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2017 BANFF-SCT
Joint Scientific Meeting
BARCELONA
27-31 March 2017

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

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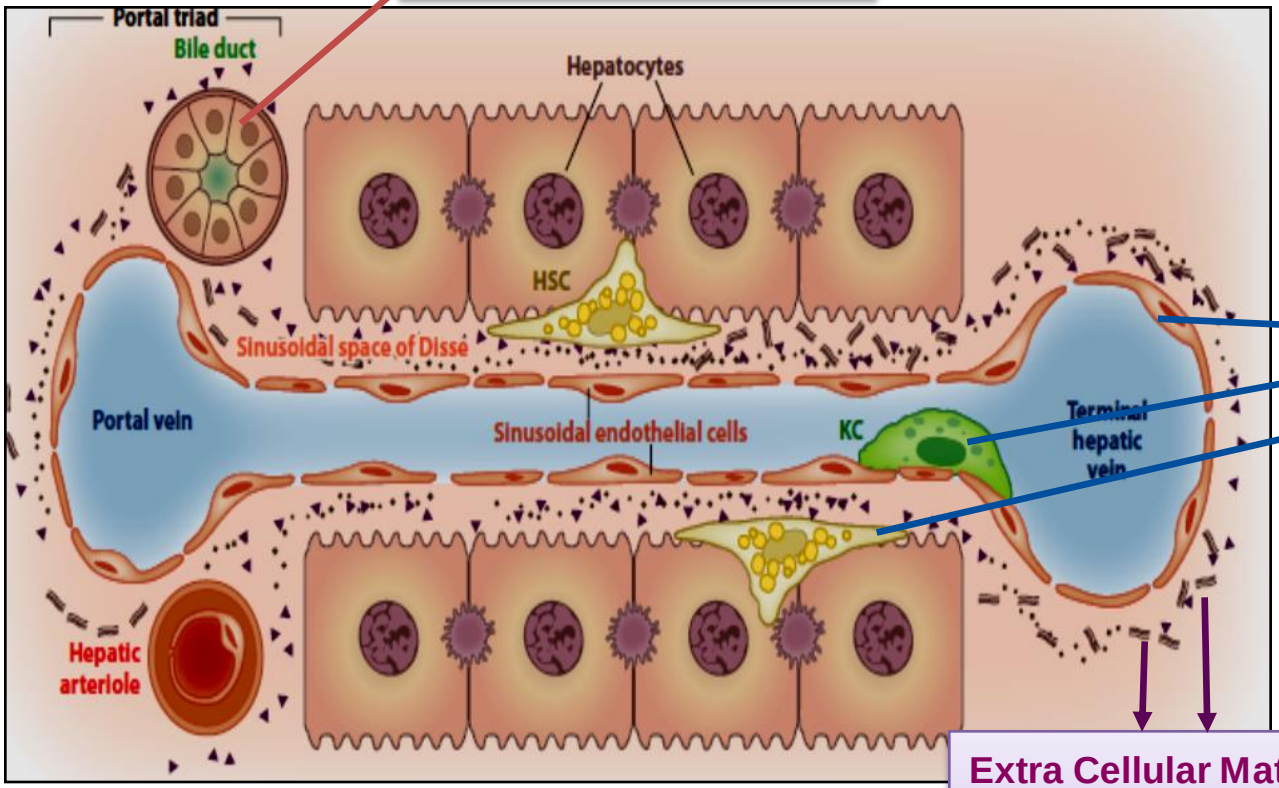
# THE NORMAL LIVER ARCHITECTURE

**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

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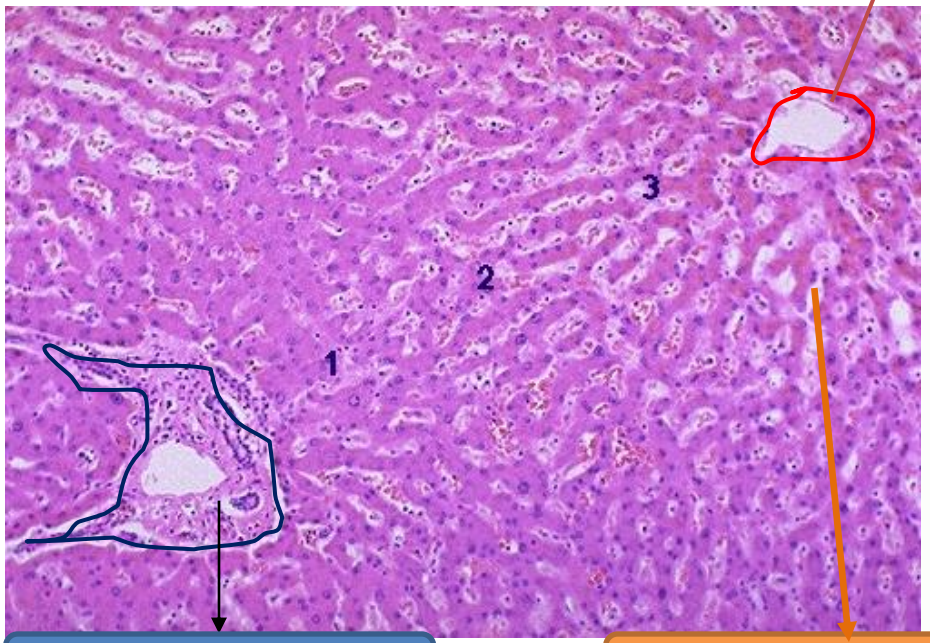
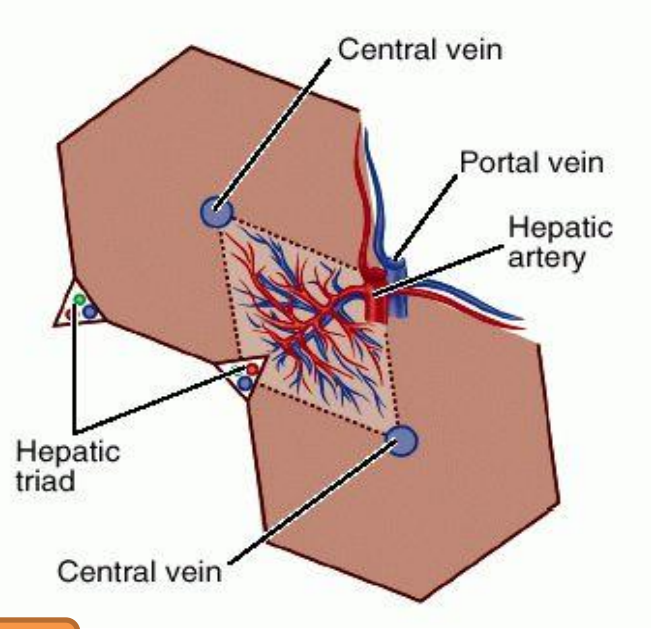
# THE NORMAL LIVER ARCHITECTURE

Central Vein

Portal Flow Distribution




Portal Triad

Sinusoids

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

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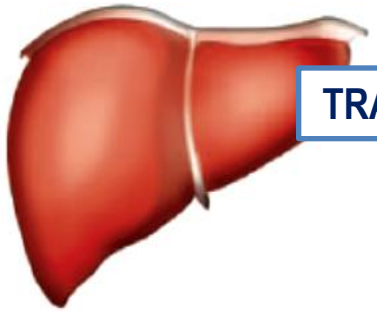
**PERSISTENT INFLAMMATORY CONDITIONS**
**Infections-Rejection- biliary / vascular complications- steatohepatitis**



