MICROBIOTA, PROBIOTICS AND CYSTIC FIBROSIS

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1. Introduction
2. Intestinal inflammation
3. Intestinal microbiota
4. Probiotics
5. Conclusions

No conflict of interest to declare
Introduction

- Cystic fibrosis - Mucoviscidosis
- Hereditary disease:
  - autosomal recessive
  - identification genetic defect on chromosome 7 in 1989
- In all populations:
  - variable frequency
  - highest in Caucasians
- Belgium:
  - carriers: 1/20 to 1/30
  - patients: 1000 to 1500 (40 new diagnoses/year)
- Europe: 50,000 patients – rare disease
Basic defect

CFTR gene
- 27 exons
- 2224 nucleotides
- 2000 mutations

CFTR protein =
- 1480 amino-acids

F508del

Discovered 1989

CFTR: Cystic Fibrosis Transmembrane Regulator
CFTR protein function

- Ion channel at the apical surface of epithelial membranes
- Impairment of chloride secretion and subsequently secretion of water and other ions
Multi-organ disease

Impaired function in all organs with exocrine glands

lower en upper airways
pancreas
liver – gal bladder
intestine
genital system
skin (sweat)
Intestinal inflammation

- Repeated and/or chronic antibiotic exposure
- Low pH due to exocrine pancreatic insufficiency
- Intestinal dysmotility

Lumen

- Thick mucus
- Bacterial overgrowth and altered bacterial composition
- Calprotectin

Mucosa

- CFTR
- CF INTESTINE

Intestinal epithelial cells

- Inflammatory stimuli (e.g., IL-1, LPS)
- Increased inflammatory response

Neutrophils

Lee 2012
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Cystic fibrosis and intestinal inflammation

- **Van Biervliet S (1999 and 2003):** mucosal damage and decreased intestinal alkaline phosphatase:
  - 19/61 CF infants at diagnosis with mucosal damage on duodenal biopsy
  - IAP important in defence against bacterial overgrowth

- **Hendriks HJE (2001):** increased intestinal permeability:
  - 13/14 stable CF children with abnormal lactulose/mannitol test
  - partly corrected with Lansoprazole

- **Werlin SL (2010):** **cystic fibrosis enteropathy**:
  - 26/41 CF adults/children without overt GI disease with small bowel abnormalities on capsule endoscopy (diffuse areas of inflammatory findings with edema, erythema, mucosal breaks and ulcerations)
  - Fecal Calprotectin (FCP) abnormal in 18/30 (60%) CF patients
Cystic fibrosis and intestinal inflammation

- **Lisowska A (2010):** intestinal inflammation and small intestine bacterial overgrowth (SIBO):
  - FCP CF patients > FCP healthy controls
  - FCP abnormal in 21/25 (84%) CF patients vs 0/30 healthy controls
  - SIBO in 40% of CF patients but FCP SIBO+ = FCP SIBO- 

- **Rumman N (2014):** intestinal inflammation and gastrointestinal symptoms:
  - no significant difference in GI symptoms in 62 CF patients with normal and abnormal FCP

- **Dhaliwal J (2015):** intestinal inflammation and growth in children:
  - FCP 30 Crohn disease > FCP 28 CF patients > FCP 47 healthy controls
  - FCP abnormal in 17/28 (61%) CF patients
  - FCP correlates significantly with poor growth
Adriaanse MP (2015): enterocyte damage - intestinal inflammation and nutritional status - CF related morbidities:

- 86 CF patients and 107 healthy controls
- serum intestinal fatty acid binding protein (I-FABP = marker enterocyte damage) and FCP
- I-FABP in CF patients > I-FABP healthy controls
- FCP abnormal in 93% CF patients
- I-FABP correlates negatively with lung function in children
- FCP correlates negatively with lung function in adults
- FCP is significantly associated with the presence of pancreatic insufficiency, CF related diabetes and use of proton pump inhibitors
- enterocyte damage and intestinal inflammation in CF patients + inverse correlation between enteropathy and lung function + association of enteropathy with CF related morbidities
Cystic fibrosis and intestinal inflammation

