Infantile Cholestasis
Metabolic and Genetic Cholestasis: An Overview

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MBBS, London University
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Inflammatory Bowel Disease
Liver Fibrogenesis and Cystic Fibrosis
Non-invasive point of care testing
Intestinal USS in IBD
Elastography in Liver Disease

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Conflicts of Interest

Speakers Fees
Janssen-Cilag, Pty Ltd
Abbvie, Pty Ltd
Ferring, Pty Ltd
Metabolic and Genetic Infant Cholestasis

START REVISING HERE

This is a big topic
You have been warned
Infant Cholestasis - Metabolic and Genetic

Big Topic
Many Disorders – big lists
Many Investigations – even bigger lists
Focus Key disorders associated with ALF / Transplant
Discuss the variable epidemiology
Evolution of Genomic sequencing for diagnosis
replacing more invasive conventional testing
Discuss several more common / important conditions
Bile Acids (BAs) - major component of bile

- Synthesized from Cholesterol / Cholesterol homeostasis
- Emulsification / Digestion / Absorption of Fat and FSVs (micelles)
- Luminal pathogen surveillance (with Secretory IgA)
- Microbiome modulation

BAs drive bile flow and recycling by

- Uptake re-absorbed BAs from portal vein
- transition through hepatocyte
- excretion into canaliculus and the biliary tree
Cholestasis = Interrupted entero-hepatic BA re-circulation
Usually in the liver

Cholestasis

Final common pathway many liver pathologies

Interrupts synthesis, secretion and modification of BAs

Most genetic causes impair / interfere with canalicular secretion
Why does Cholestasis cause liver damage?

- Not emulsifying / detergent properties of bile!
- Accumulation of intra-cellular bile acids
  - Endoplasmic reticulum (ER) stress
  - Mitochondrial damage
- Attraction and activation of neutrophils
- Hepatocyte and cholangiocyte damage
- Activation of fibrosis cascade
- Cirrhosis / HCC
- Liver failure

Li, Cai, Boyer, Mol Aspects Med 2017
Infants with Cholestasis usually present with Jaundice
Tissue Bilirubin = “flag” for Cholestasis
But Bile salts (colourless) doing the damage
Differential of Infant Cholestasis looks like?
<table>
<thead>
<tr>
<th>Condition</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extra hepatic biliary atresia / Choledochal cyst / Spontaneous perforation bile ducts</td>
<td>Extrahepatic Obstruction Tumors/masses (intrinsic and extrinsic) / Cholelithiasis or biliary sludge Neonatal sclerosing cholangitis</td>
</tr>
<tr>
<td>Infection</td>
<td>Viral Adenovirus, cytomegalovirus (CMV)…….. Bacterial Urinary tract infection, sepsis, syphilis Protozoal Toxoplasma</td>
</tr>
</tbody>
</table>

### Metabolic / genetic diseases

#### Disorders of carbohydrate metabolism
- Galactosemia
- Fructosemia
- Type IV glycogenosis

#### Disorders of amino acid metabolism
- Tyrosinemia

#### Disorders of lipid metabolism
- Wolman, Niemann-Pick C, Gaucher

#### Disorders of bile acid synthesis
- BASDs 1-4 eg BASD-1 = 3-beta-hydroxy-delta-5-C27 ....
- Zellweger Syndrome - Peroxisomal Disorder
- Smith-Lemli-Opitz syndrome

#### Inherited cholestatic disorders
- Alagille syndrome*
- ARC syndrome (arthrogryposis-renal dysfunction-cholestasis)
- Cystic fibrosis
- NISCH syndrome (neonatal ichthyosis sclerosing cholangitis)
- **Progressive familial intrahepatic cholestasis (PFIC), including Byler disease**
- Dubin-Johnson syndrome¶

#### Mitochondrial disorders

#### Other metabolic defects
- Alpha-1-antitrypsin deficiency
- Citrin deficiency
- Congenital disorders of glycosylation

### Endocrine
- Hypopituitarism
- Hypothyroidism

### Toxic
- Drugs
- Parenteral nutrition

### Alloimmune
- GALD (NNH)

### Miscellaneous
Don’t confuse LIST for Neonatal Cholestasis with List for Neonatal Liver Failure and Jaundice – much shorter

TIP

1st 24 hours

Basic Viral screen
Basic Metabolic Screen
No HSM cytopenia / blasts
No overt catastrophe