**TRANSPLANTED LIVER**

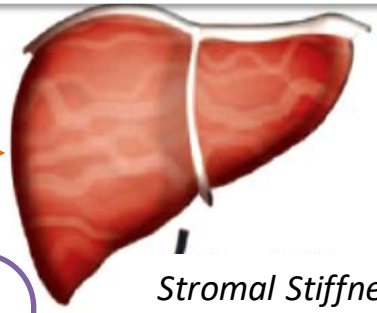
**ACTIVATE IMMUNE RESPONSE**

CHRONIC INFLAMMATION

**ALLOGRAFT FIBROSIS & CIRRHOSIS**

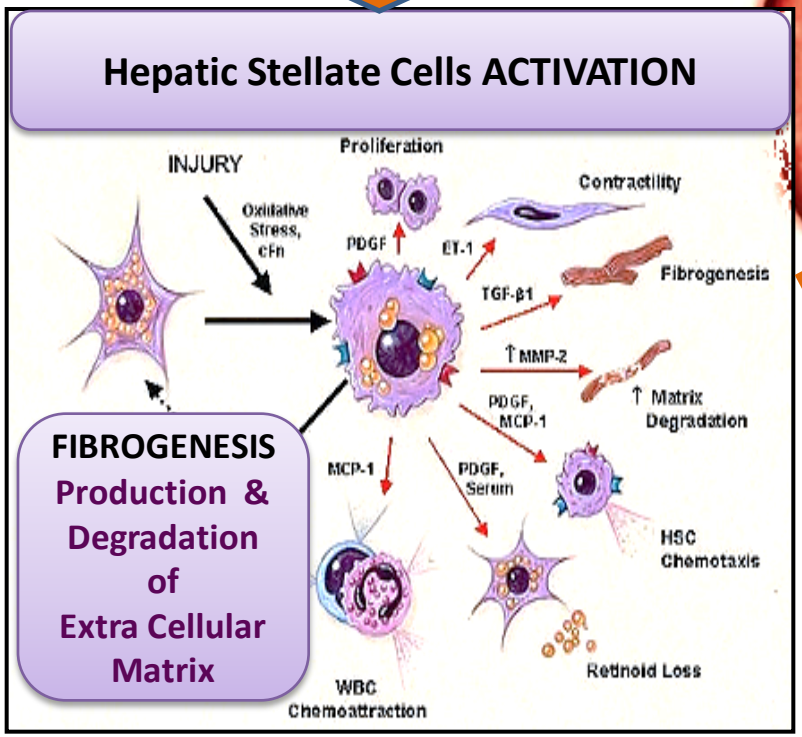
*Activated HSCs*

*Myofibroblast phenotype*



*Stromal Stiffness*

**Hepatic Stellate Cells ACTIVATION**




**FIBROGENESIS**
**Production & Degradation of Extra Cellular Matrix**

**PERPETUATION OF PRO-FIBROGENIC STATUS**

**EXTRA CELLULAR MATRIX ACCUMULATION**

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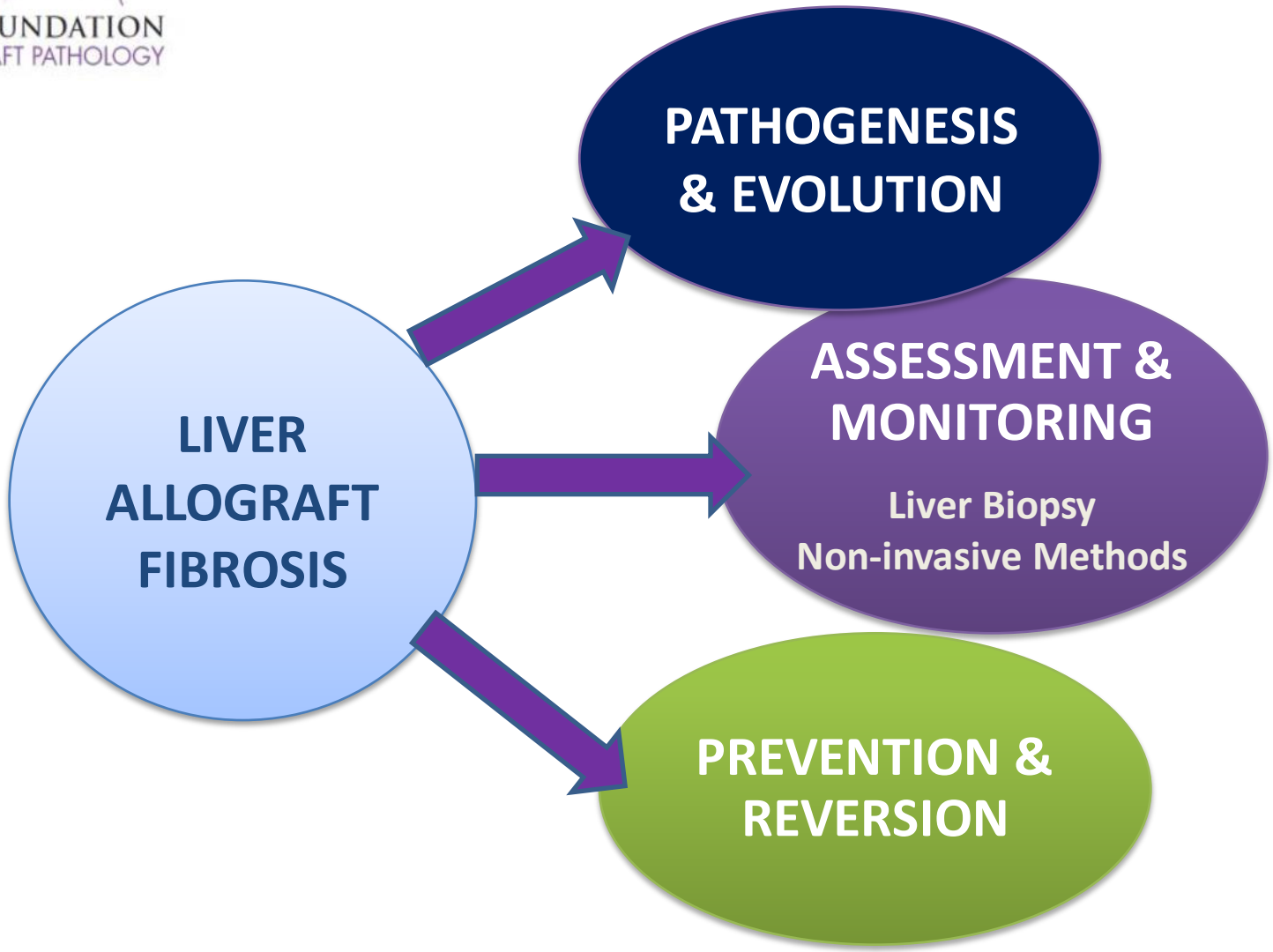
# FIBROSIS IN PEDIATRIC LIVER TRANSPLANTATION



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# FIBROSIS IN PEDIATRIC LIVER TRANSPLANTATION

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

| Reference | Center | Number of Biopsies | Time After LT | Abnormal Histology | Main Histological Diagnoses |
|---|---|---|---|---|---|
| Fouquet et al.[9] (2005) | Paris | 67 | >10 years | 73% | Chronic rejection (42%), centrilobular fibrosis (22%), biliary cirrhosis (4%), other (4%) |
| Evans et al.[3] (2006) | Birmingham | 113, 135, 164 | 1, 5, 10 years | 32% at 1 year, 55% at 5 years, 69% at 10 years | Chronic hepatitis ± fibrosis (64%), biliary fibrosis (2%), recurrent PSC (2%), other (2%)—at 10 years |
| Ekong et al.[6] (2008) | Chicago | 63 | >3 years | 97% | Fibrosis (97%), inflammation (70%) |
| Scheenstra et al.[4] (2009) | Groningen | 77, 64, 66, 55 | 1, 3, 5, 10 years | 34% at 1 year, 48% at 3 years, 65% at 5 years, 69% at 10 years | Fibrosis (69%)—at 10 years |
| Ueno et al.[5] (2011) | Osaka | 24 | >1 year | >71% | Fibrosis (71%), inflammation (58%) |
| Miyagawa-Hayashino et al.[14] (2012) | Kyoto | 67 | >5 years | >84% | Fibrosis (84%), inflammation (58%) |
| Venturi et al.[7] (2012) | Brussels | 38 | 7 years | 94% | Fibrosis (94%), inflammation (74%), ductal proliferation (26%), steatosis (26%) |
| Tomita et al.[8] (2013) | Tokyo | 59 | 0.2-15 years (median, 6 years) | >86% | Fibrosis (86%), inflammation (39%), steatosis (10%) |
| Kosola et al.[11] (2013) | Helsinki | 54 | >3 years | >43% | Steatosis (43%), ductular reaction (43%), fibrosis (39%), inflammation (22%) |
| Briem-Richter et al.[10] (2013) | Hamburg | 60 | >1 year | 40% | Fibrosis (33%), mild acute rejection (20%), steatosis (17%), early chronic rejection (3%) |
| Dattani et al.[12] (2014) | King's College Hospital, London | 56 | >1 year | 84% | Hepatitis (41%), bridging fibrosis/cirrhosis (27%), NRH (16%), biliary problem (12.5%), rejection (4%), other (11%)* |
| Sanada et al.[13] (2014) | Tochigi | 89, 55 | 2 and 5 years | >42% | Inflammation (42%), fibrosis (34.5%)—at 5 years |

**-Evolutive process? Patient predisposing condition? Could be related to post-transplant persistent injuries?**

# FIBROSIS IN PEDIATRIC LIVER TRANSPLANTATION

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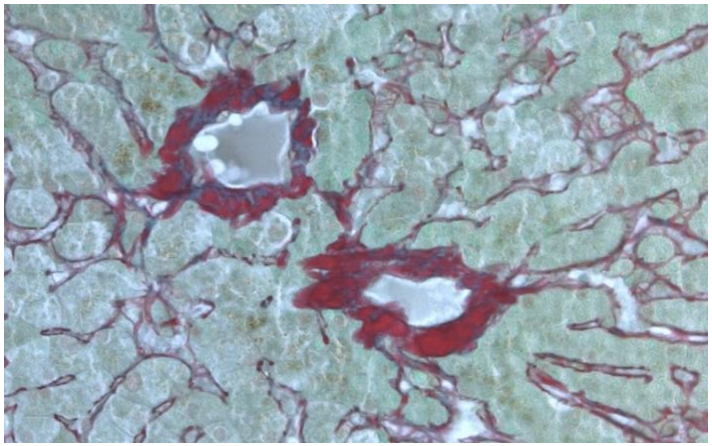
## ASSESSMENT & MONITORING

Invasive Approach-Liver Biopsy-**"GOLD STANDARD"**

**QUANTITATIVE**

**MORPHOMETRIC ANALYSIS**

Quantify the fibrosis area found in the liver biopsy specimen stained by PicroSirius-Red.