- **Flas T (2015):** intestinal inflammation, intestinal microbiome and cirrhosis:
  - 11 CF with cirrhosis (CFCIR) + 19 CF with no liver disease (CFnoLIV)
  - FCP: elevated in most patients but similar in CFCIR and CFnoLIV
  - Intestinal permeability testing by urinary lactulose/mannitol excretion ratio: elevated in most patients and slightly lower in CFCIR
  - Small bowel transit time: longer in CFCIR
  - Small bowel endoscopy: more severe intestinal mucosal lesions in CFCIR
  - Fecal microbiome: ↓ *bacteroides* - associated with lower capsule endoscopy scores in CFCIR ; ↑ *Clostridium* - associated with higher capsule endoscopy scores in CFCIR
  - Abnormal intestinal permeability and elevated FCP in CF patients + increased intestinal mucosal lesions, slower small bowel transit and alterations in faecal microbiome in CFCIR
  - Disturbances in intestinal function combined with changes in microbiome may contribute to the development of hepatic fibrosis and intestinal lesions
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Cystic fibrosis and intestinal microbiota

- **Duytschaever G (2011 and 2012):**
  - ↓ lactic acid bacteria - *Clostridia* – *Bifidobacterium spp* - *Veillonella spp* - *Bacteroides Prevotella spp*; ↑ enterobacteria
  - cross-sectional data: predominant fecal microbiota has comparable species richness in CF patients and healthy controls
  - longitudinal data: lower temporal stability and lower species richness in the predominant fecal microbiota in CF patients
  - first evidence of **general dysbiosis** in CF patients

- **Duytschaever G (2013):**
  - significantly lower abundance and temporal stability of *Bifidobacteria* and *Clostridium cluster XIVa*
  - first report of specific microbial determinants of dysbiosis in CF patients
Cystic fibrosis and intestinal microbiota

- **Scanlan PD (2012):** ↓ species richness and diversity

- **Bruzzese E (2014):** ↓ *Eubacterium rectale, Bacteroides uniformis, Bacteroides vulgatus, Bifidobacterium adolescentis, Bifidobacterium catenulatum* and *Faecalibacterium prausnitzii*

- **Del Campo R (2014):** ↑ *Proteobacteria* and *Actinobacteria* ; ↓ *Firmicutes* and *Bacteroidetes*

- **Hoffman LR (2014):** ↑ *Escherichia coli*

- **Debyser G (2015):** ↑ host proteins involved in inflammation and mucus formation → ↓ *Faecalibacterium prausnitzii* ; ↑ *Enterobacteriacea, Ruminococcus gnavus* and *Clostridia species*
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## Probiotics and CF (1)

<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td><strong>Probiotic</strong></td>
<td><em>LGG</em></td>
<td>Mixture (7 types)</td>
<td><em>LGG</em></td>
</tr>
<tr>
<td><strong>Patient type (n)</strong></td>
<td>CF (30) (10 <em>LGG</em>) Co (30)</td>
<td>CF (47) (24 probiotics)</td>
<td>CF (22) (10 <em>LGG</em>) Co (?)</td>
</tr>
<tr>
<td><strong>Study type</strong></td>
<td>Prospective (1m)</td>
<td>RDBPC (1m)</td>
<td>RDBPC (1m)</td>
</tr>
<tr>
<td><strong>Effect</strong></td>
<td>FCP (rectal NO)</td>
<td>FCP</td>
<td>FCP (rectal NO)</td>
</tr>
<tr>
<td></td>
<td>CF &gt; Co 27/30 (90 %) ▲ <em>LGG</em>: FCP▼</td>
<td>31/47 (66%) ▲ Probiotics: 21/24 FCP▼</td>
<td>CF &gt; Co 12/19 (63 %) ▲ <em>LGG</em>: FCP▼</td>
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<td></td>
<td>Microbiome:</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>dysbiosis (▲ if antibiotics) <em>LGG</em>: biodiversity ▲ Reduced microbial richness ≈ intestinal inflammation</td>
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# Probiotics and CF (2)

<table>
<thead>
<tr>
<th>Probiotic</th>
<th>Patient type (n)</th>
<th>Study type</th>
<th>Effect</th>
<th>Microbiome:</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>L Reuteri</strong></td>
<td>CF (2x30)</td>
<td>RDBPC cross-over (6m)</td>
<td><strong>FCP:</strong> ▼</td>
<td>dysbiosis</td>
</tr>
<tr>
<td><strong>L Reuteri</strong></td>
<td>CF (61) (30 <strong>L Reuteri</strong>)</td>
<td>RDBPC (6m)</td>
<td><strong>FCP:</strong> =</td>
<td><strong>L Reuteri:</strong> biodiversity ▲ bacterial density ▼</td>
</tr>
<tr>
<td><strong>LGG</strong></td>
<td>CF (20)</td>
<td>Prospective (1m)</td>
<td>50 % bacterial overgrowth</td>
<td></td>
</tr>
</tbody>
</table>

