Cholestatic liver dysfunction during critical illness

Yoo-Mee Vanwijngaerden
Lies Langouche
Dieter Mesotten
Greet Van den Berghe

Department of Cellular and Molecular Medicine
Laboratory of Intensive Care Medicine – KU Leuven
University Hospitals Leuven
“Cholate stasis”
“Cholate stasis”

Simplified scheme of hepatobiliary transporters

Uptake

Canalicular export

Bile acids
Bilirubin

NTCP

OATPs

Glycine/Taurine

Gluconic acid

BSEP

MRP2

MRP3

MRP4

BLOOD

HEPATOCYTE

BILE

Alternative export

0. Introduction – Molecular mechanisms bile flow

34th Annual Meeting SIZ – June 13th 2014
"Cholate stasis"

Simplified scheme of hepatobiliary transporters

- **Uptake**
  - BLOOD
  - Bile acids
  - Bilirubin
  - OATPs

- **Canalicular export**
  - HEPATOCYTE
  - NTCP
  - Glucuronic acid
  - BSEP
  - MRP2
  - MRP3
  - MRP4

- **Alternative export**

0. Introduction – Molecular mechanisms bile flow

34th Annual Meeting SIZ – June 13th 2014
ICU cholestasis

- No consensus
- Criteria:
  - Bilirubin total > 3 mg/dL
  - ALP > 400 U/L and gammaGT > 80 U/L
- Incidence: 20% after 10d
- ↑mortality, ↑LOS

Patel JJ. J Intens Care Med 2013. The Association of Serum Bilirubin Levels on the Outcomes of Severe Sepsis.
Biliary sludge on ICU

- Presence of sediment in the gallbladder
- Diagnosed by ultrasonography
- Prevalence: ± 50% after 5d
- Acute complications:
  - biliary colic, necrotizing cholecystitis, cholangitis and acute pancreatitis


Pazzi P. Dig Liver Dis 2003. Biliary sludge- the sluggish gallbladder
Role of parenteral nutrition

- Parenteral nutrition is assumed to aggravate both cholestatic liver dysfunction and biliary sludge formation
Central hypothesis

“Cholestasis” in the early phase of critical illness is brought about by changes in synthesis and transport of bile acids and is a protective response of the liver. Parenteral nutrition can modify this protective cholestatic response.

Hyperbilirubinemia

? Cholestasis
Part 1

Unravel the mechanisms behind cholestasis during critical illness

Data published as:
1. Cholestasis & Critical illness – Study outline

Study outline

• 130/40 prolonged critically ill vs 20/10 control patients
• Serum levels of bile acids, bilirubin (HPLC-MS)
• mRNA expression, protein expression of (real time-PCR, western blotting, immunohistochemistry)
  – Hepatobiliary transporters
  – Synthesis enzymes
  – Nuclear receptors
1. Cholestasis & Critical illness - Results

Serum bilirubin (mg/dL)

Total bilirubin = Free bilirubin + Conjugated bilirubin

Serum levels are expressed in mg/dL, and represented as median with IQR (25th – 75th percentiles) - p-values are calculated with unpaired Mann-Whitney U test
Serum levels are expressed in µM and represented as median with IQR (25th – 75th percentiles) – p-values are calculated with unpaired Mann-Whitney
Correlation bilirubin – bile acids

Correlations were calculated using Pearson’s correlation test
**Bilirubin and bile acid transporters**

Uptake

- **BLOOD**
  - NTCP

- **Bile acids**
  - OATPs

- **Bilirubin**

Canalicular export

- **HEPATOCYTE**
  - Glycine/Taurine
  - Conjugation
  - Glucuronic acid

Alternative export

- **BSEP**
- **MRP2**
- **MRP3**
- **MRP4**

Protein expression – semi-quantitative via immunohistochemistry

mRNA expression – real-time PCR
1. Cholestasis & Critical illness - Results

Bilirubin and bile acid transporters

- Uptake
- Canalicular export

Protein expression – semi-quantitative via immunohistochemistry
mRNA expression – real-time PCR

BLOOD

NTCP

BSEP

Bile acids

Bilirubin

OATPs

HEPATOCYTE

Glycine/Taurine

Conjugation

Glucuronic acid

BILE

MRP2

MRP3

MRP4

Control patients (n=20/10)