**PicroSirious-Red**

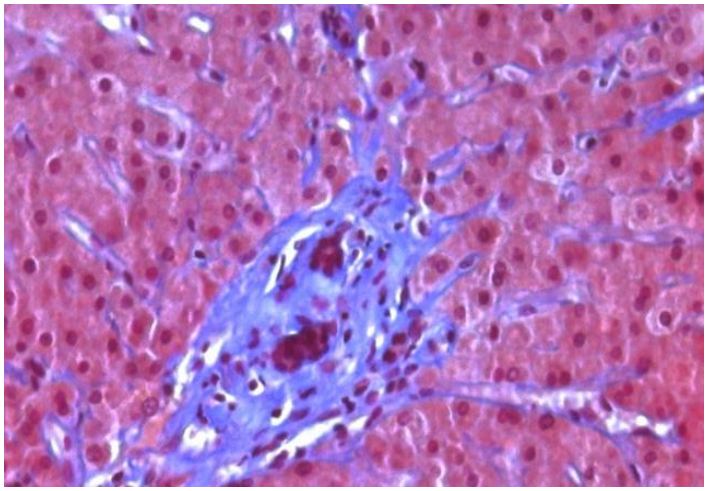
**Collagen I-II-III**

**Counterstained**

**SEMIQUANTITATIVE**

**HISTOLOGIC SCORING SYSTEMS**

Pathologists review the liver biopsy classifying fibrosis in mild moderate or severe according the scores



**Masson´s Trichrome**

**Collagen**

**Nuclei**

**Cytoplasm**

**INTRODUCTION**

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# Fibrosis Semiquantitative Assessment
## Histological Scoring Systems

**DESIGNED TO STAGE CHRONIC HEPATITIS NO FOR TRANSPLANTED LIVERS**

**ONLY REFERENCIATE PORTAL FIBROSIS**

| SCHEUER system (1991) | | |
|---|---|---|
| Combines Necroinflammation and Fibrosis grade 0-4 | | |
| Portal inflammation & necrosis | Lobular inflamma ecrosis | Portal Fibrosis |

| METAVIR (1994) | |
|---|---|
| Combines piecemeal and lobular nec ith inflammation and fibrosis | |
| Activity & Necroinflammation A 0 -3 | Portal Fibrosis 0-4 |

| HAK system (1995) | |
|---|---|
| Periportal or periseptal i nepatitis 0-4 | Confluent necrosis 0-6 |
| Focal (spotty) lytic , apoptosis and focal infla 0-4 | Portal inflammation 0-4 |
| Portal and B g Fibrosis 0-6 | |

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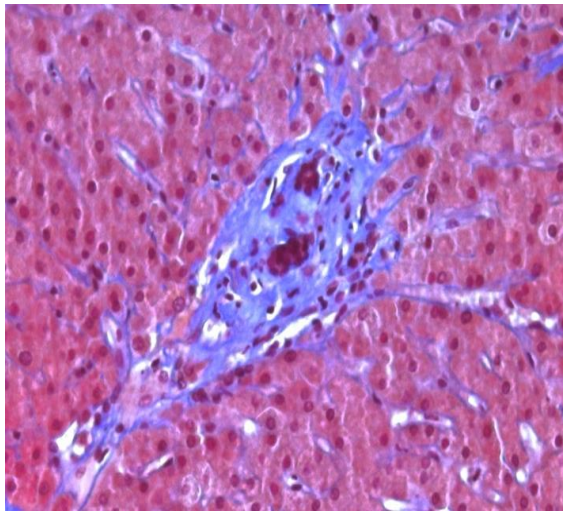
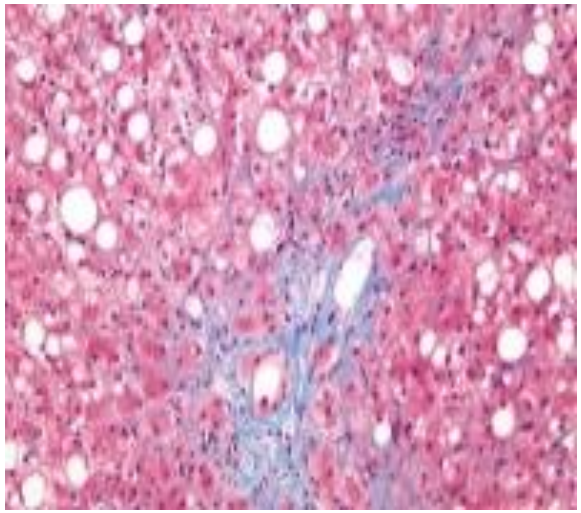
# LIVER BIOPSY
## Fibrosis at the Three Main Areas of the Liver Parenchyma

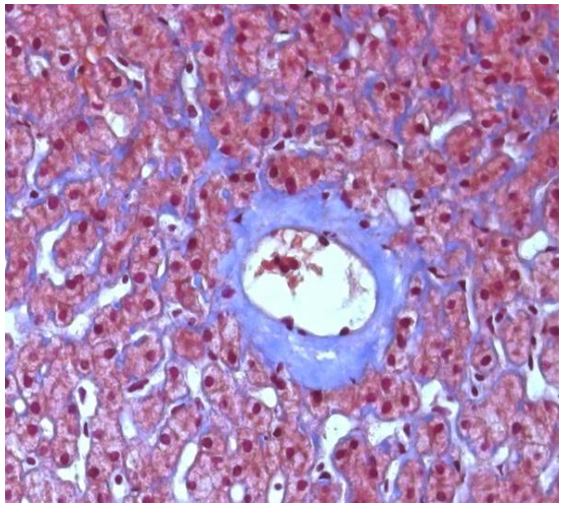
**Portal Fibrosis**



**Sinusoidal fibrosis**



**Centrilobullar fibrosis**



**Conventional systems used to stage fibrosis in the native liver fail to recognize these patterns of graft fibrosis.**

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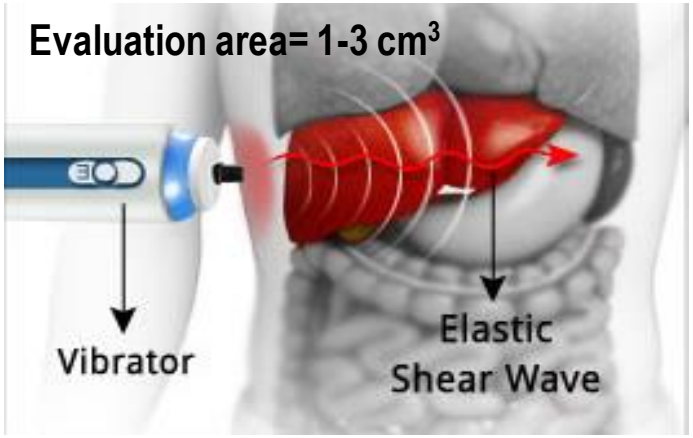
# FIBROSIS IN PEDIATRIC LIVER TRANSPLANTATION

## ASSESSMENT & MONITORING

### Non-Invasive Approach

### Hepatic Imaging

**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.

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# 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**

**ELF panel***

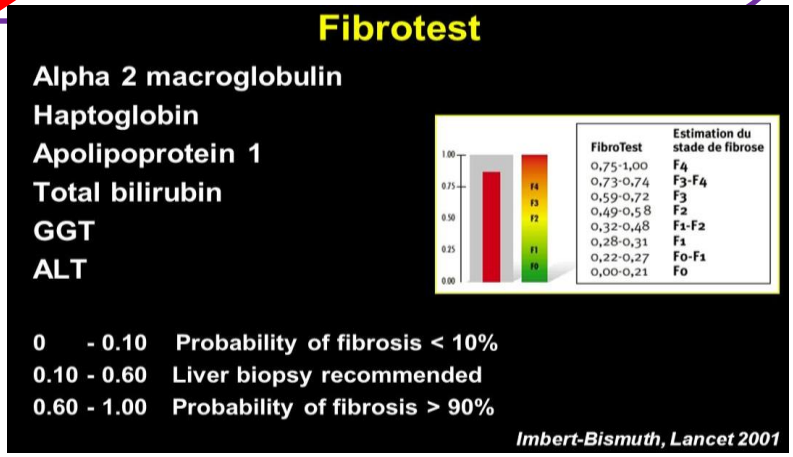
**HA**: **appeared to be a fair predictor of liver allograft fibrosis** (Hartley [illegible] GN 2006;43 217-21)

**ELF** panel***: accurate in pediatric NAFLD (AUROC 0.92); no correla[illegible]he degree of pediatric allograft fibrosis.** (Goldschmidt I, et al Ped Transpl. 2013; 17:525-34)

**Could the serum markers replace Liver Biopsy?**

**MicroRNAs:** Intrahepatic microRNAs predictive of inflammation, reje[illegible] proliferation.(need LB)

**Markers of cell Death:** [illegible]nsitive marker of fibrosis i[illegible]AFLD.



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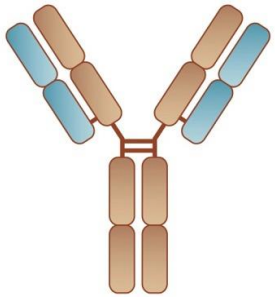
# FIBROSIS IN PEDIATRIC LIVER TRANSPLANTATION

## ASSESSMENT & MONITORING

### Non-Invasive Approach

### Immunological investigation



**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

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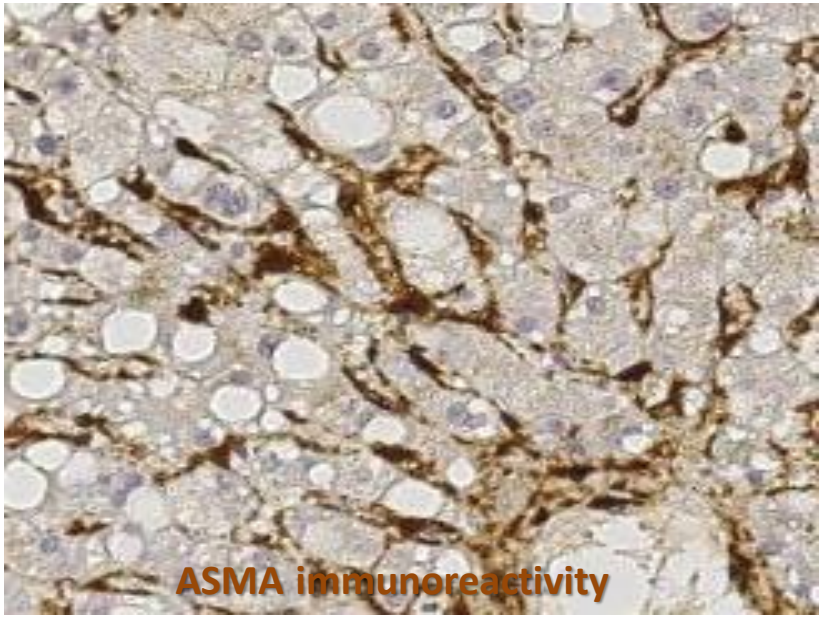
# FIBROSIS IN PEDIATRIC LIVER TRANSPLANTATION

**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?**

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# 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**