22/30 = GALD / NNH
(Always give early IVIG)

Shanmugam, Eur J Pediatr 2011
### Table 1: Aetiology of infantile cholestasis

<table>
<thead>
<tr>
<th>Disease category</th>
<th>No. patients</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Idiopathic*</td>
<td>52</td>
<td>25</td>
</tr>
<tr>
<td>Metabolic/genetic</td>
<td>46</td>
<td>23</td>
</tr>
<tr>
<td>Obstructive</td>
<td>41</td>
<td>20</td>
</tr>
<tr>
<td>TPN cholestasis</td>
<td>41</td>
<td>20</td>
</tr>
<tr>
<td>Infective</td>
<td>18</td>
<td>9</td>
</tr>
<tr>
<td>Bile duct hypoplasia</td>
<td>7</td>
<td>3</td>
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<tr>
<td>Total</td>
<td>205</td>
<td>100</td>
</tr>
</tbody>
</table>

Stormon, JPCH 2001
### Epidemiology Infant Metabolic Cholestasis in Australia

<table>
<thead>
<tr>
<th>Disease category</th>
<th>No. patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha-1-antitrypsin deficiency</td>
<td>12</td>
</tr>
<tr>
<td>Panhypopituitarism</td>
<td>10</td>
</tr>
<tr>
<td>Cystic fibrosis</td>
<td>7</td>
</tr>
<tr>
<td>Neonatal iron storage disease</td>
<td>5</td>
</tr>
<tr>
<td>Neonatal lupus erythematosus</td>
<td>3</td>
</tr>
<tr>
<td>Zellweger syndrome</td>
<td>2</td>
</tr>
<tr>
<td>Hereditary fructose intolerance</td>
<td>2</td>
</tr>
<tr>
<td>Byler syndrome</td>
<td>2</td>
</tr>
<tr>
<td>Galactosaemia</td>
<td>1</td>
</tr>
<tr>
<td>Niemann–Pick disease</td>
<td>1</td>
</tr>
<tr>
<td>Gaucher disease</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>46</strong></td>
</tr>
</tbody>
</table>

CF rare in Asia
38 Chinese Cases since 1974

Stormon JPCH 2001
## Peadiatric Acute Liver Failure Infants < 90 days

**PALF (n=148)**

<table>
<thead>
<tr>
<th>Category</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indeterminate</td>
<td>37%</td>
</tr>
<tr>
<td>Inherited Metabolic Diseases</td>
<td>19%</td>
</tr>
<tr>
<td>(Galactosemia, MRCD, NPC Tyrosinemia-I, Urea cycle)</td>
<td></td>
</tr>
<tr>
<td>Viral Hepatitis</td>
<td>16%</td>
</tr>
<tr>
<td>(HSV and Enteroviruses)</td>
<td></td>
</tr>
<tr>
<td>GALD / NNH</td>
<td>13.5%</td>
</tr>
<tr>
<td>Catastrophic / Overt Insults</td>
<td>12%</td>
</tr>
<tr>
<td>Haemophagocytic Syndrome</td>
<td>3%</td>
</tr>
</tbody>
</table>

PALF, Sundaram J Peds 2011.
# Inherited (Cholestatic) Metabolic Diseases

## Paediatric Acute Liver Failure <90 days

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>N (total=148)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metabolic Diseases</td>
<td>28</td>
<td>18.9</td>
</tr>
<tr>
<td>Galactosemia</td>
<td>12</td>
<td>8.1</td>
</tr>
<tr>
<td>Respiratory Chain Defect</td>
<td>5</td>
<td>3.4</td>
</tr>
<tr>
<td>Tyrosinemia</td>
<td>3</td>
<td>2.0</td>
</tr>
<tr>
<td>Neiman Pick Type C</td>
<td>3</td>
<td>2.0</td>
</tr>
<tr>
<td>Mitochondrial Disorder</td>
<td>3</td>
<td>2.0</td>
</tr>
<tr>
<td>Urea Cycle Defect</td>
<td>1</td>
<td>0.7</td>
</tr>
<tr>
<td>OTC Deficiency</td>
<td>1</td>
<td>0.7</td>
</tr>
</tbody>
</table>
Relevance of Metabolic / Genetic Infant Cholestasis

Approximately 20-25% Infant Cholestasis
Approximately 20% Infant Liver transplant in 1st 90 days
Approximately 28% Liver transplant in 1st 5 Years

