Early endothelial damage: pathophysiology, prevention and treatment.

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Director, Spanish Bone Marrow Donor Program
Human body contains 1-6 x 10^{13} endothelial cells weighing \approx 1.5 \text{ kg} and covering a surface of 4-7000 sqm.
Vascular endothelium

Input signals ►
- Cell interaction
- Soluble mediators
- Oxygenation
- Hemodynamic
- Temperature, pH

Output signals ► changes in:
- Vasomotor tone
- Permeability
- Haemostatic balance
- Inflammatory response
- Cell proliferation & survival

Basal  ➔  Activation

Adaptive response (physiological)
Vascular endothelium

**Output signals**
- Cell swelling
- Loss of mitochondria
- Apoptosis
- MHC antigen presentation
- Procoagulant phenotype
- Increased permeability
- ↑ leukocyte trafficking
- Secretion inflammatory mediators

**Net liability to the host**
Sepsis/SIRS
First morphological changes observed in VOD occur in the sinusoidal endothelial cells.
veno-occlusive disease

capillary leak syndrome

SCT associated thrombotic microangiopathy

diffuse alveolar haemorrhage

engraftment syndrome

Overlapping clinical manifestations
Could all them be initiated by an endothelial damage?
- fibrin-related aggregates
- platelet and leukocyte adhesion to the endothelium
- endothelial apoptosis

Organ dysfunction

endothelial dysfunction

endothelial activation

procoagulant status

inflammatory response

↑permeability

vasoconstriction

Endothelium in HSCT

Endothelium

Conditioning regimen

allo-reactivity

neutrophils

IL-1 / IL-2 / TNF-α / IFN-γ

LPS/DAMPs

CNI
thrombotic microangiopathy
capillary leak syndrome
engraftment syndrome
diffuse alveolar haemorrhage
VOD
thrombotic microangiopathy
Organ dysfunction
Endothelial dysfunction after HSCT

- HSCT performed between 2007-2010
  - **Autologous HSCT** (BEAM / MLF)
  - **Allogeneic HSCT** (Cy-TBI / Flu-MLF) (MAC vs RIC)

- **Samples** (through CVC)
  - Pre          Day 0        +7          +14        +21

- **Samples** (through CVC)
  - Conditioning
  - HSCT

- Soluble markers of endothelial damage
  - von Willebrand factor (vWF)
  - ADAMTS-13 activity
  - sVCAM-1
  - sIVAM-1
  - TNF-alfa receptor I (sTNFR I)

**ex vivo studies**
vWF

**ex vivo studies**

**BEAM**

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**MLF**

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**Cy/TBI (MAC)**

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**Flu/MLF (RIC)**

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* auto

* allo

- BEAM
- MLF
- Cy/TBI (MAC)
- Flu/MLF (RIC)

Palomo et al. BB&MT 2010
sVCAM-1/sTNFRI ex vivo studies

**sVCAM-1**

- **auto**
  - Pre 0 +7 +14 +21
  - 0,5 1 1,5 2 2,5

- **allo**
  - Pre 0 +7 +14 +21
  - 0,5 1 1,5 2 2,5

**sTNFRI**

- **auto**
  - Pre 0 +7 +14 +21
  - 0,5 1 1,5 2 2,5

- **allo**
  - Pre 0 +7 +14 +21
  - 0,5 1 1,5 2 2,5

---

**BEAM**

- **Cy/TBI**
  - Pre 0 +7 +14 +21
  - 0,5 1 1,5 2 2,5

- **Flu/MLF**
  - Pre 0 +7 +14 +21
  - 0,5 1 1,5 2 2,5

**MLF**

- **Cy/TBI**
  - Pre 0 +7 +14 +21
  - 0,5 1 1,5 2 2,5

- **Flu/MLF**
  - Pre 0 +7 +14 +21
  - 0,5 1 1,5 2 2,5

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*Palomo et al. BB&MT 2010*
Endothelial dysfunction after HSCT