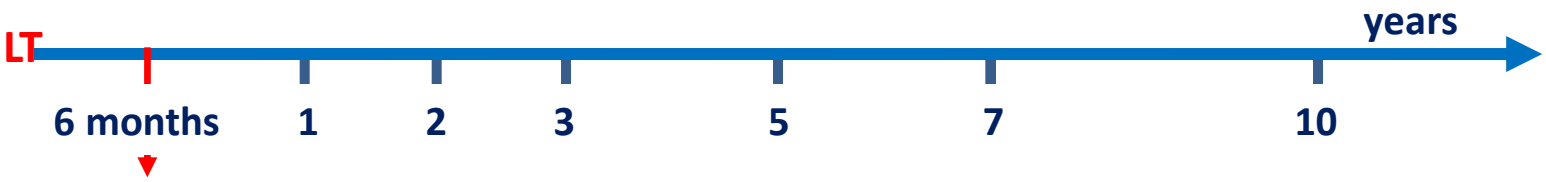
**Exclusion Criteria**: Re-transplantation; inadequate LB; incomplete follow-up(< 3 LB) = **31**

Clinical -Biochemical & Serologic Assessment

| Pre-LT factors: | Post-LT factors: |
|---|---|
| Donor Age | Vascular and biliary complications |
| Donor type | Infections (0-6 months) |
| Ischemia Time | Autoantibodies & gammaglobulins % |
| Recipient age-gender-weight | History of Post-transplant |
| height- blood pressure | lymphoproliferative disease |
| Liver Transplant indication | |
| CMV - EBV status | |

Available data of Doppler ultrasound- TE

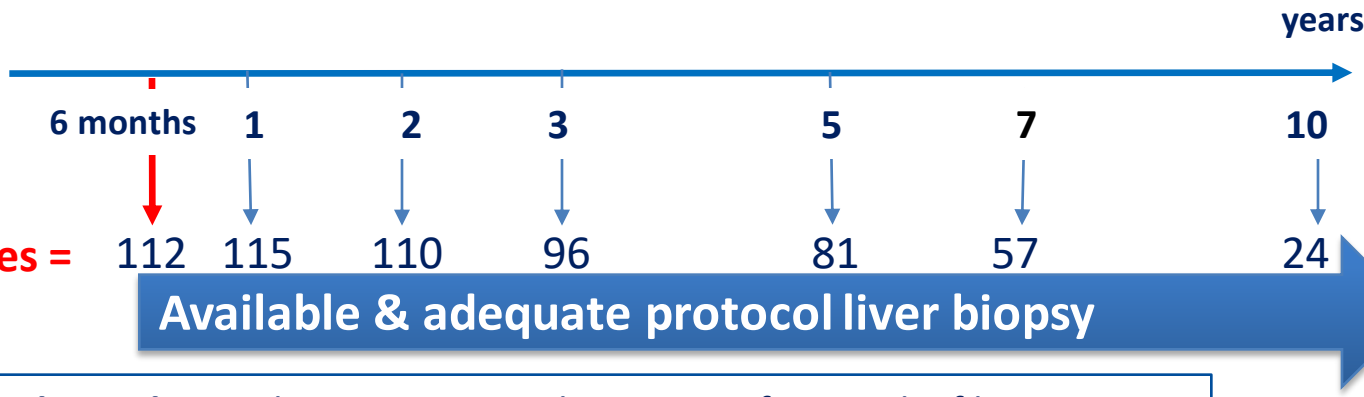
**Adequate and available protocol liver biopsy**



# Patients & Methods- Histologic Assessment

**170** Pediatric LT recipients - **31** Excluded

**139 patients**




**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)

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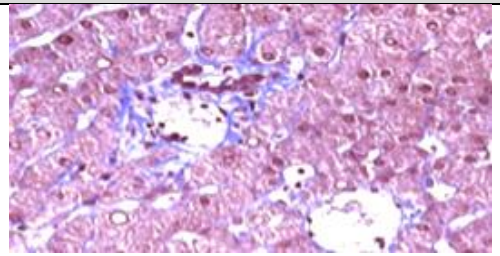
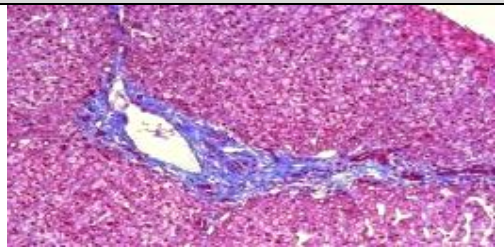
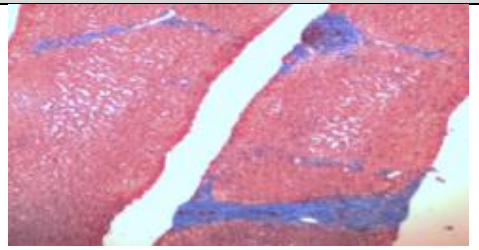
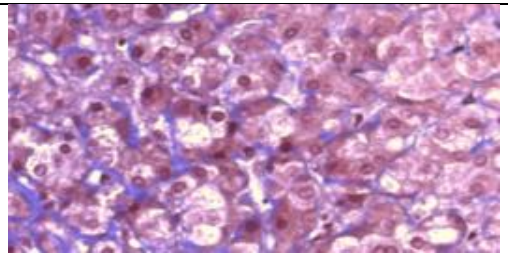
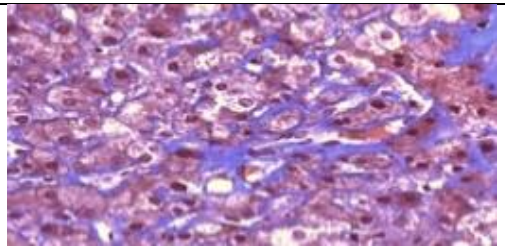
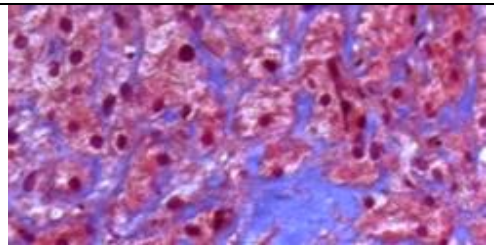
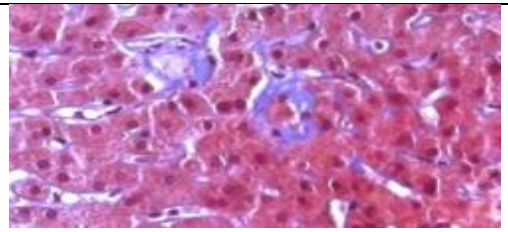
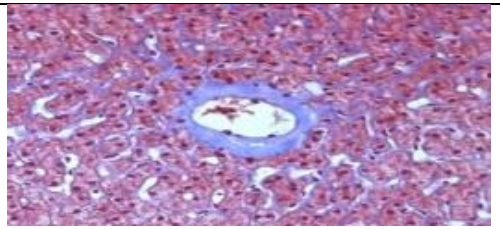
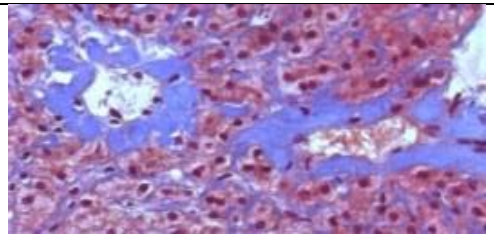
# *Design of a new histological fibrosis scoring*

# *Liver Allograft Fibrosis Score (LAFSc)*

*American Journal of Transplantation 2012; 12: 2986–2996*

# Histologic Features and Staging definitions of the Liver Allograft Fibrosis Score= 0-9 (LAFSc)

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

American Journal of Transplantation 2012; 12: 2986–2996

Design of a new histologic fibrosis scoring

# Patients & Methods

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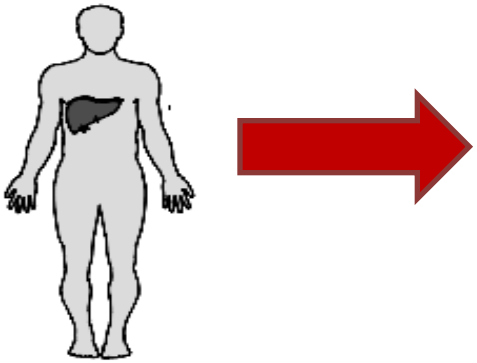
**POPULATION INCLUDED**

**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**

**38 patients/ 76 LB**

| Demographic Data | | |
|---|---|---|
| | Median LT- age (yrs) | 1.6 (r: 0.4 - 14) |
| 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**

American Journal of Transplantation 2012; 12: 2986–2996

Design of a new histologic fibrosis scoring

# Patients & Methods- Histologic Assessment


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

American Journal of Transplantation 2012; 12: 2986–2996

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# Results I

## Fibrosis staged by LAFSc- METAVIR & Ishak systems

| | 6 MONTHS | 7 YEARS |
|---|---|---|
| METAVIR F0–F4 | F0: 10 (26.3%); F1–F2: 28 (73.6%) F3: 0 | F0: 4(10.5%); F1–F2: 31 (81.5%) F3:3 (7.9%) |
| Ishak F0–F6 | F0: 11(28.9%); F1–F2: 15 (39.4%) F3–F4: 12 (31.5%) | F0: 2(5.2%); F1–F2: 11(28.9%) F3–F4: 20(52.6%); F5: 6 (15.7%) |
| LAFSc F0–F9 | 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 | 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 |

## Correlation among morphometric analysis with LAFSc- METAVIR & Ishak

| Spearman Correlation | LAFSc | METAVIR | Ishak |
|---|---|---|---|
| Morphometry (rho; p value) | 0.731** $p < 0.000$ | 0.571** $p < 0.000$ | 0.566** $p < 0.000$ |
| Ishak (rho; p value) | 0.759** $p < 0.000$ | 0.940** $p < 0.000$ | |
| METAVIR (rho; p value) | 0.739** $p < 0.000$ | | |

