Lymphangiogenesis in Bronchiolitis Obliterans Syndrome

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Background
Bronchiolitis Obliterans Syndrome

BOS is defined as a progressive airflow obstruction that cannot be explained by acute rejection, infection or other confounding complications.

Pathogenesis is still obscure, a major role of the **adaptive immune system** is indicated via both **alloimmune** & **non-alloimmune** mechanisms

- disruption of the balance between type 1, 2, 17 and Treg immune response
- alloimmune reactivity driven by HLA mismatch
- humoral immunity
- autoimmunity

Pathogenesis is still obscure, a major role of the **adaptive immune system** is indicated via both **alloimmune** & **non-alloimmune** mechanisms

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**Risk factors**

- acute rejection
- CMV pneumonitis/infection
- HLA mismatching
- primary graft dysfunction
- aspiration
- lymphocytic bronchitis/bronchiolitis
- EBV reactivation
- prolonged allograft ischemia

**Background**

**Bronchiolitis Obliterans Syndrome**

**Diagnosis** is usually made by clinical, physiological and radiographic parameters.

<table>
<thead>
<tr>
<th>2001 Classification according to the ISHLT</th>
<th></th>
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</thead>
<tbody>
<tr>
<td>BOS 0</td>
<td>FEV$<em>1$ &gt; 90% of baseline and FEF$</em>{25-75}$ &gt; 75% of baseline</td>
</tr>
<tr>
<td>BOS 0-p</td>
<td>FEV$<em>1$ 81 – 90% of baseline and/or FEF$</em>{25-75}$ ≤ 75% of baseline</td>
</tr>
<tr>
<td>BOS I</td>
<td>FEV$_1$ 66 – 80% of baseline</td>
</tr>
<tr>
<td>BOS II</td>
<td>FEV$_1$ 51 – 65% of baseline</td>
</tr>
<tr>
<td>BOS III</td>
<td>FEV$_1$ &lt; 50% of baseline</td>
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</tbody>
</table>

Sustained (≥ 3 weeks) decline of FEV$_1$ and other possible causes resulting in this decline are excluded.

**Background**

*Bronchiolitis Obliterans Syndrome*

**TBBx** does not depict a sufficient method for diagnosis

**Obliterative bronchiolitis** characterised by *subepithelial fibrosis* resulting in luminal occlusion, *atrophy of smooth muscle, destruction of the elastic part* of the airway wall are is rarely seen mostly *mucostasis* or *foamy histiocytes* may be seen

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DE JONG PA (2011). Thin-section Computed Tomography findings before and after azithromycin treatment of neutrophilic reversible lung allograft dysfunction
SABRI YY (2013). Bronchiolitis Obliterans (BO): HRCT findings in 20 patients
Background
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Radiological hallmarks
Decreased attenuation and vascularity
Mosaic perfusion pattern
Air trapping at expiratory images

DE JONG PA (2011). Thin-section Computed Tomography findings before and after azithromycin treatment of neutrophilic reversible lung allograft dysfunction
SABRI YY (2013). Bronchiolitis Obliterans (BO): HRCT findings in 20 patients
Treatment options are disappointing, a stabilisation or reduction of decline in \( \text{FEV}_1 \), but rarely an improvement has been documented:

- adjustment in immunosuppressive therapy
- azithromycin
- extracorporeal photopheresis
- retransplantation

Background

Bronchiolitis Obliterans Syndrome

Treatment options are disappointing, a stabilisation or reduction of decline in FEV$_1$, but rarely an improvement has been documented:

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By the time BOS is diagnosed irreversible damage to the airways has occurred so that the key to increasing survival is successful prevention by e.g. reduction of risk factors and regular follow-up visits.