**in vitro studies**

**Macrovascular EC**

HUVEC
MEM199 + 20% Pool of patients sera

**Endothelial cells monolayer**

**Microvascular EC**

HMEC-1
MEM199 + 20% Pool of patients sera

**Extracellular matrix**

**Immunochemistry**
Adhesion receptors: VCAM-1, ICAM-1 y E-Selectin
Adhesion proteins: vWF, TF

**Perfusion studies**
Leucocytes over endothelial cells
Platelets over extracellular matrix
Material and Methods

**HUVEC/HMEC**
20% patients sera

Signal transduction mechanisms
- p38 MAPK, SAPK/JNK and Erk 42/44

**INFLAMMATION**
- Osmotic Shock
- Genotoxic Agents
- Antisense
- Inflammatory Cytokines
- TNF-α, IL-1

**PROLIFERATION**
- Growth Factors
- EGF
- GRB
- SOS
- RAS
- p38 MAPK

**APOPTOSIS**
- Cell Integrity
- Membrane Blebbing
- DNA Fragmentation
- Apoptosis
Inflammation: Adhesion Molecules

AUTOLOGOUS SCT

ALLOGENEIC SCT

Gold particles/um²

Pre  Day 0  +7  +14  +21

VCAM-1

ICAM-1

E-Selectin

AUTOLOGOUS SCT

ALLOGENEIC SCT

P- p38MAPK

p38MAPK

Palomo et al. BB&MT 2009
Inflammation: Leukocyte adhesion to EC

CONTROL  AUTO 14  ALLO 21

Leukocyte adhesion (%)

AUTOLOGOUS SCT

ALLOGENEIC SCT

Leukocyte adhesion (%)

Palomo et al. BB&MT 2009
Thrombogenicity of the ECM (platelet adhesion)

CONTROL
AUTO 14
ALLO 21

AUTOLOGOUS SCT

ALLOGENEIC SCT

Palomo et al. BB&MT 2009
Thrombogenicity of the ECM (vWF concentration)

vWF

AUTOLOGOUS SCT

ALLOGENEIC SCT

Gold particles / um²

Palomo et al. BB&MT 2009
**Proliferation**

**HUVEC**

**CONTROL**

**AUTO 14**

**ALLO 21**

### AUTOLOGOUS SCT

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### ALLOGENEIC SCT

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*Palomo et al. BB&MT 2009*
Apoptosis

CONTROL

ALLO 21

Palomo et al. BB&MT 2009
## Endothelial changes after HSCT

### Veno-occlusive disease (SOS)
- Thrombotic microangiopathy

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Auto-HSCT</th>
<th>Allo-HSCT</th>
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<tbody>
<tr>
<td>-Proinflammatory</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>-Prothrombotic</td>
<td>±</td>
<td>+++</td>
</tr>
<tr>
<td>-Proliferation</td>
<td>++</td>
<td>++</td>
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<tr>
<td>-Proapoptotic</td>
<td>-</td>
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</table>

### Capillary leak syndrome
- Engraftment syndrome
Endothelial damage after HSCT

**AUTOLOGOUS HSCT**

- **Conditioning**
- **G-CSF**
- **Engraftment**
- **Pre**
- **Day 0**
- **Day 7**
- **Day 14**
- **Day 21**

**ALLOGENEIC HSCT**

- **Conditioning**
- **Engraftment**
- **Alloreactivity?**
- **Pre**
- **Day 0**
- **Day 7**
- **Day 14**
- **Day 21**
Endothelial damage is not only a consequence of GvHD but could also be a possible triggering event.

Penack et al. Blood 2011
Activated endothelial cells can express and present antigens.

Activated EC will be exposed twice to donor lymphocytes that could recognize the antigens presented.

