Microbioma e hígado

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Gut microbiome

- Composed by more than $10^{14}$ microorganisms including bacteria, viruses and fungi.
- Most of the bacteria are anaerobic with varying numbers and composition according to the site of the gut.
- Main phyla: Firmicutes and Bacteroidetes but species present and proportions vary between individuals.
- Analysis: evolved from cultures to sequencing of the whole genoma
- Beneficial effects on host metabolism, digestion and immune function

Anand et al, Semin Liver Dis 2016
Gut microbiome alterations: dysbiosis

- Intestinal dysbiosis: disruption in symbiosis due to an imbalance in the microbial composition.

- Quantitative (bacterial overgrowth) and/or qualitative changes in microbiota.

- Gut microbiota: major role in the gut and systemic immune system.

- Dysbiosis: potentially involved in the pathogenesis of obesity, neurologic disease, IBD, cancer and liver disease.

Anand et al, Semin Liver Dis 2016
Intestinal dysfunction: Gut dysbiosis and bacterial translocation

Arroyo, V. et al. (2016) Acute-on-chronic liver failure in cirrhosis
Nat. Rev. Dis. Primers doi:10.1038/nrdp.2016.41
Mechanisms of liver disease in dysbiosis

- Liver: particularly susceptible to the effects of dysbiosis: > 70% of the liver’s blood supply through portal vein

- Frequent exposure to gut-derived toxins and microbial products

- Main mechanisms of dysbiosis induced liver disease: increased intestinal permeability, bacterial/metabolite translocation and immune activation.

- Involved in the pathogenesis of NAFLD, alcoholic liver disease and cirrhosis.

Anand et al, Semin Liver Dis 2016
## Dysbiosis in NAFL/NASH

<table>
<thead>
<tr>
<th>Study</th>
<th>Study population</th>
<th>Comparison group</th>
<th>Intestinal microbiota changes in NAFLD group</th>
<th>Technique</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jiang et al, 2015</td>
<td>Healthy adults ($n = 32$) Clinical diagnosis or biopsy-proven NAFLD ($n = 53$)</td>
<td>Healthy vs. NAFLD</td>
<td>↑ Lactobacillales; ↑ Lactobacillus (genus); ↑ Anaerobacter (genus); ↑ Escherichia (genus); ↑ Streptococcus (genus); ↑ Clostridium XI (genus); ↓ Alistipes (genus); ↓ Odoribacter; ↓ Flavonifractor (genus); ↓ Oscillibacter (genus)</td>
<td>16s rRNA gene sequencing stool sample</td>
</tr>
<tr>
<td>Mouzaki et al, 2013</td>
<td>Healthy adults ($n = 17$) Biopsy-proven simple steatosis ($n = 11$) Biopsy-proven NASH ($n = 22$)</td>
<td>Healthy vs. NASH</td>
<td>↓ Bacteroidetes; ↓ Clostridium coccoideis (Firmicutes phylum)</td>
<td>Real-time PCR; stool sample</td>
</tr>
<tr>
<td>Raman et al, 2013</td>
<td>Healthy adults ($n = 30$) Clinically diagnosed NAFLD ($n = 30$)</td>
<td>Healthy vs. NAFLD</td>
<td>No change at phylum level; ↑ Lactobacillaceae (Family); ↓ Ruminococaceae (Family)</td>
<td>16s rRNA gene pyrosequencing; stool sample</td>
</tr>
<tr>
<td>Zhu et al, 2013</td>
<td>Healthy children ($n = 16$) Obese children ($n = 25$) Biopsy-proven NASH ($n = 22$)</td>
<td>Healthy vs. obese</td>
<td>↑ Bacteroidetes; ↓ Firmicutes; ↓ Actinobacteria; ↓ Proteobacteria</td>
<td>16s rRNA gene pyrosequencing; stool sample</td>
</tr>
<tr>
<td></td>
<td>Healthy vs. NASH</td>
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<tr>
<td></td>
<td>Obese vs. NASH</td>
<td></td>
<td>↑ Proteobacteria</td>
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</tbody>
</table>

Dysbiosis has been involved in the development of steatosis (endogenous production of alcohol, alterations in the metabolism of choline and bile acids…) and in the progression to NASH

Anand et al, Semin Liver Dis 2016
Dysbiosis in alcoholic liver disease

- Alcohol use alters intestinal microbiota (decrease in Bacteriodetes and Firmicutes and increase in Proteobacteria and Acinetobacteria).

