UNClear – TO DIAGNOSIS AND TREATMENT (FAMILIAL HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS TYPE 3)

Narinder Kaur
The Children’s Hospital at Westmead
Perinatal period

• Baby boy, 8th child of consanguineous parents (2nd degree), 33 yo mother, All 7 siblings well, one passed away at 3 m after a “cold” and bleeding.

• Antenatal scan- Foetal hydrops, abnormal dopplers, Born 35/40, 2.9 kg, Intubated

• Respiratory distress- PHTN
  • Mechanical ventilation, HFO and nitric oxide then CPAP

• Non-immune hydrops – uncertain aetiology
  • Hydrops, ascites, pericardial and plural effusions
  • Albumin infusions and Forced diuresis
  • Mother- O pos, baby O pos, Coombs- neg, G6PD-neg

• DIC
  • Haemorrhages from mouth, nose, puncture wounds, petechiae
  • APTT- >200, INR- >12, PT- >150, Raised D Dimers - 8.21
  • Blood film- microangiopathic haemolysis
  • Needed extensive blood support
Ongoing issues….

- **Conjugated hyperbilirubinemia**
  - Bili- 434, elevated transaminases and hepatosplenomegaly
  - Hepatitis B & C, TORCH (IgG-pos), CMV, EBV, HHV6 HSV-neg
  - Ursodeoxycolic acid started
  - DISIDA scan- markedly reduced hepatocellular function and marked intrahepatic cholestasis.
  - Liver Bx: Non- specific Neonatal Giant Cell Hepatitis
  - Skeletal muscle Bx- normal
  - Resp chain-Complex II and III borderline low- suggested testing in liver
  - UMS, Gag screen, transferrin Isoforms- no abnormality, pompe (alpha glucosidase activity normal)

- **HLH**
  - Persistent fevers, hepatosplenomegaly, pancytopenia, high ferritin (8649 ug/L), low fibrinogen (0.4 g/L) and high Triglycerides (3.4 mmol/L)
  - Bone marrow aspirate- no haemophagocytosis
  - Treated with Prednisone, Cyclosporin, IVIG, Etoposide
  - Igs, C3 and C4, NBT, perforin expression by flow– normal
  - **NK cell function – Absent Cytotoxicity and absent degranulation**
  - **Unc 13 D- homozygous mutation (c.118-309C>T) identified**

- **Sepsis**
  - Klebsiella Pneumoniae, Enterococcus faecalis, S. Epidermidis
  - GCSF for severe neutropenia

- **Hypertension**
  - Thought be secondary to Prednisone and cyclosporin
  - Treated with antihypertensives

- Multiple PICU admissions with sepsis/HTN invasive/non-invasive ventilation.
- Most significant - Cardiac arrest -> Pericardial drain->PICU
Patient received a Haplo-identical HSCT from his sister at age 6m
TCR αβ depletion
Pre-cond-Flud/Treo/Thiotepa/ATG
GVHD pro- TCR αβ depletion/cyclosp
HLH control before conditioning- Alemtuzumab
Post BMT journey

- Persistent HSM with deranged LFTs
- D+35 post BMT:
- Repeat DISIDA scan - significant hepatic parenchymal retention: suggestive of intrahepatic cholestasis.
- Liver Bx:
  - Cholestasis with evidence of bile duct damage, favouring GvHD
- Renal TISSUE:
  - No diagnostic features (inadvertently biopsied). Renal AV fistula
- 9 months post BMT:
  - Liver wedge Bx:
    - Prominent cholestasis, portal/perisinusoidal fibrosis, (ISHAAK GRADE 2)
  - Spleen BX:
    - Reduced white pulp, NO HLH
  - Skin rash treated as GvHD:
    - Skin BX: non-specific superficial dermatitis, no cutaneous GvHD
  - BMA:
    - 2m post trilineage engraftment, 5 m post normocellular, reduced megakaryocytes, no HLH
  - (Chimerism D30 -100% donor cells, D71 -100%, D106 -T cells 99% donor, NK cells)
  - NK cell fn:
    - Cytotoxicity assay NORMAL, Degranulation flow REDUCED. (65% lymphocytes Nk cells)
  - T reg percentage using CD 127 marker was normal.
- 9 m post BMT:
  - Open splenectomy + PEG and Splenectomy

Possibility of another genetic condition

- Refractory HTN
- LV hypertrophy, Renal failure
- Renal US-inc echogenicity of kidney
- Microcephaly
- Thrombocytopenia
- FTT
- Developmental delay-limited a
- Chromosome microarray on pr