Lymphangiogenesis, the growth of new lymphatic vessels, occurs in physiological & pathological processes in both developmental states & adult individuals

- tissue inflammation
- transplant rejection
- tumour metastases
- wound healing
- development of the corpus luteum

Lymphatic vessels display the afferent arm of the lymphatic system

Many similarities with the blood vascular system

Present in all vascularised tissues except for bone marrow, retina & CNS

Crucially involved in

- regulation of tissue fluid
- immune defence
- absorption and transportation of triglycerides & lipophilic compound

OLIVER G (2010). Endothelial cell plasticity: how to become and remain a lymphatic endothelial cell.
JELTSCH M (2003). Genesis and pathogenesis of lymphatic vessels
Centrifugal theory of the embryologic development of lymphatic vessels

originating from embryonic veins

at embryonic week 6-7

several key regulators in embryonic development are also involved in adult lymphangiogenesis

Molecular mechanisms in lymphangiogenesis

VEGFR-3/VEGF-C/-D Axis

proliferation, migration & survival of lymphatic endothelial cells

overexpression of VEGF-C/-D stimulates lymphangiogenesis (e.g. released by macrophages)

Prox-1 – the master regulator of lymphatic cell fate

Podoplanin – crucial in separation of lymphatic vessels from veins

LYVE-1 – involved transportation of leukocytes throughout lymphatics

All can serve as specific markers for lymphatic vessels

RUIZ DE ALMODOVAR C (2009). Role and Therapeutic Potential of VEGF in the Nervous System
ABTAHIAN F (2003). Regulation of blood and lymphatic vascular separation by signaling proteins SLP-76 and Syk.
JACKSON DG (2001). LYVE-1, the lymphatic system and tumor lymphangiogenesis.
Background

Lymphangiogenesis in Transplantation

It is suggested that lymphatic vessels are involved in the pathogenesis of graft rejection by **presenting antigens, trafficking immune response** and **regulation of fluid homeostasis** and **tissue edema** in either a beneficial or harmful way.

**Previous results in...**

renal transplantation: increase of lymphatic vessels in kidney grafts – **indicator for superior outcome?**

controversial role in acute rejection – **possible exit route for mononuclear cells?**

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DIETRICH T (2010). Cutting edge: lymphatic vessels, not blood vessels, primarily mediate immune rejections after transplantation.
It is suggested that lymphatic vessels are involved in the pathogenesis of graft rejection by **presenting antigens**, **trafficking immune response** and **regulation of fluid homeostasis** and **tissue edema** in either a beneficial or harmful way.

**Previous results** in...

- **renal transplantation**: increase of lymphatic vessels in kidney grafts – *indicator for superior outcome*?
- controversial role in acute rejection – *possible exit route for mononuclear cells*?

- **corneal transplantation**: experimental corneal transplantation as a model for allogeneic transplantation
  inhibition of lymphangiogenesis induces alleviation of graft rejection

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DIETRICH T (2010). Cutting edge: lymphatic vessels, not blood vessels, primarily mediate immune rejections after transplantation.
Previous results in...

liver transplantation: lymphangiogenesis is induced in acute rejection - *involvement in resolution?*

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Background

Lymphangiogenesis in Transplantation

**Previous results in...**

liver transplantation: lymphangiogenesis is induced in acute rejection - *involvement in resolution*

heart transplantation: increased lymphatic vessels density in severe acute rejection

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Background
Lymphangiogenesis in Transplantation

Previous results in...

- liver transplantation: lymphangiogenesis is induced in acute rejection - involvement in resolution

- heart transplantation: increased lymphatic vessels density in severe acute rejection

- lung transplantation: increased LVD in acute rejection, induction of lymphangiogenesis in a rat model for obliterative airway disease in a VEGF-C dependent manner, inhibition by Cyclosporin A

Aims of the Study

Evaluation of podoplanin positive lymphatic vessels indicating lymphangiogenesis in BOS patients and control subjects

→ increased lymphangiogenesis in BOS patients?
→ correlation of lymphatic vessels with time to BOS III diagnosis?
**Study collective**

36 patients
- 23 BOS patients
- 13 control subjects

**Inclusion Criteria**

<table>
<thead>
<tr>
<th>BOS patients</th>
<th>Control subjects</th>
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<tbody>
<tr>
<td>retransplantation at the Division of Thoracic Surgery, MUW</td>
<td>Surgery at the Division of Thoracic Surgery, MUW</td>
</tr>
<tr>
<td>BOS as indication of retransplantation</td>
<td>healthy lung tissue could be obtained from this surgical operation</td>
</tr>
<tr>
<td>verification of BOS by lung function testing</td>
<td>confirmation by a pathologist</td>
</tr>
<tr>
<td></td>
<td>sufficient peribronchial tissue</td>
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</table>
Material & Methods