- Alcohol abuse increases intestinal permeability (disruption of the tight junctions through metabolic products and intestinal inflammation).

- Frequent exposure to gut-derived toxins and microbial products (LPS) that stimulate innate immune system: liver inflammation and damage

Anand et al, Semin Liver Dis 2016
Intestinal bacterial overgrowth in patients with cirrhosis

Intestinal permeability and liver function in cirrhosis*

%  

Child A  Child B  Child C

p <0.05

Test: EDTA- Cr$^{51}$

Scarpellini et al. Am J Gastroenterol 2009
Bacterial translocation to MLN in advanced cirrhosis

Cirera et al. J Hepatol 2001

n = 101

p < 0.05
Dysbiosis in cirrhosis

HEPATIC ENCEPHALOPATHY

BACTERIAL INFECTION

Alterations in colonic mucosal and fecal microbiota in patients with hepatic encephalopathy or infection associated to inflammation and cognition

Bajaj et al, Am J Physiol Gastrointest Liver Physiol 2012; J Hepatol 2014
Bacterial DNA Translocation Is Associated With Systemic Circulatory Abnormalities and Intrahepatic Endothelial Dysfunction in Patients With Cirrhosis

Bellot et al, Hepatology 2010
Bacterial DNA Translocation Is Associated With Systemic Circulatory Abnormalities and Intrahepatic Endothelial Dysfunction in Patients With Cirrhosis

<table>
<thead>
<tr>
<th>Patients With Ascites (n = 55)</th>
<th>bactDNA(+) (n = 21)</th>
<th>bactDNA(−) (n = 34)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP (mmHg)</td>
<td>76 ± 10*</td>
<td>86 ± 10*,†</td>
</tr>
<tr>
<td>CO (L/minute)</td>
<td>8.1 ± 2.0</td>
<td>7.5 ± 1.9</td>
</tr>
<tr>
<td>CI (L/minute/m²)</td>
<td>4.5 ± 1.1</td>
<td>4.2 ± 0.7</td>
</tr>
<tr>
<td>SVR (dyne/second/cm⁻⁵)*</td>
<td>717 ± 241*,†</td>
<td>909 ± 253*,†</td>
</tr>
<tr>
<td>SVRI (dyne/second/m²/cm⁻⁵)</td>
<td>1262 ± 412*,†</td>
<td>1580 ± 333*,†</td>
</tr>
<tr>
<td>PVRI (dyne/second/m²/cm⁻⁵)</td>
<td>115 ± 35†</td>
<td>124 ± 44</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>79 ± 16†</td>
<td>80 ± 14†</td>
</tr>
<tr>
<td>FHVP (mmHg)</td>
<td>10.8 ± 3</td>
<td>11.8 ± 4</td>
</tr>
<tr>
<td>WHVP (mmHg)</td>
<td>30.3 ± 5†</td>
<td>31.5 ± 6†</td>
</tr>
<tr>
<td>HVPG (mmHg)</td>
<td>19.5 ± 4.3†</td>
<td>19.7 ± 4.7†</td>
</tr>
<tr>
<td>HBF (mL/minute)</td>
<td>1161 ± 620</td>
<td>966 ± 630</td>
</tr>
<tr>
<td>TNFα (pg/mL)</td>
<td>390 ± 99*,†</td>
<td>175 ± 6*</td>
</tr>
<tr>
<td>NOx (nmol/L)</td>
<td>36.3 ± 10*,†</td>
<td>28.2 ± 12*</td>
</tr>
<tr>
<td>PRA (ng/mL/hour)</td>
<td>2.8 ± 1.8*,†</td>
<td>1.7 ± 2.1*</td>
</tr>
<tr>
<td>IL-12 (pg/mL)</td>
<td>600 ± 179*,†</td>
<td>359 ± 15*</td>
</tr>
</tbody>
</table>

![Graph showing postprandial increase in HBF and HVPG](image)

Bellot et al, Hepatology 2010
ORGAN DYSFUNCTION IN DECOMPENSATED CIRRHOSIS

1. Liver dysfunction
2. Brain dysfunction.
3. Renal dysfunction
4. Circulatory dysfunction
5. Lung dysfunction: Hepatopulmonary syndrome.
6. Immune dysfunction
7. Cardiac dysfunction: Cirrhotic cardiomiopathy.
8. Adrenal dysfunction: Hepatoadrenal syndrome.
9. Thyroid dysfunction
10. Gut dysfunction
PERIPHERAL ARTERIAL VASODILATION HYPOTHESIS