Relative contribution depends on region
**Investigations Neonatal / Infant Cholestasis – Metabolic ?**

**O/E- Dysmorphology / Iccthyosis / Splenomegaly (storage)**

**Bloods**
- Ammonia
- Lactate
- Acylcarnitine profile
- Amino Acids
- Gal-1-phosphatase
- VLCFAs
- Lysosphingomyelin 509(NPC)
- Alpha-1-Antitrypsin phenotype
- Alpha-fetoprotein
- Transferrin Isoforms for CDG

**Urine**
- Organic Acids
- Amino Acids
- Succinyl acetone
- Bile acid profile
- Sugars / polyols (TALDO-PPP)
Infant Cholestasis Metabolic / Genetic – 2nd Tier Changing Practice

Liver Biopsy – traditional cornerstone of investigation
   Less discriminating (many conditions = cholestasis, ductular reaction)
   Poor sensitivity for many IMDs

Skin biopsy for Fibroblast culture and white cell enzymes?
Muscle biopsy for Respiratory Chain Enzymology?
   Invasive, cumbersome, slow results, confounded by liver damage

Now Genome / Exome Sequencing - equivalent / better information
   - Increasingly popular / less invasive / better sensitivity
   - Cost dropping
   - Turnaround time improving – weeks / months
Conundrum of Mitochondrial (Respiratory Chain) disorders

Molleston, JPGN 2013
Mitochondrial disorders - diagnostic problem
Over-represented in Acute Liver Failure / Transplant setting

Typically difficult to diagnose if multi-organ dysfunction undeclared
Alerted - low blood sugars, high lactate / pyruvate
    - Especially if cardiac or neurological issues
Pages and pages potentially useful investigations
Muscle biopsy / enzymology confounded by liver dysfunction
    Low sensitivity and long delay for result
Genetic Mutations including mtDNA deletions found in 17% of Mitochondrial disorders (DGUOK, POLG, TRMU)
    Quicker / Much less invasive
Genomic / exome sequencing becoming standard for suspected Mitochondrial diseases

McKiernan J inherited Metab disease 2017
Infant Paediatric Cholestasis in Era of Genomic sequencing

When obvious surgical /other causes are excluded

Frequency of detection of genetic mutations

Patient origin

Number / gene mutations on Gene panel (GWAS)

18 genes – 25-30% pick up in Western Society

Alpha-1 Antitrypsin

Metabolic Disorder (Mitochondrial etc)

Alagille’s

PFIC

Bile Acid Synthetic disorders

Cystic Fibrosis

Shagrani, Clin Genet 2017
<table>
<thead>
<tr>
<th>Gene Exome Sequencing 24 genes – Reducing turnaround time</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Neonatal ALF ? = No ! Infant Cholestasis ? = Yes</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Condition</th>
<th>Gene(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alagille Syndrome</td>
<td>Jagged Genes</td>
</tr>
<tr>
<td>Alpha-1-Antitrypsin Deficiency</td>
<td>Serpina</td>
</tr>
<tr>
<td>Arthrogryphosis, Renal dysf, Cholestasis</td>
<td>ARC gene</td>
</tr>
<tr>
<td>Bile Acid Synthetic Disorders 1, 2, 3</td>
<td>Range of Aldolase genes</td>
</tr>
<tr>
<td>Cerebrotendinous Xanthomatosis</td>
<td>CTX</td>
</tr>
<tr>
<td><em>PFIC</em> 1, 2, 3, 4, 5</td>
<td><em>ATP8B1, ABCB1, ABCB4, TJP2, NR1H4</em></td>
</tr>
<tr>
<td>Citrullinemia (Neonatal and adult)</td>
<td></td>
</tr>
<tr>
<td>Hereditary Fructose Intolerance</td>
<td></td>
</tr>
<tr>
<td>Familial Hypercholanemia</td>
<td>(TJP2)</td>
</tr>
<tr>
<td>Iccthyosis, Leukocyte Vacuoles, Alopecia, Sclerosing Cholangitis</td>
<td></td>
</tr>
<tr>
<td>Niemann Pick C</td>
<td></td>
</tr>
<tr>
<td>North American Indian Childhood Cirrhosis</td>
<td></td>
</tr>
<tr>
<td>Zellweger Peroxisomal Biogenesis Disorder 1a / 5a</td>
<td></td>
</tr>
</tbody>
</table>
Saudi - Advanced Pediatric Cholestasis – 60% pick up Contribution Individual Genes (189 genes - NGS)