Immunolabelling of lymphatic vessels via immunohistochemistry with *podoplanin* as a marker for lymphatic epithelium

http://www.leinco.com/immunohistochemistry (July 24th, 2015)
TissueFAXS

automatic scanning of a whole slide
selection of regions of interests with TissueFAXS Viewer

Material & Methods

Evaluation of regions of interest

- Lymphatic vessels per bronchiole
- Lymphatic vessels per mm bronchial epithelium
- μm lymphatic endothelium per mm bronchial epithelium
- Correlation of lymphatic vessels with time to BOS III diagnosis

Statistics

- SPSS Statistics 21
- GraphPad Prism 5
### Results

**Demographics**

<table>
<thead>
<tr>
<th></th>
<th>BOS</th>
<th>Control</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>23</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Presence of infiltrates</td>
<td>15/20</td>
<td>3/13</td>
<td>0.0946</td>
</tr>
<tr>
<td>(with/without)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex (ma/fe) (%)</td>
<td>12 (52) / 11 (48)</td>
<td>7 (54) / 6 (46)</td>
<td>0.923</td>
</tr>
<tr>
<td>Diagnosis (%)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>α1-AT deficiency</td>
<td>1 (4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bronchiectasis</td>
<td>1 (4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>COPD</td>
<td>5 (22)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTEPH</td>
<td>1 (4)</td>
<td></td>
<td></td>
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<tr>
<td>Cystic fibrosis</td>
<td>8 (35)</td>
<td></td>
<td></td>
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<tr>
<td>LAM</td>
<td>1 (4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pulmonary hypertension</td>
<td>1 (4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pulmonary fibrosis</td>
<td>5 (22)</td>
<td></td>
<td></td>
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<tr>
<td>LuTX type (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DLuTX</td>
<td>17 (74)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SLuTX</td>
<td>6 (26)</td>
<td></td>
<td></td>
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<tr>
<td>Mean ischemic time, min</td>
<td>303 ± 74</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mean ± SD)</td>
<td></td>
<td></td>
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<tr>
<td>ECMO bridging to</td>
<td>2 (11) / 16 (89)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>transplantation (y/n) (%)</td>
<td></td>
<td></td>
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<tr>
<td>Intraoperative ECMO</td>
<td>10 (59) / 7 (41)</td>
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<tr>
<td>support (y/n) (%)</td>
<td></td>
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<tr>
<td>Mechanical ventilation,</td>
<td>3 ± 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>d (mean ± SD)</td>
<td></td>
<td></td>
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<tr>
<td>ICU stay, d (mean ± SD)</td>
<td>8 ± 5</td>
<td></td>
<td></td>
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<tr>
<td>Hospital stay, d (mean ±</td>
<td>24 ± 11</td>
<td></td>
<td></td>
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<tr>
<td>SD)</td>
<td></td>
<td></td>
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<tr>
<td>Immunosuppression</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Cyclosporine A (y/n)</td>
<td>8/13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mycophenolic acid (y/n)</td>
<td>19/2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tacrolimus (y/n)</td>
<td>13/8</td>
<td></td>
<td></td>
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<tr>
<td>Azathioprine (y/n)</td>
<td>2/19</td>
<td></td>
<td></td>
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<tr>
<td>Time to ReTX, m (mean ±</td>
<td>84.13 ± 51.76</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SD)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Time to BOS III diagnosis, m (mean ± SD)</td>
<td>62.65 ± 45.28</td>
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</table>
Results
Lymphatic Vessel Density

BOS

Control
Results

Lymphatic Vessel Density

Lymphatic vessels per mm bronchial epithelium

µm lymphatic endothelium per mm bronchial epithelium

[Graphs showing comparison between BOS and Control groups with and without infiltrates.]
Results

Lymphatic Vessel Density

Correlation of LVs per bronchiole with time to BOS III diagnosis
Lymphangiogenesis does not seem to play a role in bronchiolitis obliterans syndrome

increase of lymphatic vessels found in acute rejection of lung allografts at day 14, however no further increase at day 90 – formed lymphatic vessels are already sufficient

equal distribution of inflammatory infiltrates in both groups may indicate a similar inflammatory situation

Cyclosporin features an inhibitory effect on VEGF-C and leads to a decrease of LYVE-1+ cells in an obliterative bronchiolitis rat model – patients in this study have received Cyclosporin A
Thank you for your attention!