- **GI comfort:** ▲
- **Pulmon F exacerbations:** ▼
- **URTI:** ▼
- **pulm function:** =
- **hospitalisation:** =
- **TNF alfa/IL8:** =

**GI comfort:** ▲
**Stool appearance:** ▲
**Stool number:** ▼
**Stool fat and sugar:** ▼
# Probiotics and CF (3)

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<tbody>
<tr>
<td>Patient type (n)</td>
<td>CF (2x19)</td>
<td>CF (10)</td>
<td>CF (37) (20 probiotics)</td>
<td>CF (61) (30 L Reuteri)</td>
</tr>
<tr>
<td>Study type</td>
<td>RDBPC cross-over (6m)</td>
<td>Prospective (6m)</td>
<td>RDBPC (1m)</td>
<td>RDBPC (6m)</td>
</tr>
<tr>
<td>Effect</td>
<td>Pulmon F: exacerbations ▼ FEV1 ▲ Hospitalisations ▼ Weight ▲</td>
<td>Pulmon F: exacerbations ▼ pulm function = Sputum bacteria/neutrophils /IL8 =</td>
<td>Pulmon F: exacerbations▼ QOL ▲ at 3 m but not at 6m</td>
<td>Pulmon F: exacerbations▼ URTI ▼ pulm function = Hospitalisation = TNF alfa/IL8 = FCP =</td>
</tr>
</tbody>
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Probiotics and CF: Belgian study

- **Type**: RDBPC cross-over (2 x 4 months)

- **Patients**: mild to moderate disease; 4 - 14 years; 100 → 25

- **Probiotics**: 10.10⁹ CFU 1 capsule / day
  
  *Lactobacillus rhamnosus vésalius 001 LMG S-28148*
  
  *Bifidobacterium animalis subsp. lactis vésalius 002 LMG 23512*

- **Primary outcomes**: pulmonary exacerbations

- **Secondary outcomes**: gastro-intestinal symptoms, general well-being, growth, hospitalisations, intestinal inflammation – permeability – microbioma, pulmonary function – sputum cultures
Flow chart

Period A
4 months
- Physical
- W, H, BMI s.d.
- Sputum culture
- Gut permeability
- Calprotectine
- Laboratory
- QoL

Wash out
1 month

Period B
4 months
- Physical
- W, H, BMI s.d.
- Sputum culture
- Gut permeability
- Calprotectine
- Laboratory
- QoL
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Conclusions

- **Cystic fibrosis:**
  1) Chronic intestinal inflammation and abnormal balance of the microbiota
  2) Chronic intestinal inflammation may be a driver for systemic inflammation

- **Studies with probiotics show:**
  
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<tr>
<th></th>
<th>LGG</th>
<th>LR</th>
<th>Mixtures</th>
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<tbody>
<tr>
<td>1) Restoration of intestinal microbiota</td>
<td>+</td>
<td>+</td>
<td>NS</td>
</tr>
<tr>
<td>2) Reduction intestinal inflammation</td>
<td>+</td>
<td>+/-</td>
<td>+</td>
</tr>
<tr>
<td>3) Reduction pulmonary exacerbations</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4) Improvement lung function</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4) Improvement gastro-intestinal health</td>
<td>+</td>
<td>+</td>
<td>NS</td>
</tr>
<tr>
<td>5) Improvement general well-being</td>
<td>NS</td>
<td>NS</td>
<td>+</td>
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<tr>
<td>6) Reduction hospitalisation</td>
<td>+</td>
<td>-</td>
<td>NS</td>
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- **Mandatory before general use of probiotics in cystic fibrosis:**
  1) Proven efficiency in well designed clinical trails
  2) Proven safety
Conclusions

The clinical significance of the gut microbiota in cystic fibrosis and the potential for dietary therapies

*Li L, Somerset S. Clin Nutr 2014; 33:571-80*