Passenger Lymphocyte Syndrome (case presentation)

Dr. Namal Bandara
Kings College Hospital
Case history

• 24-year Female

• Known Patient with Wilsons Disease

• DBD donor Liver Transplantation done on 15/08/2016

• Indication acute on chronic liver failure following Wilsons Disease.
Case history

- Haemoglobin level immediate post OP 76g/L
- Improved to 124g/L at POD 6
- Gradually declined thereafter 70g/L at POD 12

- At POD 10 patient developed signs and symptoms suggestive of acute graft rejection laboratory tests and imaging studies confirmed the same
- Methyl Prednisolone Pulse Therapy started
Case History

• Cause for drop in Haemoglobin level?

<table>
<thead>
<tr>
<th>Date</th>
<th>15\textsuperscript{th} Aug (imm PO)</th>
<th>21\textsuperscript{st} Aug (POD 6)</th>
<th>27\textsuperscript{th} Aug (POD 12)</th>
<th>29\textsuperscript{th} Aug (POD 14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb level (g/L)</td>
<td>76</td>
<td>124</td>
<td>70</td>
<td>80</td>
</tr>
<tr>
<td>LDH</td>
<td></td>
<td></td>
<td>▲ 566</td>
<td>(&lt;240IU/L)</td>
</tr>
<tr>
<td>Con Bilirubin</td>
<td>▲ 151</td>
<td></td>
<td></td>
<td>(&lt;4umol/L)</td>
</tr>
<tr>
<td>Haptoglobin</td>
<td>▼ &lt;0.1</td>
<td></td>
<td></td>
<td>(0.8-2.2g/L)</td>
</tr>
</tbody>
</table>

• Haemolytic causes
  • Acute or delayed haemolytic transfusion reactions
  • Autoimmune haemolysis
  • MAHA
  • Drug induced haemolysis
## Findings of the transfusion laboratory

<table>
<thead>
<tr>
<th></th>
<th>Pre Transplant</th>
<th>Post-Transplant</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Antibody screening</strong></td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td><strong>Antibody detected</strong></td>
<td>None</td>
<td>Anti D</td>
</tr>
<tr>
<td><strong>DAT</strong></td>
<td>-</td>
<td>+4 with IgG specificity</td>
</tr>
<tr>
<td><strong>Eluate from DAT positive cells</strong></td>
<td>-</td>
<td>Anti D</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Blood Group</th>
<th>Patient</th>
<th>Donor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B Rh D positive</td>
<td>B Rh D negative</td>
</tr>
</tbody>
</table>
Management

• “Passenger lymphocyte syndrome” was considered as the reason for haemolysis
• Recommended to transfuse B Rh D negative red cells if further red cell transfusions required
• Patient was already on methyl prednisolone pulse therapy
• Haemoglobin level improved without further transfusions.
Passenger Lymphocyte Syndrome (PLS)

• **Definition**
  The PLS refers to the clinical phenomenon of alloimmune haemolysis resulting from the antibodies produced by viable donor B lymphocytes “passenger lymphocytes” in a primary or secondary immune response against the recipient’s red blood cell antigens.

• **History**
  • The appearance of unexpected antibodies of A and B specificity in recipients of kidney allografts from ABO minor mismatched donors was first reported in the early 1980s.
  • Then, more than 100 cases involving liver, kidney, pancreas, spleen, heart, lung, and heart-lung were published in 1991.
Passenger Lymphocyte Syndrome (PLS)

- PLS can occur after:
  - Solid organ transplant
  - Stem cell transplant
  - Administration of B cell rich cellular therapeutic infusions
- PLS most often seen in solid organ transplantation with minor ABO mismatch:
  - Donor Group O / Recipient Group A,
- Also reported with other blood group systems like Rh, K, Fy, Kidd:
  - Donor lacks recipient’s antigens
  - Donor can form antibodies against recipient’s red blood cell antigens
Passenger Lymphocyte Syndrome (PLS)

• Pathophysiology
  • PLS can be considered as a type of graft vs host disease
  • Viable donor lymphocytes passively transferred to the recipient
  • Immunocompetent donor memory B lymphocytes produce antibodies in a secondary immune response against the recipient’s red cells.
  • The massive red cells destruction is thought to be complement-mediated
Primary and secondary immune response

Passenger Lymphocyte Syndrome (PLS)

• Pathophysiology
  • PLS is heterogeneous,
    • Donor B-lymphocytes are detected in recipients but antibody production is either delayed or is never detectable
    • Both donor B-lymphocytes and antibody are detected but haemolysis may not occur
  • The triggers for antibody production and haemolysis is still incompletely understood.
PLS – risk factors