GvHD and endothelium

Transplant

Engraftment

GvHD
Effect of immunosuppressants in the endothelia

Carmona et al. (submitted)
Pharmacological prevention

- Sodium heparin by continuous infusion.
  - 1 RND + prospective study = VOD
  - 2 RND ↓ VOD
- Prostaglandin E1 by continuous infusion.
  - no beneficial effect, important toxicities
- Ursodeoxycholic acid per os.
  - 4 RND trials and 2 historically controlled studies have shown ↓ VOD and TRM.
- LMWH: no RND studies, effective?
- Defibrotide. 1 RND in children ↓ VOD and GvHD

The only agent with activity on the endothelium
Inflammation: Adhesion Receptors

**HUVEC**

- Gold particles/µm²

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**HMEC**

- Gold particles/µm²

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- DF: - DF, + DF

**HMEC + DF**

- Images showing cellular adhesion with gold particle labeling.
Thrombogenicity: Platelet adhesion onto ECM

**HUVEC**

- **HUVEC + DF**

**HMEC**

- **HMEC + DF**

---

**HUVEC**

- **Pre**
- **Day 0**
- **+7**
- **+14**
- **+21**

**HMEC**

- **Pre**
- **Day 0**
- **+7**
- **+14**
- **+21**

---

- **- DF**
- **+ DF**
Thrombogenicity: Expression of VWF / TF

HMEC

**TF**

**VWF**

**TF + DF**

**VWF + DF**

**Thrombogenicity**:

Expression of VWF / TF

HMEC

Partículas de oro/μm²

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<td>60</td>
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**Legend**

- *: Statistical significance
- #: Statistical trend

**Statistical Significance**

- *: Significant difference
- #: Trend towards significance
Signal transduction mechanisms

HUVEC

P-p38MAPK

DF

C Day 14

0 15’ 30’

0 15’ 30’

P-Akt

DF

C Day 14

0 15’ 30’

0 15’ 30’

HMEC

P-p38MAPK

DF

C Day 14

0 15’ 30’

0 15’ 30’

P-Akt

DF

C Day 14

0 15’ 30’

0 15’ 30’
Effect of immunosuppressants

Mean Gray Value (MGV)

- DF
+ DF

C, CSA, TAC, SIR, TAC+SIR

Carmona et al. (submitted)
VOD prophylaxis with DF in children


- 365 pts (65% allo / 31% auto)
- Median age: 6.6 years
- RAND: DF to day +30 vs. control arm
  - VOD incidence 12% | 20% p=0.049
  - GvHD incidence 45% | 63% p=0.004
  - Mortality 6% | 24%
Treatment

Corticosteroids

- Veno-occlusive disease → some favorable report
- Capillary leak syndrome → low effectiveness
- Engraftment syndrome → effective at low doses
- Diffuse alveolar hemorrhage → effective at high doses
- Thrombotic microangiopathy → ineffective
# Treatment

Defibrotide in severe VOD

<table>
<thead>
<tr>
<th>Author (Journal, year)</th>
<th>Patients (n)</th>
<th>CR rate (%)</th>
<th>Day +100 Survival (%)</th>
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<td>Richardson et al (Blood, 1998)</td>
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<td>Sucak et al (Transplant Proc, 2007)</td>
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<tr>
<td>Richardson et al (BB&amp;MT, 2010)</td>
<td>149</td>
<td>46</td>
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What about TMA? or GvHD?
Conclusions I

1. Both allo- and auto-HSCT produce endothelial activation and damage.

2. The degree of endothelial activation and damage correlates with the intensity of the conditioning and the type of immunosuppressant used.

3. Endothelial activation and damage are especially significant after the conditioning and during engraftment. Possible long-term role of the allo-reactivity.

4. Activation of endothelial cells in HSCT is characterized by an inflammatory reaction (allo- & auto-HSCT). A prothrombotic phenotype can be observed in allo-HSCT.
5. Nowadays, defibrotide is the only agent that prevents the activation and damage of endothelial cells. In a single randomized study in children, DF reduce the incidence of VOD and GvHD!!

6.- Similarly, defibrotide is the only effective agent to treat patients with severe VOD
Many thanks for your attention