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

*American Journal of Transplantation 2012; 12: 2986–2996*

Design of a new histologic fibrosis scoring

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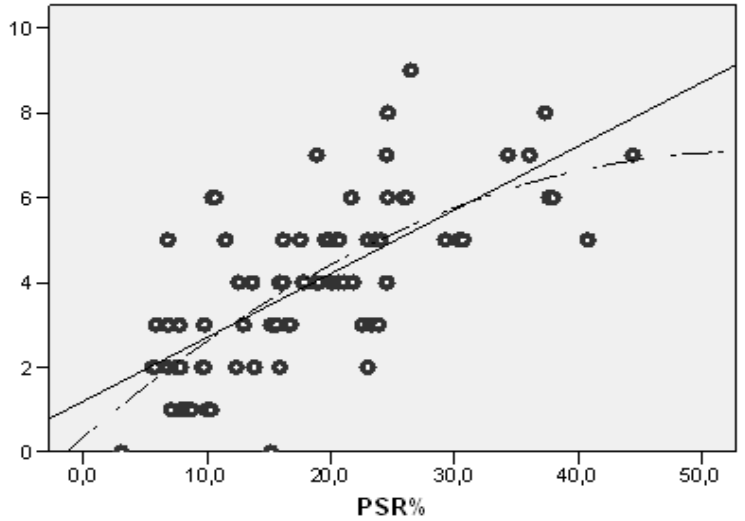
# Results I

## Correlation between collagen deposits (morphometric analysis) & LAFSc

| Equation | R2 | F | gl1 | gl2 | p= |
|---|---|---|---|---|---|
| Linear Regression | 0.493 | 73.78 | 1 | 76 | 0.000 |
| Quadratic Regression | 0.508 | 38.78 | 2 | 75 | 0.000 |




## 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

*American Journal of Transplantation 2012; 12: 2986–2996*

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# Results I

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

| | | Invasive methods | | | |
|---|---|---|---|---|---|
| Noninvasive methods | | PSR% | LAFSc | METAVIR | Ishak |
| TE (FibroScan®) | rho | –0.126 | –0.225 | 0.132 | 0.036 |
| | p value | p = 0.47 | p = 0.19 | p = 0.44 | p = 0.83 |
| APRI | rho | –0.155 | –0.245 | –0.308 | 0.168 |
| | p values | p = 0.36 | p = 0.14 | p = 0.06 | p = 0.34 |

TE = transient elastography; APRI index = (AST × upper normal limit) × 100/platelet count ($10^9$/L).

**No correlation was found among TE or APRI index with morphometric analysis, METAVIR, Ishak & LAFSc**

*American Journal of Transplantation 2012; 12: 2986–2996*

Design of a new histological fibrosis scoring

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# *Dynamics Of Allograft Fibrosis In Pediatric Liver Transplantation*

*American Journal of Transplantation 2014; 14: 1648–1656*

# Patients & Methods

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**POPULATION INCLUDED**

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

**139** Pediatric LT receptors

**54**

*Protocol liver biopsies- long-term follow-up*

1 2 5 10 years

**54** LB 6 | **54** LB 3 years | **54** LB 7 years

| Demographic Data | | |
|---|---|---|
| | Median age at LT (years, range) | 1.28 (0.2-15.7) |
| | Median Weight at LT (kg, range) | 7.66 (3.8-53.7) |
| Liver Transplant Indication | Biliary Atresia | 30 (55%) |
| | P.I.F. Cholestasis | 9 (16%) |
| | Metabolic diseases | 8 (15%) |
| | Tumors | 5 (9%) |
| | Alagille Syndrome | 2 (4%) |
| Donor Type | Living Related Donor/ Deceased Donor (n,%) | 29 (53%) - 25 (47%) |
| | Median donor age (years, range) | 30.1 (0.4- 50.3) |
| | Median Ischemia time (minutes, range) | 169.5 (68- 892) |
| Immunosuppression received at LT | TAC+ Steroids | 24 (44%) |
| | TAC+ Basiliximab | 23 (43%) |
| | TAC monotherapy | 7 (13%) |

*American Journal of Transplantation 2014; 14: 1648–1656*

Dynamics of liver allograft fibrosis

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# Patients & Methods

American Journal of Transplantation 2014; 14: 1648–1656

**54** Pediatric LT receptors

*Protocol liver biopsies*

1 — 2 — 5 — 10 years

- **54** LB 6 months
- **54** LB 3 years
- **54** LB 7 years

**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 162 LB**

- -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.

Dynamics of liver allograft fibrosis

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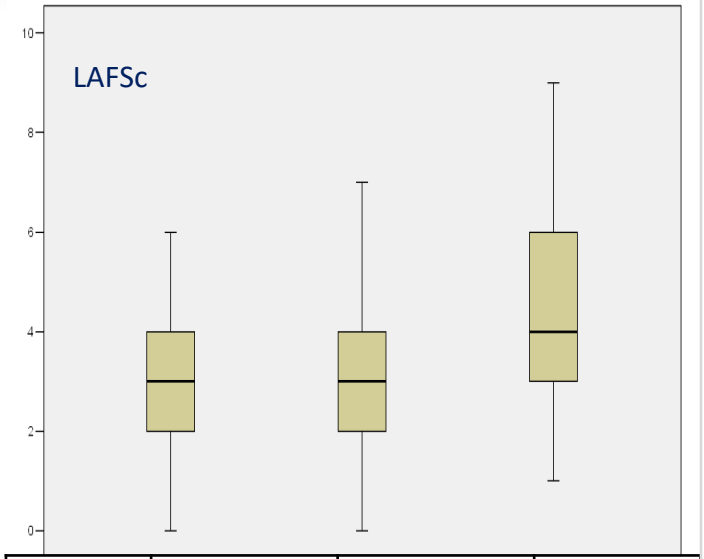
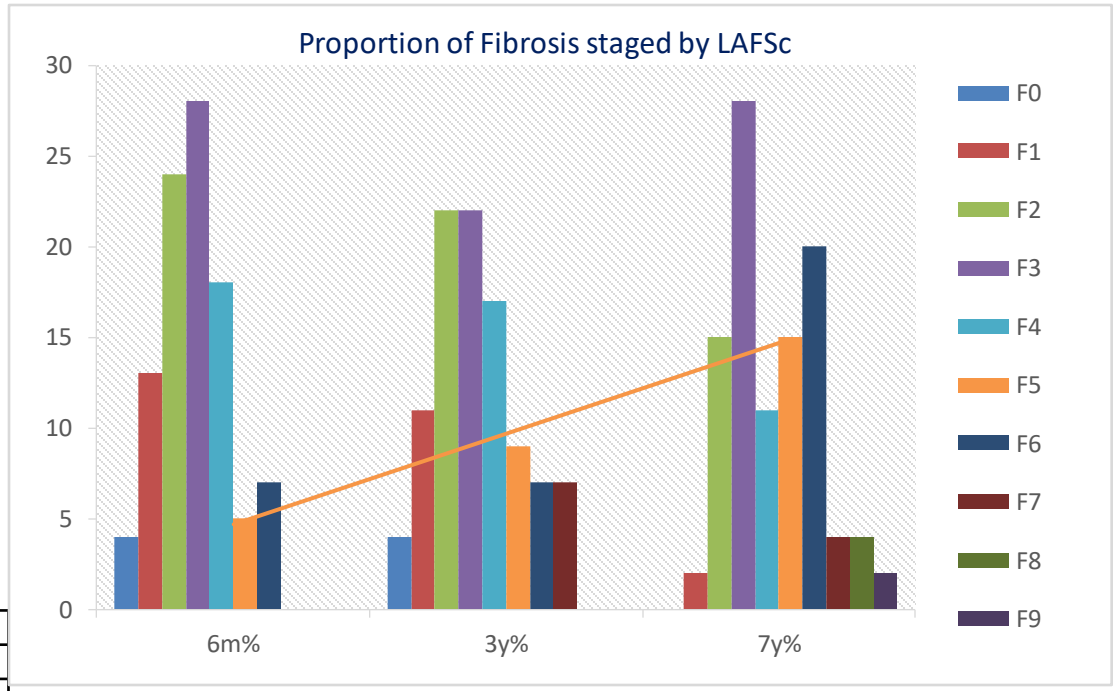
# Results II- Histologic Assessment of Allograft Fibrosis




| LAFSc | 6 months | 3 years | 7 years |
|---|---|---|---|
| X±SD | 2.9 (0.5) | 3.3( 1.8) | 4.3 (1.8) |
| IC 95% | 1.2- 1.8 | 1.5- 2.1 | 1.6- 2.1 |




**Fibrosis progressed along the time in 40 (74%) patients.**

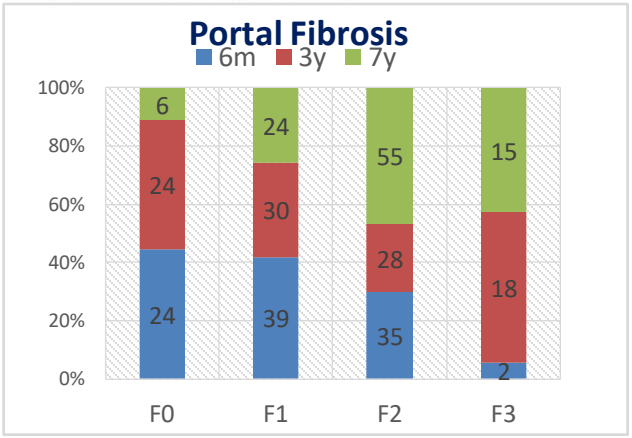
**Stable or reduced fibrosis was found in 14 (26%) patients.**