ICU patients (n=130/40)

p-values are calculated with either unpaired Mann-Whitney or Fisher’s exact test

represents p<0.05 for mRNA and/or protein expression
represents p<0.1 for mRNA and/or protein expression

1. Cholestasis & Critical illness - Results

34th Annual Meeting SfZ – June 13th 2014
Bile acid synthesis enzymes

Protein expression – quantitative via western blotting
mRNA expression – real-time PCR

‘De novo’ synthesis

Cholesterol

CYP7A1

CA

CDCA

Conjugation

Glycine/Taurine

Representative p<0.05 for mRNA and/or protein expression

p-values are calculated with unpaired Mann-Whitney test
Nuclear receptors

Protein expression – semi-quantitative via immunohistochemistry
mRNA expression – real-time PCR

Uptake

Canalicular export

BLOOD

HEPATO CYTE

NTCP

FXR

VDR

PXR

CAR

BSEP

BILE

OATPs

Bile acids

Bilirubin

Glycine/Taurine

Bile acids

Glucuronic acid

OATPs

NTCP

FXR

PXR

VDR

CAR

αRXR

αRXR

αRXR

αRXR

Response Element

MRP2

MRP3

MRP4

Alternative export

1. Cholestasis & Critical illness - Results

34th Annual Meeting SIZ – June 13th 2014
Conclusion

• **Failing** hepatobiliary system during critical illness
  Failure to inhibit bile acid synthesis, upregulate canalicular bile acid export and localize pivotal nuclear receptors in the hepatocytic nuclei may indicate dysfunctional feedback regulation by increased circulating bile acid levels

• **Beneficial** response to critical illness
  Critical illness may result in maintained bile acid synthesis (CYP7A1), reversal of normal bile acid transport (BSEP/MRP3) and suppression of nuclear receptors (FXR/RXRα) to increase serum bile acid levels
Part 2

Compare the impact of caloric restriction or nutritional support with parenteral nutrition on “cholestasis” during critical illness.

Data published as:
### 2.1. Rabbits study - Outline

#### Study outline

- Serum levels of **liver enzymes, bile acids**
  - (enzymatic colorimetric assays, HPLC-MS)
- Protein expression, mRNA expression of
  - Synthesis enzymes
  - Hepatobiliary transporters
  - Nuclear receptors

<table>
<thead>
<tr>
<th>Time</th>
<th>Activity</th>
<th>Fed 280 kCal/kg/day</th>
<th>Fasted 1.4 kCal/kg/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>Blood sampling</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Randomization, Anesthesia,</td>
<td>Fed n=10</td>
<td>Fasted n=11</td>
</tr>
<tr>
<td></td>
<td>Placing iv lines, Third degree</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>burn wound, Fluid resuscitation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 1-6</td>
<td>Euthanasia, Blood sampling,</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Harvesting liver tissue</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
2.1. Rabbits study - Results

Serum liver enzymes

AST AUC (IU/L)  
ALT AUC (IU/L)

AUC is area under the curve using dialysis measurements. Levels are expressed as median with IQR. p-values are calculated with unpaired Mann-Whitney U test.
2.1. Rabbits study - Results

Profile serum bile acids

G-DCA/DCA-ratio

- ILL - fed (n=10)
- ILL - fasted (n=11)

* represents p≤0.05 for comparison of changes over time (baseline vs day 7 levels) using Wilcoxon Signed Rank test
2.1. Rabbits study - Results

**Hepatobiliary transport system**

Protein expression – quantitative via western blotting
mRNA expression – real-time PCR

**Uptake**

BLOOD

Conjugated bile acids

**HEPATO CYTE**

‘De novo’ synthesis

Cholesterol → Bile acids

NTCP

OATPs

** alternative export**

BSEP

Bile

Bile acids

FXR

RXRα

CYP7A1

Glycine

Response Element

**Canalicular export**

MRP2

MRP3

MRP4

Typical expression

ILL - fed (n=10)

ILL - fasted (n=11)

Unpaired Mann-Whitney U test was used for comparison of mRNA/protein expression between fed and fasted critically ill rabbits

represents p<0.05 for mRNA and/or protein expression
Early versus Late Parenteral Nutrition in Critically Ill Adults

