Circulating miRNAs as biomarker for liver fibrosis in chronic hepatitis B and C virus infection

P.J. Poortmans¹, J. Lambrecht², L.A. van Grunsven², R. Reynaert¹,²

(1) Department of Gastro-Enterology and Hepatology, UZ Brussel, Brussels, Belgium
(2) Liver Cell Biology Lab, Department of basic (bio-)medical sciences, Vrije Universiteit Brussel (VUB), Brussels, Belgium
Liver fibrosis

Image source: Adapted from Ramos-Lopez et al. Genetic, metabolic and environmental factors involved in the development of liver cirrhosis in Mexico. World J Gastroenterol, 2015; 21(41):11552-66
Liver fibrosis

- **Key player:** Hepatic stellate cell
- Frequent complications, high mortality rates
- **Diagnosis and staging:** Liver biopsy, FibroScan®, ALT, AST, ...

→ More accurate non-invasive tools are needed

MicroRNA

→ Stably present in ECM, blood, urine, etc.


Circulating miRNAs in liver fibrosis

**HBV**
- Increased: miR-19b, miR-192, miR-22, miR-92a, miR-122, miR-125b, miR-150, miR-181b
- Decreased: miR-29a, miR-451, miR-572, miR-575, miR-638, miR-1974

**HCV**
- Increased: miR-16, miR-192, miR-320, miR-22, miR-34a, miR-1207, miR-122, miR-1275, miR-146a
- Decreased: miR-29a, miR-652, miR-451, miR-572, miR-122, miR-575

**Alcohol**
- Increased: miR-135b, miR-571, miR-490, miR-761, miR-513
- Decreased: miR-29a, miR-652, miR-214, miR-203, miR-652

**NAFLD**
- Decreased: miR-630, miR-762, miR-744
- Increased: miR-16, miR-192, miR-19a, miR-223, miR-375, miR-375, miR-122, miR-572, miR-125b, miR-638
Aim of the study

To identify circulating miRNAs correlated to the degree of liver fibrosis in patients with chronic liver disease due to chronic viral hepatitis
Materials and methods

Patients with chronic viral hepatitis
UZ Brussels

NucleoSpin® miRNA Plasma procedure

- Sample preparation and protein precipitation:
  Mix sample and lysis buffer. Add precipitation buffer and centrifuge to remove contaminating proteins.

- RNA and DNA binding:
  Add isopropanol to the cleared lysate and bind RNA and DNA to binding column.

- Optional DNA digestion:
  For complete removal of DNA background perform DNase digestion on column. Omit this step for DNA isolation.

- Washing:
  Efficient washing with two different wash buffers.

- Elution:
  Elute pure small RNA/DNA with RNase-free water.

RT-qPCR

Spiked-in
cel-miR-39-3p

Metavir
F0-F1
Absent or mild fibrosis
Significant fibrosis
Severe fibrosis
Cirrhosis
## Study population

**Total population:** 42 patients  
**Healthy controls:** 11 individuals

<table>
<thead>
<tr>
<th>Fibrosis F-stage</th>
<th>HBV induced</th>
<th>HCV induced</th>
</tr>
</thead>
</table>
| **F0-F1**  
No/minimal liver fibrosis | 15          | 13          |
| **F2**  
Significant liver fibrosis | 3           | 3           |
| **F3**  
Advanced liver fibrosis | 1           | 3           |
| **F4**  
Cirrhosis | 0           | 4           |
| **TOTAL** | **19**      | **23**      |
MiRNAs involved in HSC activation

miRNA linked to pathways involved in HSC activation?
# Selected miRNAs

<table>
<thead>
<tr>
<th>miRNA</th>
<th>Expression in aHSCs</th>
<th>Expression in blood</th>
<th>Associated liver disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-19b-3p</td>
<td>decrease</td>
<td>increase</td>
<td>HBV infection, NAFLD</td>
</tr>
<tr>
<td>miR-150-5p</td>
<td>decrease</td>
<td>increase</td>
<td>HBV infection</td>
</tr>
<tr>
<td>miR-192-5p</td>
<td>decrease</td>
<td>increase</td>
<td>HBV infection, HCV infection, NAFLD</td>
</tr>
<tr>
<td>miR-200b-3p</td>
<td>increase</td>
<td>increase</td>
<td>NAFLD (murine model)</td>
</tr>
<tr>
<td>miR-142-3p</td>
<td>decrease</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>miR-219a-1-5p</td>
<td>increase</td>
<td>?</td>
<td>?</td>
</tr>
</tbody>
</table>
Circulating miR-150, -192 and -200b are upregulated in chronic viral hepatitis.
Conclusion

Chronic HBV

Chronic HCV

miR-150 ↑
miR-192 ↑
miR-200b ↑
1. Validation in large cohorts of patients

miR-150 ↑
miR-192 ↑
miR-200b ↑

miR-150 ↑
miR-192 ↑
miR-200b ↑
Perspectives and potential relevance

1. Validation in large cohorts of patients

2. Biomarker panels

- miR-150
- miR-192
- miR-200b
- ...

Non-invasive diagnosis and staging of liver fibrosis
Perspectives and potential relevance

1. Validation in large cohorts of patients

2. Biomarker panels

3. Prognostic: fibrosis progression markers?
Perspectives and potential relevance

1. Validation in large cohorts of patients

2. Biomarker panels

3. *Prognostic:* fibrosis progression markers?

4. Therapeutic target
Acknowledgements

• Prof. Dr. Hendrik Reynaert

• Prof. Dr. Leo van Grunsven

• Mr. Joeri Lambrecht

• The entire staff of the LIVR-lab

• The study-nurse, Mrs. Rita Vertommen
Back-up slides
The propagation of the low-frequency vibration was measured by the vibrator. An ultrasonic single-element transducer operating at 5 MHz was used to generate the vibration. The elastic wave velocity, VS, is the slope of the wave pattern.

Transient elastography in liver

L. SANDRIN et al. 1707
Validation of used methods

miR-122

HBV infected

HCV infected

Healthy

F0-F1 F2 F3

F-stage
dCT over cel-miR-39

***

****

HBV infected

HCV infected

****

**

****

*

Healthy

F0-F1 F2 F3 F4

F-stage
dCT over cel-miR-39

Vrije Universiteit Brussel