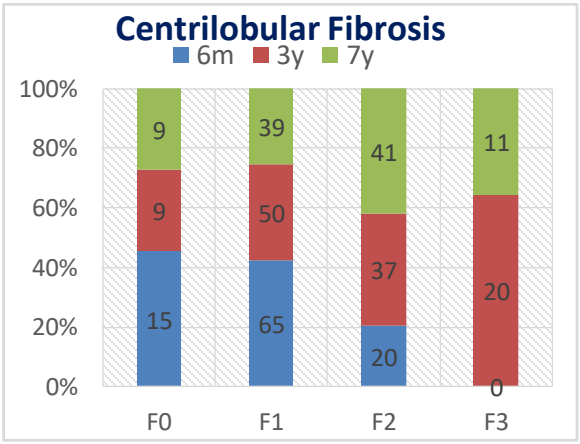
**Patients with increased liver enzymes show similar amount of fibrosis than those with normal liver function**

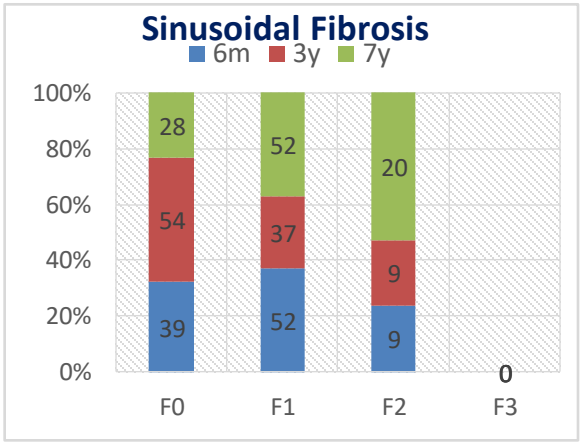
American Journal of Transplantation 2014; 14: 1648–1656

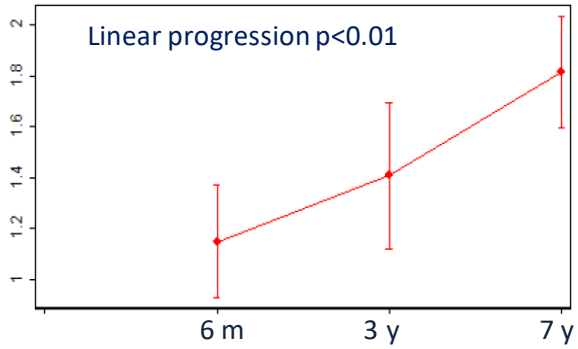
Dynamics of liver allograft fibrosis

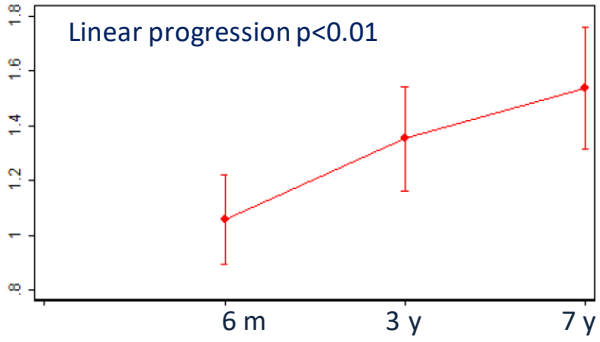
# Results II- Fibrosis evolution at parenchymal areas

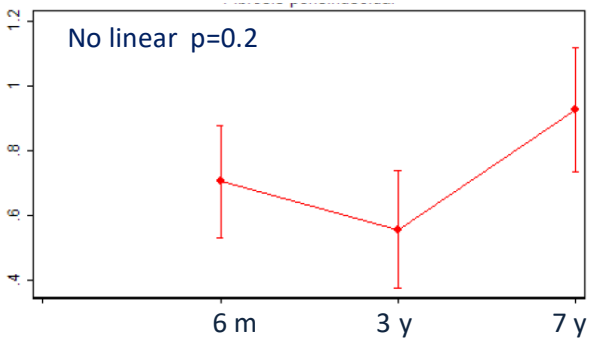




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Dynamics of liver allograft fibrosis

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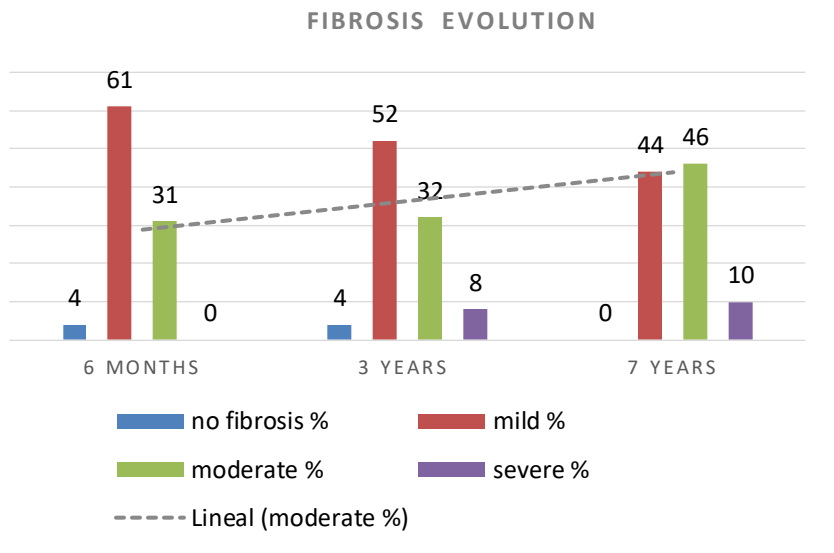
# Results III- Evolution of Fibrosis & Activated-HSCs (ASMA)

## Fibrosis progressed along the time p<0.001

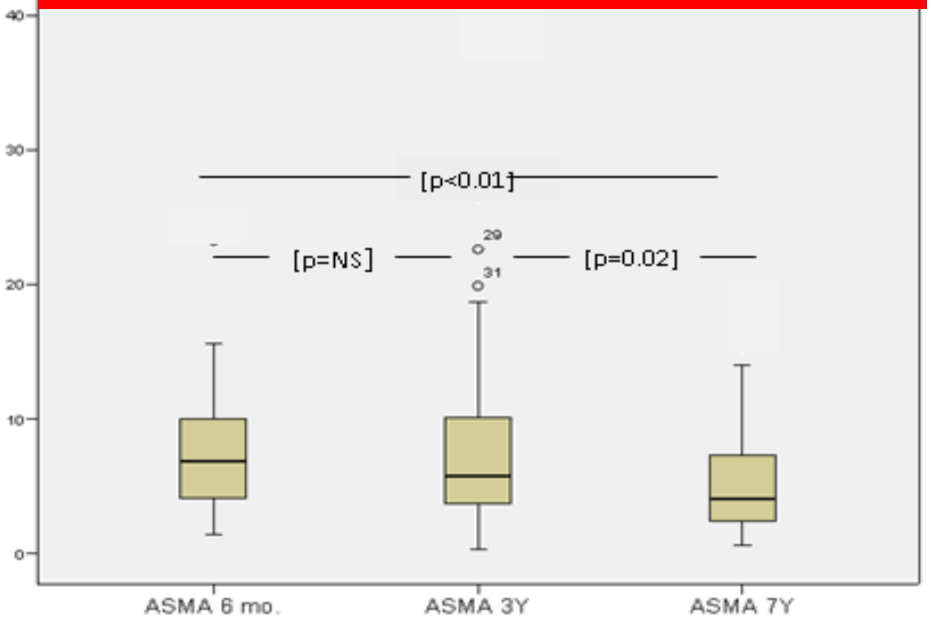



LAFSc : mild = 1- 3; moderate= 4-6; severe = 7- 9

**Increment by areas in the long-term**

| | |
|---|---|
| **Sinusoidal** | **33%** |
| **Centrilobular** | **45%** |
| **Portal** | **57%** |

## Activated HSCs decreased along the time p< 0.01




| | 6 months | 3 years | 7 years |
|---|---|---|---|
| **Median ASMA** | **6.9 (1.5-23.3)** | **5.7 (0.4-38)** | **4.1 (0.7-18)** |
| IC 95% | 6.4- 9.0 | 6.0 - 9.8 | 4.3 - 6.5 |

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

*Liver Transplantation 2016 Jun;22(6):822-9*

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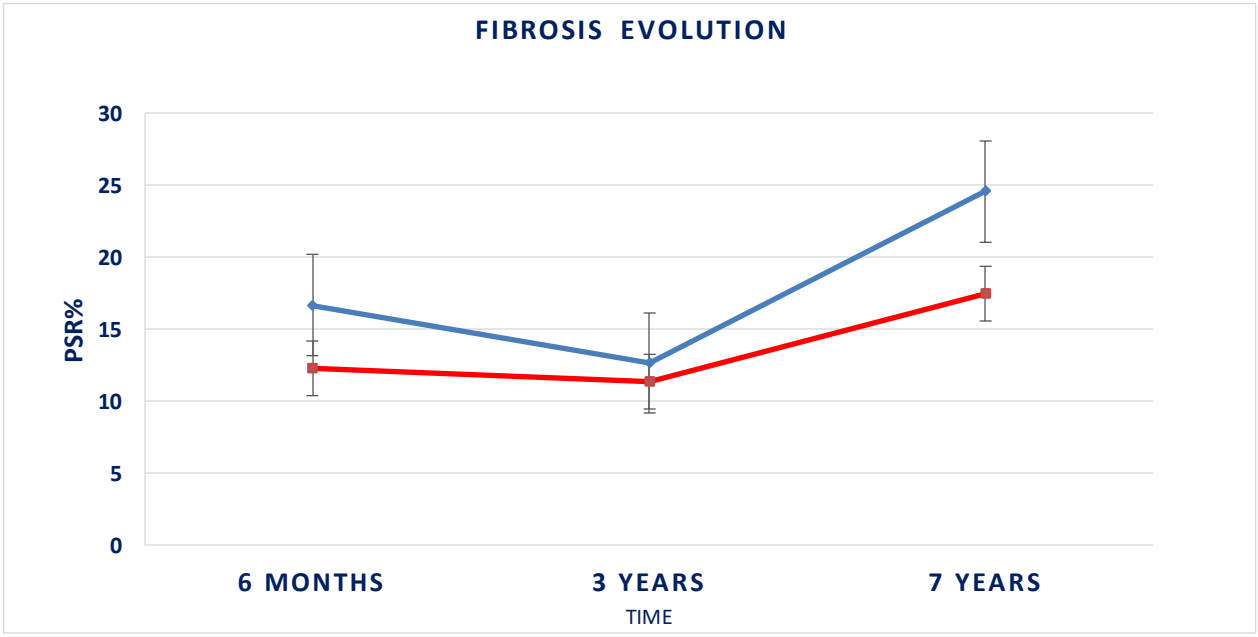
# Results III- Evolution of Fibrosis according to Activated -HSCs at 6m