Top 10
PFIC-2
PFIC-3
PFIC-4
Crigler-Najjar
Wilsons
Tyrosinemia
Dubin - Johnson
Alagille
BASD
PFIC-1

Shagrani, Clin Genet, 2017
Saudi - Clinical Algorithm for Advanced ICholestasis

High Liver Enzymes
High Direct Bilirubin

Low or Normal G.G.T
Cholestasis

High Serum Bile Acid
1) ATP8B1 (PFIC1)
2) ABCB11 (BSEP) Deficiency
3) Familial Hypercholanemia (TJP2)
4) (ARC) Arthrogryposis - Renal dysfunction,
Cholestasis Syndrome*
5) Microvillus inclusion disease

Normal Bile Acid
Bile Acid synthesis defect

High G.G.T Cholestasis

1) ABCB4(MPR3) deficiency
2) Alagille Syndrome
3) Biliary Atresia
4) Choledochal cyst
5) Caroli Disease

*(ARC) Syndrome cased by VPS33B mutation can also present with high G.G.T

Shagrani, Clin Genet, 2017
Infant Cholestasis
In the era of Genomic sequencing

Once overt surgical, metabolic, dysmorphology and endocrine causes excluded
Genomic sequencing is now a reliable rapid technique
Not in time to help Neonatal Liver failure
But for the older child with more time
Permits personalised management, especially for Mitochondrial disorders
Highlight some specific disorders

Commonest Inherited Cholestasis
  Alagille’s
  Alpha 1 antitrypsin

Commonest Metabolic Cholestatic Disorders
  Galactosemia
  Hereditary Fructoseemia
  Tyrosinemia 1
Infant with Cholestasis - ? Alagille’s
Infant Face may not be obvious
Other dysmorphology helps and sibs / parents
Major issues

Multiple growth dysmorphology issues
Cardiac issue determines infant mortality
Biggest issue is retention of bile salts in Liver / skin (itch)
Liver 15-25% will progress

- Portal Hypertension / Cirrhosis / Liver transplant
- Most transplanted for severe itch / prior to ESLD
- Dermal deposits of Bile salts and cholesterol
- Failure of FSV (Vitamins A,D,E) absorption
- Often require parenteral Vit A, D
Treatment Itch (Huge) in Alagille Syndrome

Skin Care (not too dry not to greasy)
Temperature control (reduce ambient heat)
UDCA – no benefit
Rifampicin standard therapy for bile acid itch
  Antagonise opiate receptors?
  Impair re-uptake BAs from Portal Vein?
Surgery to exclude Bile salt reuptake
  Partial external or internal biliary diversion
External biliary diversion – messy but effective in 60-70%

Yang, JPGN 2009)
Alpha – 1- Antitrypsin Deficiency

Serine protease inhibitor (Pi) encoded by SERPINA1

   Autosomal Recessive

   200 variants for dysfunctional Pi protein (limit tissue damage)

   Abnormal Pi accumulates / polymerises in ER

   Damages liver cells and bile acid recirculation

Lung – un-opposed neutrophil elastases – lung disease

10% patients develop Infant Cholestasis usu self limited

   Diagnosed by Serum Pi deficiency / Phenotype (electrophoresis)

20-30% progress to Cirrhosis and risk of HCC

No therapies change natural history Liver disease

Common indication for Liver Transplant (infancy-young adult)
Pittsburgh- Paediatric Liver Transplants (25-30%) for Metabolic Disorders

