Monitoring of human uterus transplantation with cervical biopsies - a provisional scoring system for rejection.

Johan Mölne

Department of Pathology and Genetics, Sahlgrenska Academy, University of Gothenburg, Sweden
Disclosures

• None
Introduction

• Absolute uterine factor infertility (AUFI) has until now been the major untreatable form, of female infertility (~1:10,000).
  – uterine agenesis (Mayer-Rokitansky-Küster-Hauser syndrome)
  – hysterectomy

• Recently uterus transplantation has proven to be the first option for treatment of AUFI, with the demonstration of successful live births.
History of uterus transplantation

• First transplant in Saudi Arabia 2000
  – vascular thrombosis after ≈ 3 months

• Uterus tx from deceased donor in Turkey 2011
  – technical success but no childbirth

• First child born in Gothenburg 2014
  – Utx trial started 1999, small and large animals, primates and finally humans
  – 9 women transplanted, 2 early failures
  – 6 healthy babies born so far
Today

• Several centers have started Utx trials
  – Nov 2015 a living donor in China
  – 2016-17 eleven living donor cases
    • 6 in Dallas, USA
    • 3 in Prague, Czech Republic
    • 1 in Tubingen, Germany
    • 1 in China
  – 2016-17 five deceased donors
    • 3 in Prague, Czech Republic
    • 1 in Cleveland, USA
    • 1 in Sao Paulo Brazil

• No children born yet
• Early failures reported in USA
Immunosuppressive therapy

• Kidney-type regimen used in Gothenburg

• Induction therapy
  – ATG (2,5 mg/kg body weight) and steroids (500 mg Solu-Medrol) day 0 and day 1

• Maintenance therapy
  – mycophenolate mofetil (MMF) and tacrolimus for six to eight months
  – thereafter tacrolimus monotherapy - if possible
Immunosuppression - protocol

- Thymo 5mg/kg
- Solu-Medrol 500mg

Legend:
- red: tac
- yellow: pred
- gray: MMF

Timeline:
- tx
- 3m
- 6m
- 12m
Biopsies

• Cervical punch biopsies were used
  – needle biopsies for endometrium/myometrium on 2 occasions

• Protocol schedule
  – 1, 2 and 4 weeks
  – thereafter monthly for at least a year
  – after one year, 1-3 month intervals
Histology

• Biopsies were fixed in formalin for > 2h under constant shaking, embedded in paraffin, sectioned and stained with H&E

• Immunoperoxidase was used in selected cases
  – CD3, CD56, CD20, CD68 (C3c, C5b-9) all from Dako
  – C4d (ab 58781; Abcam, Cambridge, UK)
Grading of rejection

• Adopted schedule from a baboon Utx trial

• The schedule was revised (at 36 months post transplantation) and a borderline category added
Normal cervical biopsy

Borderline changes

Grade 1 rejection
Grade 2 rejection

Grade 3 rejection
• **Borderline changes**

At least two, small, nested foci of interface inflammation in the basal epithelial cell layers, dominated by lymphocytes.
Focal intercellular edema.
Minimal stromal inflammation, particularly in the papillary stroma.

• **Grade 1 rejection**

Mild mixed inflammatory cell infiltrate in the basal epidermal layer, dominated by lymphocytes.
Single epithelial apoptotic bodies.
Low-grade stromal inflammation and edema.
Grade 2 rejection

Moderate inflammatory cell infiltrate with intraepidermal influx (exocytosis), dominated by lymphocytes and with some neutrophils.
May show reduced surface epithelial thickness, some epithelial apoptotic bodies.
Marked, mixed stromal inflammation and edema

Grade 3 rejection

Significant diffuse, mixed inflammatory cell infiltrate dominated by lymphocytes and presence of neutrophils and eosinophils.
Apoptotic bodies.
Epithelial erosions/ulcerations, focal to complete.
Focal necrosis can be seen.
Dense and continuous stromal infiltrate (mixed).
Normal biopsy
Borderline changes
Borderline change CD3
Borderline change CD20
Borderline change CD68
Grade 1 rejection
Grade 1 rejection CD3
Grade 1 rejection CD20
Grade 2 rejection
Grade 2 rejection
Grade 2 rejection CD3
Grade 2 rejection CD20
Grade 2 rejection CD68
Grade 3 rejection
Grade 3 rejection CD20
Grade 3 rejection CD68
Summary

- Normal
- Borderline changes
- Grade 1 rejection
- Grade 2 rejection
- Grade 3 rejection
- After treatment (G1)
Results

163 biopsies were performed within the first 36 months in 7 patients.

13 biopsies (8.0%) in five patients showed rejection.

15 biopsies (9.2%) in five patients were evaluated as borderline changes.

135 (82.8%) were normal.
• Staining for CD3, CD20, and CD68 showed a T-cell dominant inflammation but staining was not considered necessary for routine clinical practice.

• C4d staining was negative in all biopsies investigated and none of the patients had DSA – (complement staining was not useful)
Table 4 Maximal immune cell infiltrate.

<table>
<thead>
<tr>
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<th>CD3</th>
<th>CD20</th>
<th>CD68</th>
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<tbody>
<tr>
<td><strong>Baseline biopsies, n=5</strong></td>
<td>42 (25-50)</td>
<td>0</td>
<td>21 (10-25)</td>
</tr>
<tr>
<td><strong>Normal transplant biopsies, n=8</strong></td>
<td>50 (25-100)</td>
<td>6.2 (0-25)</td>
<td>49 (10-100)</td>
</tr>
<tr>
<td><strong>Borderline changes, n=11</strong></td>
<td>115 (50-200)</td>
<td>8.8 (0-100)</td>
<td>48 (10-100)</td>
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<tr>
<td><strong>Grade 1 rejection, n=9</strong></td>
<td>122 (50-500)</td>
<td>61 (0-200)</td>
<td>41 (10-100)</td>
</tr>
<tr>
<td><strong>Grade 2 and 3 rejection, n=4</strong></td>
<td>425 (200-500)</td>
<td>137 (50-200)</td>
<td>32 (10-100)</td>
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Mean values and (range)
No serious adverse effects of the IS, no infections
Thanks to all coworkers!

- **Mats Brännström** and his large multidisciplinary team!
- Verena Bröcker – Pathology
- Ola Nilsson – Pathology
- Jana Ekberg – Nephrology and transplantation
Banff CTA 2007 classification of skin containing CTAs

• Grade 0
  No or rare inflammatory infiltrates.

• Grade I
  Mild. Mild perivascular infiltration. No involvement of overlaying epidermis.

• Grade II
  Moderate. Moderate-to-severe perivascular inflammation with or without mild epidermal and/or adnexal involvement. No epidermal dyskeratosis or apoptosis.

• Grade III
  Severe. Dense inflammation and epidermal involvement with epithelial apoptosis, dyskeratosis and/or keratinolysis.

• Grade IV
  Necrotizing acute rejection. Frank necrosis of epidermis or other skin structures.
Rejections (first 36 months)

- G1-G3 on 13 biopsies (7 rejection episodes)
- 2 patients had 0 rejections
- 3 patients had 1 rejection
- 1 patient had 2 rejection episodes
- 1 patient had 3 rejections
- 2 early failures (thrombosis, ischemia/infection)

All treated with steroids (S-M / pred)
One rejection - steroid-resistant, treated with Thymoglobulin