Michael P. Casaer, M.D., Dieter Mesotten, M.D., Ph.D.,
Greet Hermans, M.D., Ph.D., Pieter J. Wouters, R.N., M.Sc.,
Miet Schetz, M.D., Ph.D., Geert Meyfroidt, M.D., Ph.D.,
Sophie Van Cromphaut, M.D., Ph.D., Catherine Ingels, M.D.,
Philippe Meersseman, M.D., Jan Muller, M.D., Dirk Vlasselaers, M.D., Ph.D.,
Yves Debaveye, M.D., Ph.D., Lars Desmet, M.D., Jasperina Dubois, M.D.,
Aime Van Assche, M.D., Simon Vanderheyden, B.Sc.,
Alexander Wilmer, M.D., Ph.D., and Greet Van den Berghe, M.D., Ph.D.*

Casaer MP. NEJM 2011. Early versus Late Parenteral Nutrition in Critically Ill Adults.
Study outline

Preplanned subanalysis of EPaNIC

– Total bilirubin, daily, n=4640; Conjugated bilirubin n=3216; (standard routine automated laboratory assays)
– Liver enzymes (GGT, ALP, ALT and AST), twice weekly, n=3216; (standard routine automated laboratory assays)
– Bile acids, BL-D3-D5, n=280; (HPLC-MS)
– Ultrasonography gallbladder, D5, n=776
2.2. EPaNIC - Results

Daily total bilirubin (mg/dL)

Plasma total bilirubin levels of all patients in ICU are presented as mean ± standard error of the mean.

* represents p≤0.05 after comparison using the unpaired Student's t-test after logarithmic transformation.

Plasma total bilirubin levels of all patients in ICU are presented as mean ± standard error of the mean.

* represents p≤0.05 after comparison using the unpaired Student's t-test after logarithmic transformation.

Intervention window
Peak levels of plasma liver enzymes GGT, ALP are presented as boxplots (median with IQR). P-values are calculated after comparison using the Mann-Whitney U test.
Free bile acids  Conjugated bile acids

Chenodeoxycholic Acid (μM)

Glyco-chenodeoxycholic Acid (μM)

Tauro-chenodeoxycholic Acid (μM)

Plasma levels of bile acids on admission, on day 3 and day 5 of ICU stay are presented as median with IQR.

§ represents p≤0.05 using Wilcoxon signed rank test for comparison with admission values.
### Ultrasonography gallbladder D5

<table>
<thead>
<tr>
<th></th>
<th>Early PN</th>
<th>Late PN</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=420</td>
<td>n=356</td>
<td></td>
</tr>
<tr>
<td>Sludge - n(%)</td>
<td>175 (44.8)</td>
<td>124 (37.3)</td>
<td>0.04</td>
</tr>
<tr>
<td>Wall thickening - n(%)</td>
<td>24 (6.2)</td>
<td>19 (5.7)</td>
<td>0.8</td>
</tr>
<tr>
<td>Double wall - n(%)</td>
<td>24 (6.1)</td>
<td>11 (3.3)</td>
<td>0.08</td>
</tr>
</tbody>
</table>
Conclusion

- Withholding parenteral nutrition improved markers of hepatocellular injury in association with the reversal of normal bile acid trafficking and increased bile acid detoxification through conjugation.

- Patients in the late PN group revealed higher bilirubin levels, but lower levels of “cholestatic” liver enzymes (ALP/GGT) in the first week intervention window, coinciding with better outcome.

- Withholding parenteral nutrition and accepting a large caloric deficit during the first week of critical illness reduced the incidence of gallbladder sludge and thus appears to be in part a preventable complication of critical illness.
3. General conclusion

**General conclusion**

### Uptake
- **BLOOD**
  - NTCP
  - OATPs
- Bile acids
- Bilirubin

### Canalicular export
- HEPATOCYTE
  - ‘De novo’ synthesis
    - CYP7A1
  - Glycine
  - Bile acids
  - BSEP
- BILE
  - MRP2