**Activated-HSCs at 6 months = ≥ 8% = 20 patients**
**= ≤ 8% = 34 patients**




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

| | | |
|---|---|---|
| PSR% | $r^2$ 0.48 | p<0.01 |
| LAFSc | $r^2$ 0.30 | p=0.03 |

Statitistical method: Mixed regression

| | | Fibrosis 6m | Fibrosis 3 y | Fibrosis 7 y | p-value |
|---|---|---|---|---|---|
| **ASMA ≥ 8** | **20** | 16.7 ± 8 | 11.9 ± 7 | 24.6 ± 8 | <0.001 |
| **ASMA ≤ 8** | **34** | 12.3 ± 7 | 11.4 ± 6 | 17.5 ± 7 | = 0.04 |
| p-value | | =0.03 | =0.8 | < 0.01 | |

Note: p-values represent the significance between means

*Liver Transplantation 2016 Jun;22(6):822-9*

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# Results IV Demographic data of the 139 LT recipients

| | |
|---|---|
| Number of patients / Liver biopsies (n) | 139 (69 boys) /595 |
| Median age at LT (years, range, range) | 1.4 (0.2- 16.8) |
| Median Weight at LT (kg, range) | 8.4 (3.7- 63.2) |
| LT Indication: (n, %) Biliary Atresia | 75 (54%) |
| Metabolic diseases | 21 (15%) |
| Progressive Intrahepatic Familial Cholestasis | 17 (12%) |
| Tumors | 11 (8%) |
| Alagille Syndrome | 11 (8%) |
| Others | 4 (4%) |
| Living Related Donor/ Deceased Donor (n, %) | 66 (47 %) / 66 (47%) |
| Split Liver/ Reduced Deceased Donor | 4 (3%) / 3 ( 2.5%) |
| Median donor age (years, range) | 29 (0.4- 56.6) |
| Median Ischemia time (minutes, range) | 232.0 (66- 892) |
| Immunosuppression at LT (n, %):TAC+ Basiliximab | 42 (30%) |
| TAC+ Steroids | 33 (24%) |
| TAC monotherapy | 28 (20%) |
| TAC+MMf+Steroids | 13 (9%) |
| TAC+MMF+Daclizumab | 8 (6%) |
| TAC+Basiliximab+MMf | 6 (4%) |
| TAC+MMf | 6 (4%) |
| TAC+Steroids+Daclizumab | 3 (2%) |

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# Results IV Evolution of clinical variables studied

| | Time of follow-up | | | | | | |
|---|---|---|---|---|---|---|---|
| **Clinical Variables** | **0-6m.** | **1 yrs** | **2 yrs** | **3 yrs** | **5 yrs** | **7 yrs** | **10 yrs** |
| **LB:595/ Patients 139** | **115** | **112** | **110** | **96** | **81** | **57** | **24** |
| Vascular complications | 12 (10%) | 1 (1%) | 2 (2%) | 1 (1%) | 1 (1%) | 1 (2%) | -------- |
| Biliary complications | 19 (16%) | 2 (2%) | 3 (3%) | 1 (1%) | 1 (1%) | ---------- | 1 (4%) |
| Post-LT AA | 26 (23%) | 30 (27%) | 29 (26%) | 15 (15%) | 8 (10%) | 6 (10%) | 3 (12%) |
| AR Steroids treated | 64 (56%) | 8 (7%) | 8 (7%) | 5 (5%) | 4 (5%) | 5 (9%) | --------- |
| PTLD (EBER +) n=28 | 8 (7%) | 9 (8%) | 7 (6%) | 2 (2%) | 1 (1%) | --------- | 1 (4%) |
| Gammaglobulins> 15% | 40 (35%) | 38 (34%) | 48 (44%) | 32 (33%) | 59(73%) | 43 (75%) | 16 (67%) |
| Gammaglobulins (X) | 13.6 | 14.3 | 16.3 | 16.2 | 17.3 | 17.2 | 16.2 |

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

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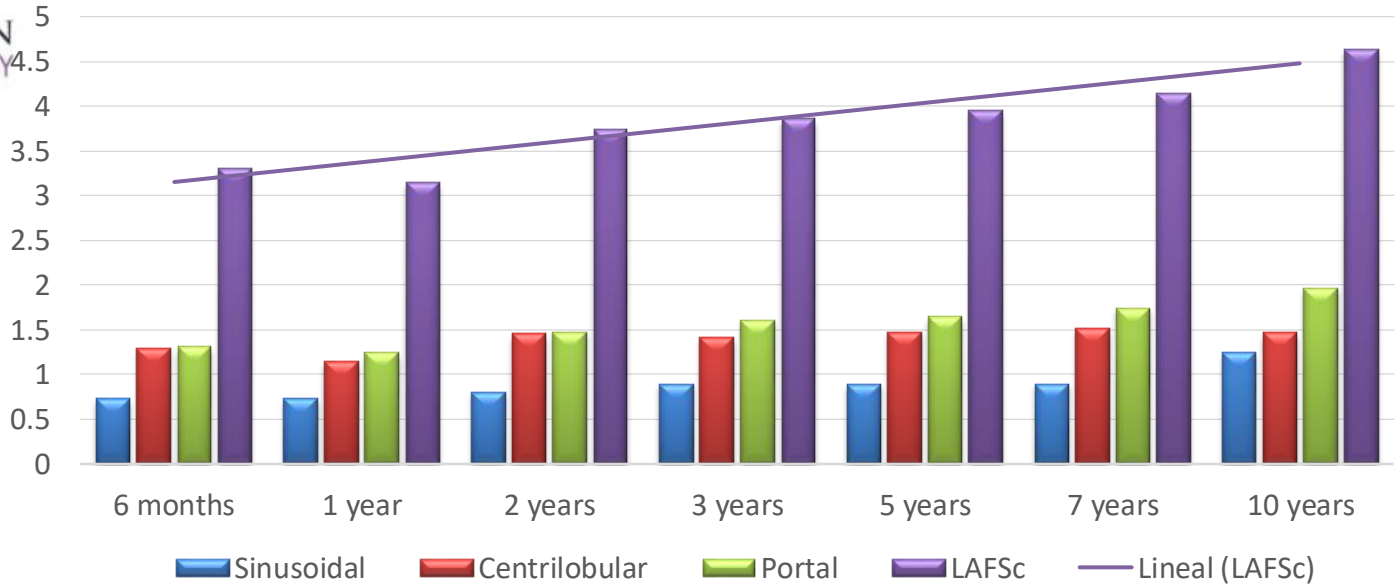
# Results IV Fibrosis evolution over time

**N=139pts.**

**595LB**




| | Fibrosis mean values ± SD | | | | |
|---|---|---|---|---|---|
| **Time** | **Sinusoidal** | **Centrilobular** | **Portal** | **LAFSc** | **N** |
| **6 m** | **0.72 (0.1)** | **1.29 (0.6)** | **1.30 (0.5)** | **3.30 (0.6)** | **115** |
| **1 y** | **0.72 (0.1)** | **1.14 (0.6)** | **1.24 (0.5)** | **3.14 (1.6)** | **112** |
| **2 y** | **0.80 (0.0)** | **1.45 (0.7)** | **1.47 (0.8)** | **3.73 ( 1.7)** | **110** |
| **3 y** | **0.89 (0.4)** | **1.41 ( 0.6)** | **1.60 (0.9)** | **3.85 (1.8)** | **96** |
| **5 y** | **0.89 (0.4)** | **1.47 (0.7)** | **1.64 (0.9)** | **3.94 (1.9)** | **81** |
| **7 y** | **0.89 (0.7)** | **1.51 (0.6)** | **1.74 (0.8)** | **4.14 (1.8)** | **57** |
| **10 y** | **1.21 (0.6)** | **1.46 (0.7)** | **1.96 (0.7)** | **4.63 (1.8)** | **24** |

| Fibrosis Increment by areas over time | | |
|---|---|---|
| **Sinusoidal** | **Centrilobular** | **Portal** |
| **68%** | **13%** | **50%** |