<table>
<thead>
<tr>
<th>Condition</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha-1-antitrypsin deficiency</td>
<td>73</td>
</tr>
<tr>
<td>Maple syrup urine disease</td>
<td>38</td>
</tr>
<tr>
<td>Familial cholestasis</td>
<td>37</td>
</tr>
<tr>
<td>Wilson’s disease</td>
<td>28</td>
</tr>
<tr>
<td>Tyrosinemia</td>
<td>20</td>
</tr>
<tr>
<td>Cystic fibrosis</td>
<td>19</td>
</tr>
<tr>
<td>Glycogen storage diseases</td>
<td>15</td>
</tr>
<tr>
<td>Crigler-Najjar syndrome I</td>
<td>15</td>
</tr>
<tr>
<td>Urea cycle disorders</td>
<td>14</td>
</tr>
<tr>
<td>Oxalosis</td>
<td>10</td>
</tr>
<tr>
<td>Histiocytosis</td>
<td>5</td>
</tr>
<tr>
<td>Hemochromatosis</td>
<td>4</td>
</tr>
<tr>
<td>Type II hyperlipidemia</td>
<td>2</td>
</tr>
<tr>
<td>Niemann-Pick disease</td>
<td>1</td>
</tr>
<tr>
<td>Neurovisceral storage disease</td>
<td>1</td>
</tr>
<tr>
<td>Acyl-CoA dehydrogenase deficiency</td>
<td>1</td>
</tr>
<tr>
<td>Indian childhood cirrhosis</td>
<td>1</td>
</tr>
<tr>
<td>Erythropoietic protoporphyria</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>285</strong></td>
</tr>
</tbody>
</table>
Successful Hepatocyte Cell Transplant

*Inborn error diseases*

- Alpha1-antitrypsin (34)
- Crigler–Najjar syndrome type I (41–43)
- Familial hypercholesterolemia (45)
- Factor VII deficiency (10)
- Glycogen storage diseases (11,44)
- Infantile Refsum’s disease (12)
- Primary oxalosis (14)
- Phenylketonuria (13,40)
- Progressive familial intrahepatic cholestasis (Dhawan *et al.*, unpublished data)
- Urea cycle defects (7–9,36–40)

Iansante, Ped Research 2018
Galactosemia

1908 = “breast milk induced neonatal nutritional toxicity”
1917 = “Galactosuria” – reducing sugars in urine
    Improved on removing lactose (still best therapy)
1937 - Gal-1-phosphate uridyltransferase deficiency (GALT)
    Failure to convert galactose to glucose

Autosomal Recessive
Presentation – well established / picked up on NB screen
    Early Neonatal period Poor feeding, hypotonia, vomiting, jaundice, hepatomegaly, hypoglycemia. Later cataracts.
Diagnosed looking for serum Gal-1-UDP in serum
Hereditary Fructose Intolerance

Autosomal Recessive Disorder
Deficiency of Aldolase (inability to metabolise fructose)
Onset after fruit or sugar intake
   Usually at weaning from breast milk/formula
   Fruit / solids introduction- unable to tolerate Fructose
      – presentation can be delayed (even to adulthood)
Nausea, vomiting, hypoglycemia, failure to thrive, jaundice
Untreated = multiple metabolic derangements
   = hepatic failure and death
Treatment = avoidance of fructose, sucrose, sorbitol before liver failure
Tyrosinemia - I

Chinsky, Gen in Med, 2017
Tyrosinemia - I

Plasma Tyrosine > 120μmol/L (“flag” not the toxicity)

Autosomal Recessive

Defective activity of Fumarylacetoacetate hydrolase (FAH)

Accumulation of FA in liver cells

- Hepatocyte apoptosis
- Liver damage, fibrosis, cirrhosis
- Liver Failure or chronic disease / HCC

Accumulation in Renal cells renal dysfunction

Presents in Infancy – Neonatal Screening is imminent
Tyrosinemia type I

Majority present < 6 months, usually neonatal period

Acute Liver failure

- Jaundice, INR > 2, hypoalbuminemia, ascites, hypoglycemia
- Unimpressive LFTs ALT/AST 100-200

Investigations

- Urine succinyl acetone
- Plasma amino acids
- AFP >150,000

Treatment

- Dietary restriction phenylalanine / Tyrosine
- Nitisinone (NTBC) blocks Tyrosine pathway
Prognosis for Tyrosinemia I - Quebec Study

Pre 1992 and NTBC
- Death < 2 years “common”
- Survivors - High Mortality from HCC (40%) / Renal failure

Post NTBC
- More than 20 years follow up
- Treatment < 3 months = NO Cirrhosis / No HCC
- Treatment starting >2 years high risk of HCC (13x SMR)

Regular HCC screen
- AFP, USS MRCP
- Transplant for HCC development

Halal, Adv in Exp Med Dis 2017
Metabolic and Genetic Cholestasis

Big Topic
Many disorders
Many Investigations
Focus Key disorders associated with ALF / Transplant
Discussed the variable epidemiology
Evolution of Genomic sequencing for diagnosis
Discussed several key conditions