- Compensated cirrhosis
- Hyponatremia
- Time (years)
- Ascites
- Cardiac output
- Splanchnic arterial vasodilation
- Systemic vascular resistance
- Extra-splanchnic vasoconstriction
- Degree of activation of RAAS, SNS, ADH
- Effective arterial hypovolemia
- Normal effective blood volume

Changes

Compensated cirrhosis

Ascites

Hyponatremia

Type-2 HRS
CHANGES IN VASOACTIVE SYSTEMS AT THE DIFFERENT PHASES OF DECOMPENSATION

Phases: 1,2: Ascites and moderate or intense Na retention; 3: Dilutional hyponatremia; 4: Type-2 HRS.
INFLAMMATORY MARKERS AND DEVELOPMENT OF ACLF AT ENROLLMENT OR DURING HOSPITALIZATION

Leucocyte count

C-reactive Protein

* p<0.05 with respect to No ACLF
** p<0.001 with respect to No ACLF
INTESTINAL DYSBIOSIS, LOST OF INTEGRITY OF GUT BARRIER, BACTERIAL TRANSLOCATION

CIRRHOSIS: PROGRESSIVE LIVER DYSFUNCTION & PORTAL HYPERTENSION

SYSTEMIC INFLAMMATION AFFECTING NON-SPLANCHNIC ORGANS

MICROCIRCULATORY DYSFUNCTION, MITOCHONDRIAL AND CELL DYSFUNCTION, CELL DEATH

LOCAL INFLAMMATION AT THE LAMINA PROPRIA

SPLANCHNIC ARTERIAL VASODILATION, SYSTEMIC CIRCULATORY DYSFUNCTION, ORGAN HYPOPERFUSION

ACUTE DECOMPENSATION AND MULTI-ORGAN DYSFUNCTION

PATHOGENESIS OF ACUTE DECOMPENSATION AND MULTIORGAN DYSFUNCTION IN CIRRHOSIS

Bernardi et al, J Hepatol 2015
MECHANISMS AND VERY EARLY CLINICAL COURSE OF ACLF

Bernardi et al, J Hepatol 2015
PLASMA RENIN CONCENTRATION (PRC), IL-8 AND IRREVERSIBLY OXIDIZED ALBUMIN (HNA2) LEVELS IN ACLF
<table>
<thead>
<tr>
<th>Therapy</th>
<th>Effect on intestinal microbiota</th>
<th>Examples</th>
</tr>
</thead>
</table>
| Prebiotic            | Complex carbohydrates; digested by colonic microbes to form short-chain fatty acids and lactate, which stimulate the growth of beneficial bacteria                                                                                   | Fructo-oligosaccharide (FOS)  
Galacto-oligosaccharide (GOS)  
Lactulose  
Inulin                                                                 |
| Probiotic            | Living microorganisms that confer a health benefit on their host through antimicrobial effects, enhancement of mucosal barrier integrity, and immunomodulation                                                                       | Lactobacillus GG (LGG)  
Lactobacillus casei  
Lactobacillus plantarum  
Lactobacillus johnsonii  
Bifidobacterium lactis  
Saccharomyces boulardii  
VSL#3                                                                 |
| Synbiotic            | Contain prebiotic and probiotic; augment the activity and prolong the survival of potentially beneficial probiotics                                                                                                            | Bifidobacterium + FOS  
Protexin  
Lactobacillus + inulin                                                                 |
| Antibiotic           | Antimicrobial effects; changes in bacterial populations and composition; alterations in bacterial metabolic function and virulence                                                                                               | Rifaximin  
Norfloxacin  
Neomycin  
Metronidazole                                                                 |
| Fecal microbiota transplant | Colonization resistance (limiting the colonization of pathogens); modulation of bacterial metabolic function                                                                                                            |                                                                 |

Anand et al, Semin Liver Dis 2016
MODULATION OF MICROBIOMA AND METABIOMA BY RIFAXIMIN IN PATIENTS WITH HEPATIC ENCEPHALOPATHY

Bajaj et al. Plos One 2013
Conclusions

- Patients with NAFLD, alcoholic liver disease and cirrhosis present important alterations in gut microbiota that could be involved in their pathogenesis.

- The frequent exposure to gut-derived toxins and microbial products (LPS) stimulates innate immune system: liver inflammation and damage (NASH) and systemic inflammation (decompensated cirrhosis) and multiorgan failure (ACLF).

- Gut microbiota was, is and will be a therapeutic target in liver diseases.