### Alternative export
- FXR
- RXRα
- Response Element
- MRP3
- MRP4

**Bile acids**
**Cholesterol**
**Glycine**

**34th Annual Meeting SIZ – June 13th 2014**
3. General conclusion

General conclusion

Uptake

Canalicular export

BLOOD

HEPATOCYTE

‘De novo’ synthesis

Bile acids

Cholesterol

CYP7A1

Glycine

Bile acids

ATP

HEPATOCYTE

Response Element

BSEP

FXR

RXRα

OATPs

BSE

MRP2

MRP3

MRP4

BILE

Bilirubin

Bile acids

Uptake

Bilirubin

Cholesterol

‘De novo’ synthesis

ATP

Response Element

MRP2

MRP3

MRP4
3. General conclusion

General conclusion

Uptake

Canalicular export

BLOOD

HEPATOCYTE

‘De novo’ synthesis

Energy saving

Bile acids

Cholesterol

Glycine

Bile acids

BSEP

BILE

OATPs

MRP2

MRP3

MRP4

OATPs

Response Element

FXR

RXRα

FXR

CYP7A1

Glycine

Cholesterol

‘De novo’ synthesis

Bile acids

Bilirubin

34th Annual Meeting SIZ – June 13th 2014
3. General conclusion

General conclusion

Rabbit study

EPaNIC trial

<table>
<thead>
<tr>
<th>Early PN</th>
<th>Late PN</th>
</tr>
</thead>
<tbody>
<tr>
<td>45%</td>
<td>37%</td>
</tr>
</tbody>
</table>

ALT AUC (IU/L)  

AST AUC (IU/L)  

Peak GGT plasma D1-LD (IU/L)  

Peak ALP plasma D1-LD (IU/L)
3. General conclusion

General conclusion

Rabbit study

EPaNIC trial

G-DCA/DCA-ratio

![Graph showing G-DCA/DCA-ratio with data points for BL and d7, indicating a significant difference (*).](image)

Peak CBil plasma D1-LD (mg/dL)

![Bar graph showing peak CBil plasma D1-LD for Early PN and Late PN with a p-value of 0.0003.](image)
General conclusion

Uptake

Canalicular export

BLOOD

Bile acids

NTCP

OATPs

HEPATOXYTE

‘De novo’ synthesis

CYP7A1

Cholesterol

Glycine

Bile acids

BSEP

BILE

FXR

RXRα

Response Element

MRP2

MRP3

MRP4

Alternative export

3. General conclusion

Bile acids

Uptake

‘De novo’ synthesis

CYP7A1

Cholesterol

Glycine

Bile acids

BSEP

BILE

FXR

RXRα

Response Element

MRP2

MRP3

MRP4

Alternative export

Bile acids

Uptake

‘De novo’ synthesis

CYP7A1

Cholesterol

Glycine

Bile acids

BSEP

BILE

FXR

RXRα

Response Element

MRP2

MRP3

MRP4

Alternative export

3. General conclusion

Bile acids

Uptake

‘De novo’ synthesis

CYP7A1

Cholesterol

Glycine

Bile acids

BSEP

BILE

FXR

RXRα

Response Element

MRP2

MRP3

MRP4

Alternative export

Bile acids

Uptake

‘De novo’ synthesis

CYP7A1

Cholesterol

Glycine

Bile acids

BSEP

BILE

FXR

RXRα

Response Element

MRP2

MRP3

MRP4

Alternative export

Bile acids

Uptake

‘De novo’ synthesis

CYP7A1

Cholesterol

Glycine

Bile acids

BSEP

BILE

FXR

RXRα

Response Element

MRP2

MRP3

MRP4

Alternative export

Bile acids

Uptake

‘De novo’ synthesis

CYP7A1

Cholesterol

Glycine

Bile acids

BSEP

BILE

FXR

RXRα

Response Element

MRP2

MRP3

MRP4

Alternative export

Bile acids

Uptake

‘De novo’ synthesis

CYP7A1

Cholesterol

Glycine

Bile acids

BSEP

BILE

FXR

RXRα

Response Element

MRP2

MRP3

MRP4

Alternative export

Bile acids

Uptake

‘De novo’ synthesis

CYP7A1

Cholesterol

Glycine

Bile acids

BSEP

BILE

FXR

RXRα

Response Element

MRP2

MRP3

MRP4

Alternative export
3. General conclusion

General conclusion

Hyperbilirubinemia  =  Protective

Hyperbilirubinemia  ≠  Cholestasis
Acknowledgements

Lies Langouche
Dieter Mesotten
Greet Van den Berghe

Laboratory of intensive care medicine – KULeuven
Intensive Care Unit - Universitaire ziekenhuizen Leuven
Storr Liver Unit – University of Sydney