- Chromosome copy number: an
  - Heterozygous deletion within c
  - 2 genes, GLRA1 and CTB-1202.1
  - Father carrier of same deletion

- (rare genetic ds. Gene codes for neurons in brain stem and spinal cord. Causes stiff baby syndrome/startle ds. Rarely seizures. Resolves by 12 m)
2007- Dx FHL PRF mutation in 62 yo Japanese man (Nogafuji)
2011- US- retroc- n=1531-175 >18y. Missense/splice site mutations in 25
<table>
<thead>
<tr>
<th>Year</th>
<th>Study</th>
<th>Country, Type of Study, Duration</th>
<th>N</th>
<th>Diagnosis</th>
<th>Genetic Testing</th>
<th>Mortality, Year Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>2015</td>
<td>Kaya, Zuhre</td>
<td>Turkey, Retrospective chart RV, PICU/3 haem units</td>
<td>52</td>
<td>28-FHL- 65%-18 genetic testing (14-P/U/S/SB, 4 RAB/LYST)</td>
<td></td>
<td></td>
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<tr>
<td>2014</td>
<td>Kyung-nam Koh</td>
<td>Korea-Retrospective data analysis, Korea- 15 years</td>
<td>251-25 FHL 64-SHLH 162-unspe</td>
<td>19- Unc 13D/4-PRF 188-medical Rx</td>
<td>32-BMT-5 yr survival 64%</td>
<td></td>
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<tr>
<td>2010</td>
<td>Tunc Fisgin FU 1995-2005</td>
<td>Turkey CM-100% Siblingdeath-81%</td>
<td>22</td>
<td>Genetic testing in 4, STX 11 in 1</td>
<td></td>
<td>86% died 18 died 1-BMT-died GVHD</td>
</tr>
<tr>
<td>2006</td>
<td>Udo Zur Stadt</td>
<td>Turkey/Germany/others</td>
<td>63-32/23/8</td>
<td>38-mutations-14-PRf/6-Unc/6-STX</td>
<td></td>
<td>FHL2,3,4- 80% Turkish 30% Germany</td>
</tr>
</tbody>
</table>
Response to Rx

- **HLH-94 protocol:**
  - Mortality before HLH 94- 91%
  - Remission induction rate 55%

- **HLH-2004 protocol:** (Early Cyclosporin and IVIG)- no published results but earlier combined treatment experience.

- **Hybrid immunotherapy:** HLH (ATG + steroids in induction) – phase II clinical trial in USA
  - Short term complete response 82%

- **Alemtuzumab:**
  - Partial response in 64%

BMT for Familial HLH

**Without BMT:**
- Median survival 1-2 months, 3 year survival <10%

Figure 2. Kaplan-Meier survival curves. (A) All eligible study patients treated with HLH-94 (n=249). (B) All patients with an affected sibling (n=80). (C) All patients who received transplants (n=124). (D) Survival related to HLH disease activity at HSCT (nonactive disease: black line; active disease: grey line; the time in both panels C and D is shown as the time from HSCT).
Stockholm-Annacarine Horne 2005- 86 pts (29FHL) bw 1995 to 2000, Overall 3 yr survival- 64% +/- 10%, Matc relatd (MRD)- 71% +/- 18% Mat URD- 70% +/- 16%, Mismat URD- 54% +/- 27%, Haplo- 50% +/- 24%

Fig 1. Kaplan–Meier survival curves for 86 children with HLH, starting at the time of SCT. (A) The estimated overall probability of survival at 3-years was 64% (±10%). (B) The estimated overall probability of survival according to the donor groups were for matched related donors (MRD) 71% (±18%) (3-year probability of survival, ±95% CI), matched unrelated donors (MUD) 70% (±10%), mismatched unrelated donors (MMUD) 54% (±27%) and for family haploidentical donors (HAPLO) 50% (±24%).
Original Article

Fludarabine-based reduced-intensity conditioning regimen for hematopoietic stem cell transplantation in primary hemophagocytic lymphohistiocytosis

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Into the future..