• Donor B-lymphocytes stimulation after transfer
  • Through infections
  • Relative recipient T-lymphocyte inhibition (increased specific immunosuppression such as depleting antibodies, ATG)

<table>
<thead>
<tr>
<th>Table 2: Risk factors for PLS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Risk factors</td>
</tr>
<tr>
<td>------------------------------</td>
</tr>
<tr>
<td>• Blood group O to A transfer</td>
</tr>
<tr>
<td>• Possible sensitizing events: Pregnancy, blood transfusions</td>
</tr>
<tr>
<td>• Cyclosporin use</td>
</tr>
<tr>
<td>• Infection in the immediate posttransplantation period</td>
</tr>
</tbody>
</table>

PLS – Clinical course

- PLS is a self-limiting condition
- Often overlooked as a potential cause of post transplantation anaemia
- Most reported cases of PLS after liver transplant resolve within 3 weeks (median, 20 days), yet positive DAT reportedly persist for over 1 year. *(Gniadek et al. Transfusion 2017;57;1262–1266)*
PLS – Clinical course

• As donor B-lymphocytes fail to self renew, or tolerance of graft lymphoid tissue towards the recipient antigen develops, haemolysis ceases as antibody production declines.

• However, cases of PLS occurring up to 182 days after the start of haemolysis have been reported implying that once transferred and stimulated, B- lymphocytes or plasma cells are capable of sustained new antibody production.

• PLS following solid organ transplantation has been reported to be:
  • 09% for kidney transplants
  • 29% for liver transplants
  • 70% for heart-lung transplants


• These differences are likely to reflect the amount of lymphoid tissue implanted with the corresponding organ transplant
Biochemical PLS in liver transplantation is 30%–40%.

In almost all the cases (68–100%) the antibodies are directed against Rh blood group antigens.

Only 30–40% of these antibodies led to immune haemolysis.

(Maxime Audet et al. Clinical & Developmental Immunology 2008; ID 715769)
Male 50y ESLD $2^{\text{ry}}$ to hepatitis C–induced cirrhosis underwent liver transplantation.

- blood group Patient O Rh+ / Donor O Rh negative female

- procedure occurred without any immediate perioperative complications

Developed severe anaemia with hyperbilirubinemia requiring increasing RBC transfusion support

- 2U group O Rh+ RBC on POD 2
- 2U group O Rh+ RBC on POD 5

- Hb level transient increase, Gradually declined 5.7g/dl on POD 11
Patient had no prior transfusion history

- His antibody screens were negative until POD 11,
- At POD 11 demonstrated the presence of anti-D and anti-K
- The DAT was positive (4+ IgG; no C3d binding detected)
- The elution showing anti-D only

The medical records of the organ were reviewed.
- The donor was found to have antibodies against D, C, and K.
- The donor was C−, E−, and K− (rr, Kell neg)

_Fung et al. Transfusion 2004;44:1635-1639_
PLS in liver transplantation exceptional cases

Fung et al. Transfusion 2004;44:1635-1639

• POD 321, the patient underwent a splenectomy.
• POD 327, started epoetin alfa
• POD 343, Hb level 15.5 g/dL & t bilirubin dropped to 6.9mg/dL
• POD 373,
  • antibody screen remained positive with 1+ reactivity against K, but not against either D or C. The DAT and eluate were negative
PLS in liver transplantation exceptional cases

Gniadek et al. Transfusion 2017;57;1262–1266

- 34-year-old male patient, blood group O pos
- History of hepatitis C and alcoholic hepatitis.
- Underwent a liver transplant from a group O, neg donor,
- Proceeded without intraoperative complications,
- Required 20 units of group O pos RBCs during surgery
PLS in liver transplantation exceptional cases

Gniadek et al. Transfusion 2017;57;1262–1266

• Stable until POD 5,

• Developed signs and symptoms consistent with RTI

• By POD 7, his anaemia had worsened,

• Antibody screen became weakly positive against some, but not all, Rh D+ screening and panel cells.

