</th>
<th>1 y</th>
<th>2 y</th>
<th>3 y</th>
<th>5 y</th>
<th>7 y</th>
<th>10 y</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>122</td>
<td>115</td>
<td>110</td>
<td>96</td>
<td>81</td>
<td>57</td>
<td>24</td>
</tr>
<tr>
<td>PROPE T</td>
<td>------</td>
<td>13 (11%)</td>
<td>26 (24%)</td>
<td>44 (46%)</td>
<td>36 (44%)</td>
<td>39 (68%)</td>
<td>17 (71%)</td>
</tr>
<tr>
<td>NO PROPE T</td>
<td>122 (100%)</td>
<td>98 (89%)</td>
<td>84 (76%)</td>
<td>52 (54%)</td>
<td>45 (56%)</td>
<td>18 (32%)</td>
<td>7 (29%)</td>
</tr>
</tbody>
</table>

Prope-tolerance did not contribute to increase fibrosis
Discussion & Future Perspectives
Pediatric liver allograft fibrosis could be seen as a dynamic process with gradual progression over time.

Fibrosis progression does not mean abnormal liver function, irreversible cirrhosis or re-transplant indication.

LAFSc identified fibrosis at portal, centrilobular and sinusoidal areas, being the most accurate score for evaluating allograft fibrosis.

Fibrosis placed at specific areas of the liver parenchyma could be related to clinical complications or transplant events.
To date, the non-invasive methods for fibrosis assessment have been unable to replace LB.

The steroids could not prevent fibrosis development.

No evidence of higher fibrosis was found in patients with low immunosuppression.

A high proportion of activated-HSCs found at early stages of LT seems to be a risk factor for early and long-term fibrosis development.
**Future Perspectives**

- Pediatric liver allograft fibrosis need to be categorized by an accurate method specifically designed to stage allograft fibrosis.

- Centralized studies are needed to confirm pediatric allograft fibrosis evolution.

- Studies evaluating the antifibrogenic properties of IS are mandatory, to adequate the treatment to fibrosis stage.

- To develop accurate non-invasive tools for fibrosis assessment to avoid the liver biopsy.
Thanks for your attention