Dynamics of liver allograft fibrosis

# Results IV Fibrosis evolution over time

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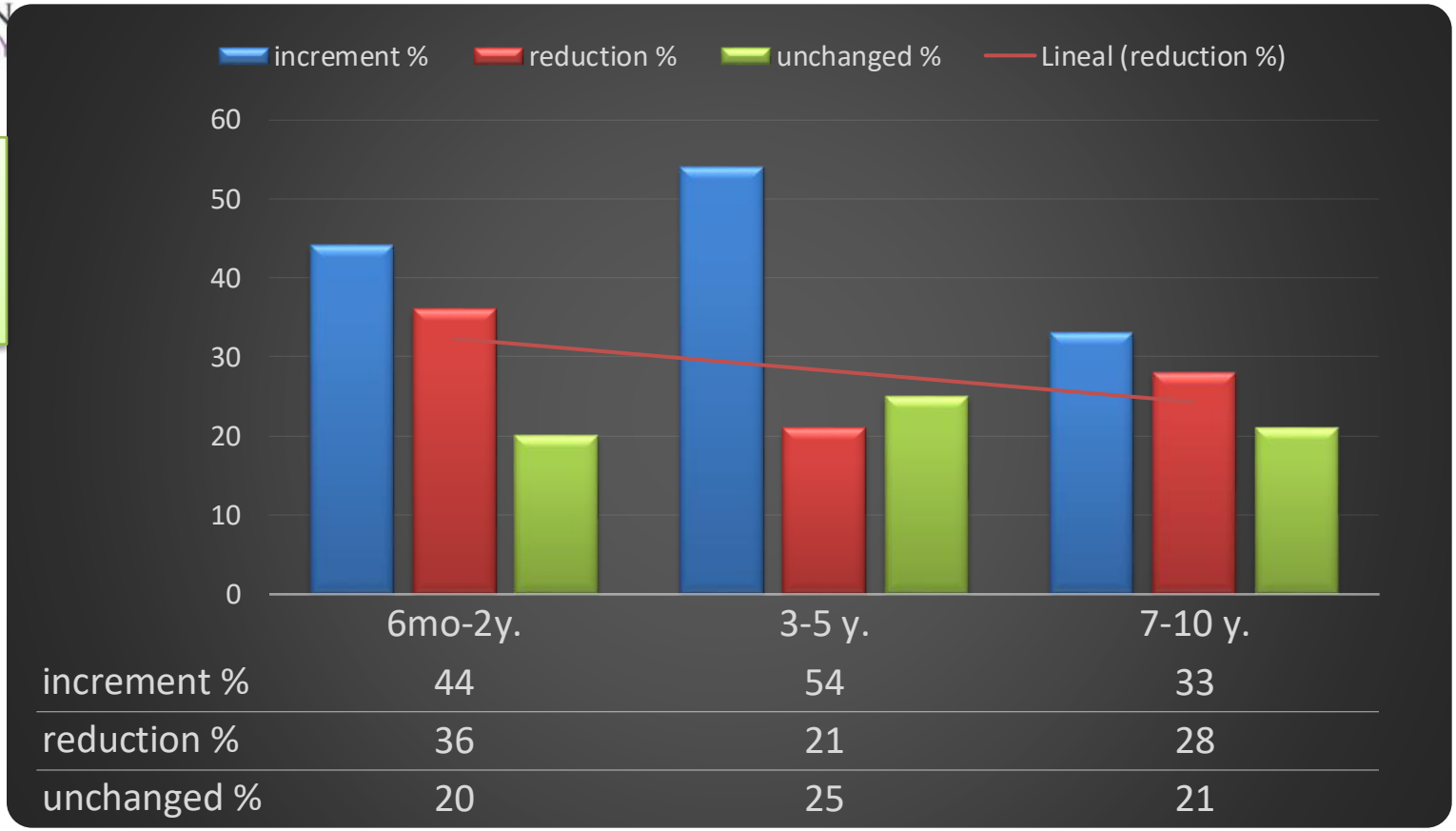
**N=139pts.**

**595LB**




| | 6mo-2y. | 3-5 y. | 7-10 y. |
|---|---|---|---|
| increment % | 44 | 54 | 33 |
| reduction % | 36 | 21 | 28 |
| unchanged % | 20 | 25 | 21 |

**LIVER ALLOGRAFT FIBROSIS IS A DYNAMIC PROCESS**

Dynamics of liver allograft fibrosis

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# Results IV Association between fibrosis & clinical variables

| Clinical Variables | Fibrosis | Fibrosis | Fibrosis Location |
|---|---|---|---|
| | N=54 | N=139 | |
| Deceased donor grafts | p<0.001 (46.3%) | p<0.02 (47.5%) | Portal p=0.001- p=0.003- p=0.01 |
| Lymphoproliferative disease | p=0.001 (18.5%) | p=0.01 (20%) | Portal p=0.01(7y) |
| Ischemia time > 400 min | p<0.01 | p<0.03 | Portal p=0.06 (6m), p<0.01(3y) |
| | | | |
| Vascular complications (0-6m) | p=0.04 (11%) | p=0.04 (10%) | Centrilobular p=0.04 (7y) |
| Gammaglobulins > 15% | p=0.02 | p=0.03 | Centrilobular p=0.02 (7y) |
| Positives AutoAntibodies (>1/40) | p=0.01 | p=0.01 | Centrilobular p=0.01 (3y) |
| | | | |
| Biliary complications 0-6 m | p=0.01 (24%) | p=0.03 (16%) | Sinusoidal p=0.05 (6m) p=0.01 (3y) |
| | | | |
| Male gender | p=0.01 (50%) | p=0.002 (50%) | Sinusoidal p=0.001<br>Centrilobular p=0.04 (7y) |

American Journal of Transplantation 2014; 14: 1648–1656

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# 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)**

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

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# 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

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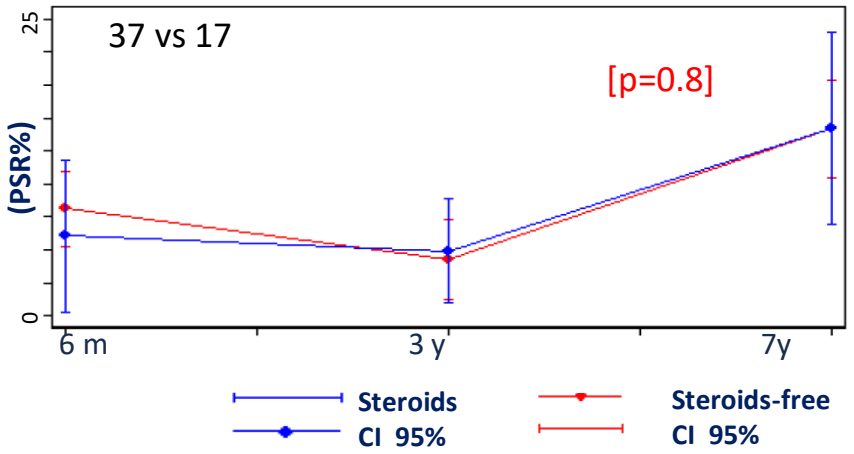
# Results IV Immunosuppression-Fibrosis evolution over time
## Steroids vs Steroids-free patients

**N=54**

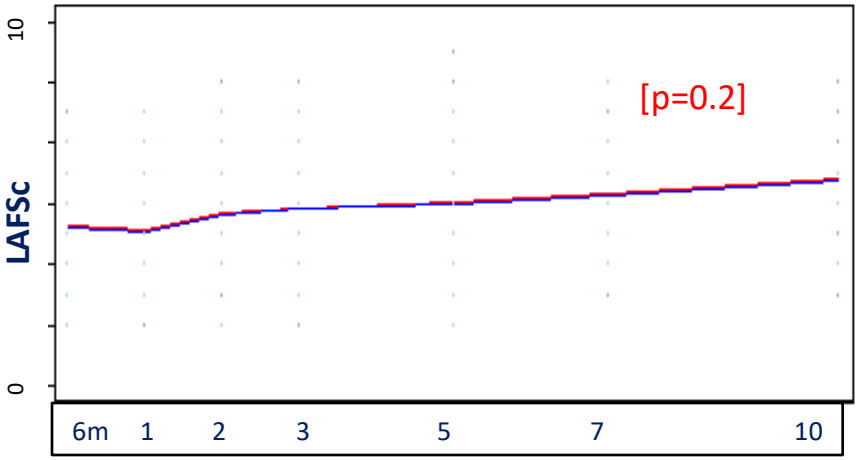
Steroids at LT

- 24 yes
  - 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
- 30 no
  - 13 (43%)Further ST for AR
    x dose: 0.3 ± 0.1 mg/kg




**N=139**

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




**Steroid therapy was not associated with reduced fibrosis in this population**

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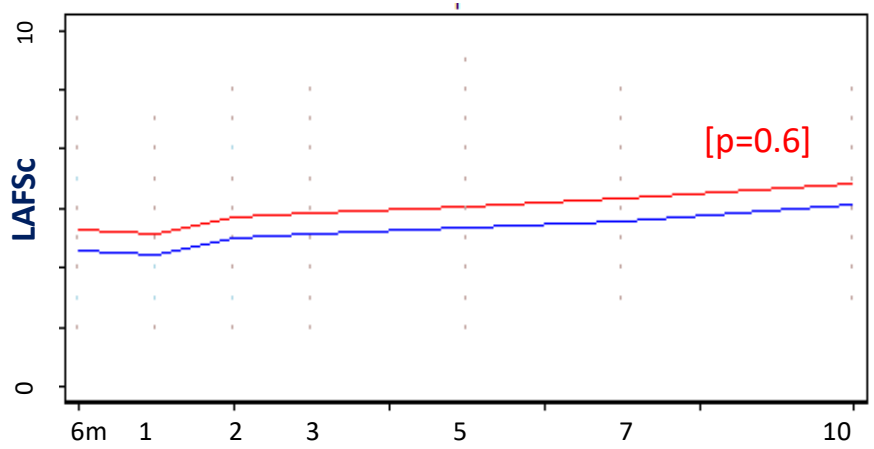
# Results IV Immunosuppression-Fibrosis according to Prope-Tolerance status

| Patients at 7 years | | Prope-T (n=18) | Non Prope-T (n=36) | |
|---|---|---|---|---|
| **N= 54** | Mean PSR% = | 19.0 ± 9.7 | 18.8 ± 9.4 | [p=0.5] |
| | Mean LAFSc = | 3.9 ± 1.7 | 4.1 ± 1.7 | [p=0.8] |

**Fibrosis evolution in Prope-Tolerance vs Non Prope-Tolerance LB (n=595)**




| | |
|---|---|
| **Total LB** | **595** |
| ***PROPE T*** | ***175*** |
| ***NO PROPE T*** | ***420*** |

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

**Prope-tolerance did not contribute to increase fibrosis**

American Journal of Transplantation 2014; 14: 1648–1656

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# *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

**DISCUSSION**

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.

**DISCUSSION**

# 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

**DISCUSSION**

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# 2017 BANFF-SCT
## Joint Scientific Meeting

**BARCELONA**
27-31 March 2017

THE CATALAN TRANSPLANTATION SOCIETY
SOCIETAT CATALANA DE TRASPLANTAMENT

*Thanks for your attention*