- Survival improved with HSCT

- Survival better with matched donor, controlled disease and RIC before Tx

- Role of gene therapy

- Correcting defective T cells safer than gene therapy and may allow early control of the HLH through the engraftment of functional gene modified effector T cells.
Acknowledgements

• Dr Melanie Wong, Paediatric Allergist and Immunologist / Pathologist, The Children’s Hospital at Westmead

• Andrew Williams, Principal hospital scientist, CHW (Gene sequencing)

• NK cell function, RPA Hospital

• BMT and PICU team CHW

Thank you
• GLRA1 mutation, 5q32
• Mutation in the gene coding for inhibitory glycine receptor alpha 1 subunit
• Rare genetic disorder
• GR most abundant in neurons in brain stem and spinal cord
• 29 mutations in GLRA1 gene found to cause Hereditary hyperekplexia.
• Aka Stiff baby syndrome or startle disease (hypertonia and exaggerated startle reflex), rarely seizures
• S/S typically fade by 1 year of age
<table>
<thead>
<tr>
<th>HLH Subtype</th>
<th>Gene/Protein</th>
<th>Function</th>
<th>Location</th>
<th>% of Familial Cases</th>
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<tbody>
<tr>
<td>FHL1</td>
<td>Unknown</td>
<td>Unknown</td>
<td>9q21.3-locus 6</td>
<td>~10(^\text{a})</td>
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<tr>
<td>FHL2</td>
<td>PFR1/perforin 1</td>
<td>Cell lysis, membrane pore formation</td>
<td>10q21-22</td>
<td>20-50</td>
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<tr>
<td>FHL3</td>
<td>UNC13D/Munc 13-4</td>
<td>Cytolytic granule exocytosis</td>
<td>17q25</td>
<td>~23</td>
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<tr>
<td>FHL4</td>
<td>STX11/syntaxin 11</td>
<td>Intracellular vesicle trafficking</td>
<td>6q24</td>
<td>~1</td>
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<tr>
<td>FHL5</td>
<td>STXB2/syntaxin binding protein 2 or UNC18B</td>
<td>Intracellular vesicle trafficking</td>
<td>19p13</td>
<td>Unknown</td>
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<tr>
<td>Griscelli syndrome type 2</td>
<td>RAB27A/Rab27a</td>
<td>Vesicle docking on microtubules</td>
<td>15q21</td>
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<tr>
<td>Chédiak-Higashi syndrome</td>
<td>LYST</td>
<td>Vesicle maturation and sorting</td>
<td>1q42.1-q42.2</td>
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<tr>
<td>Hermansky-Pudlak syndrome type 2</td>
<td>AP3B1</td>
<td>Encoding b subunit of AP3, vesicle maturation and transport</td>
<td>5q14.1</td>
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<tr>
<td>XLP type 1</td>
<td>SHD2D1A/SAP protein</td>
<td>Polarization of cytolytic granules for transport to the immunological synapse</td>
<td>Xp25</td>
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<tr>
<td>XLP type 2</td>
<td>BIRC4/XIAP protein</td>
<td>Unclear</td>
<td>Xp25</td>
<td></td>
</tr>
</tbody>
</table>

XLP, X-linked proliferative syndrome.

Table 2
HLH Subtypes and Common Disease Associations

<table>
<thead>
<tr>
<th>Infection</th>
<th>Reported Associations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viral</td>
<td>Herpesviruses (EBV, CMV, HHV-8, HSV), HIV, HTLV, adenovirus, HAV, HBV, HCV, measles, mumps, rubella, dengue, hantavirus, parvovirus B19, enterovirus, influenza</td>
</tr>
<tr>
<td>Bacterial</td>
<td>Staphylococcus aureus, Campylobacter spp, Fusobacterium spp, Mycoplasma spp, Chlamydia spp, Legionella spp, Salmonella typhi, Rickettsia spp, Brucella spp, Ehrlichia spp, Borrelia burgdorferi, Mycobacterium tuberculosis</td>
</tr>
<tr>
<td>Fungal</td>
<td>Candida spp, Cryptococcus spp, Pneumocystis spp, Histoplasma spp, Aspergillus spp, Fusarium spp</td>
</tr>
<tr>
<td>Parasitic</td>
<td>Plasmodium falciparum, Plasmodium vivax, Toxoplasma spp, Babesia spp, Strongyloides spp, Leishmania spp</td>
</tr>
<tr>
<td>Malignancy</td>
<td>Peripheral T-cell/NK-cell lymphomas, ALCL, ALL, Hodgkin lymphoma, multiple myeloma, acute erythroid leukemia, prostate and lung cancer, hepatocellular carcinoma</td>
</tr>
<tr>
<td>Hematologic</td>
<td>Systemic-onset juvenile idiopathic arthritis, Kawasaki disease, systemic lupus erythematosus, seronegative spondyloarthropathies</td>
</tr>
<tr>
<td>Nonhematologic</td>
<td></td>
</tr>
</tbody>
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\(^{a}\) Data based on familial cases.