• A DAT was positive, and IgG anti-D was identified in eluate studies

• Switched to group O neg transfusion protocol
PLS in liver transplantation exceptional cases

*Gniadek et al. Transfusion 2017;57;1262–1266*

<table>
<thead>
<tr>
<th>POD 10</th>
<th>Haptoglobin decreased to less than 6 mg/dL</th>
</tr>
</thead>
<tbody>
<tr>
<td>POD 11</td>
<td>Indirect bilirubin rose to 1.7 with a total bilirubin of 3.4 mg/dL</td>
</tr>
<tr>
<td>POD 12</td>
<td>Erythropoietin elevated to 1025 ng/mL</td>
</tr>
<tr>
<td>POD 14</td>
<td>LDH peaked at 649 U/L</td>
</tr>
</tbody>
</table>
PLS in liver transplantation exceptional cases

Gniadek et al. Transfusion 2017;57;1262–1266

<table>
<thead>
<tr>
<th>POD</th>
<th>ABO/Rh</th>
<th>Antibody screen, panel</th>
<th>DAT</th>
<th>Eluate</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>O pos</td>
<td>Negative</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>O pos</td>
<td>Negative</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>O pos</td>
<td>Inconclusive</td>
<td>Positive (IgG only)</td>
<td>Anti-D</td>
</tr>
<tr>
<td>10</td>
<td>O pos</td>
<td>Anti-D</td>
<td>Positive (IgG only)</td>
<td>Not tested</td>
</tr>
<tr>
<td>12</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>O neg</td>
<td>Anti-D</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>O neg</td>
<td>Anti-D</td>
<td></td>
<td></td>
</tr>
<tr>
<td>48</td>
<td>O neg</td>
<td>Anti-D</td>
<td></td>
<td></td>
</tr>
<tr>
<td>63</td>
<td>O pos</td>
<td>Anti-D</td>
<td></td>
<td></td>
</tr>
<tr>
<td>76</td>
<td>O pos</td>
<td>Inconclusive</td>
<td>Positive (IgG only)</td>
<td>Anti-D</td>
</tr>
<tr>
<td>168</td>
<td>O pos</td>
<td>Negative</td>
<td>Positive (IgG only)</td>
<td>Not tested</td>
</tr>
<tr>
<td>233</td>
<td>O pos</td>
<td>Negative</td>
<td>Positive (IgG only)</td>
<td>Anti-D</td>
</tr>
</tbody>
</table>

* The table shows blood bank serologic test results after liver transplantation.
PLS in liver transplantation exceptional cases

Gniadek et al. Transfusion 2017:57:1262–1266

The reticulocyte index decreased markedly and remained suppressed until POD 33.
To exclude an infectious aetiology extensive infectious disease testing was performed, all were negative:

- blood and sputum cultures;
- Histoplasma, cryptococcal, and Aspergillus (galactomannan) antigen testing;
- cytomegalovirus, Epstein-Barr virus, and HIV nucleic acid testing.

Preoperative serology for IgG anti-CMV indicated that the donor was negative, but the recipient was positive.

Parvovirus B19 nucleic acid tests of plasma and marrow were negative.

Medications modified suspecting drug induced anaemia/haemolysis.
PLS in liver transplantation exceptional cases

Gniadek et al. Transfusion 2017;57;1262–1266

- Reticulocytopenia / anaemia /normal erythropoietin levels,
- Needed excluding an intrinsic marrow or RBC disorder.
  - Flow cytometry-based testing for PNH was unremarkable
  - A marrow biopsy revealed erythroid hyperplasia, but no signs of MDS
  - MDS FISH panel and marrow cytogenetic studies - no evidence of a myeloproliferative neoplasm or MDS
  - Prussian blue iron-stained marrow aspirate & Plasma iron studies were unremarkable
  - Other pertinent laboratory test results included normal folate and B12 levels
- It was concluded that haemolysis due to PLS together with reticulocytopenia
Be vigilant about ABO and minor blood group incompatibilities

Identify the risk factors

Daily DAT starting 3 – 4 days post op aids early detection

Use of donor-compatible RBCs prophylactically in the perioperative and immediate postoperative setting might reduce the development of graft associated immune haemolysis, but remains an untested hypothesis.

_Fung et al. Transfusion 2004;44:1635-1639_
Proposed algorithm for the detection and treatment of PLS.


1. Fall in haemoglobin 1-4 weeks post renal transplant
   - Imaging i.e. ultrasound to exclude bleeding
   - No bleeding source identified
   - Investigations: Hb, LDH, Reticulocytes, bilirubin, haptoglobins, DAT, blood film, CNI level
   - Elution studies to detect antibody to suspected RBC antigen (e.g. Anti-A)
   - PLS Confirmed

2. Treatment:
   - Conservative if haemoglobin stable
   - Transfusion support with DONOR blood group if required (e.g. Hb<8 and/or symptomatic anaemia)
   - Increase steroids – prednisolone 1mg/kg
     - If not resolving
     - Stop CNI
     - Plasmapharesis / Rituximab
References

- Maxime Audet et al. Clinical & Developmental Immunology 2008; ID 715769
- Fung et al. Transfusion 2004;44:1635-1639
- Gniadek et al. Transfusion 2017;57;1262–1266
Acknowledgments

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  *Kings College Hospital*
Thank you

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