The Bone Marrow in Type 1 Diabetes

Feasibility of Flow Cytometric Analysis of nPOD Bone Marrow specimens

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Cardiovascular complications are responsible for about 50% of deaths in diabetic patients.

Clinical outcome is worsened because of impairment of cellular and molecular mechanisms of vascular repair.

Unravelling the causes of defective repair mechanisms may lead to new therapies.

124,000 heart attacks in UK every year
In the DDCT/EDIC study, prevalence of myocardial scar was 4.3% by cardiac MRI and 1.4% by clinical adjudication of MI

BHF data and Circulation 2011
The central role of bone marrow in cardiovascular repair

1. Slow, circadian release under homeostasis
2. Rapid, protease-dependent following injury

Spnetti et al Cardiovasc Res 2011
The cause of all complications?
Reversing the paradigm

- Macro angiopathy
- Micro angiopathy
- Neuropathy

BM impairment
BM as a target of microangiopathy

Oikawa et al, ATVB 2010
Human BM as a target of micro-macro-angiopathy (a prospective study on tissue leftovers)

Spinetti et al, unpublished

B

Volume Fraction (%)

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>T2D</th>
<th>T2D+CLI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marrow</td>
<td></td>
<td></td>
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<tr>
<td>Bone</td>
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<td>Fat</td>
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Spinetti et al, unpublished
Enumeration of stem cells in T2D BM

Spinetti et al, unpublished
Diabetic BM neuropathy

Normal Bone Marrow

Bone Marrow in Patients with Diabetes

CXCL12 expression

Loss of osteoblasts

Nestin-positive CAR cell

Ferraro et al Sci Transl Med 2012
Di Persio NEJM 2012
Consequences of BM remodelling

- Infection
- Delayed healing
- Altered immunity
- Delayed engraftment

BM

MicroAng

MacroAng

NeuroPathy
To characterize BM remodelling at histological cellular and molecular level in nPOD diabetic patients
The freshness and quality of the samples are relevant for cell enumeration and function.

Cui et al PlosOne 2012
Use of lysis buffer wash/fixative

- Lyse/wash
- Lyse/no wash

frequently blocked the fluid system

Cui et al. PlosOne 2012

Lyse/wash

Lyse/no wash

CD133 - cells

Fixable Viability Dye eFluor® 780

nPOD
Network for Pancreatic Organ Donors with Diabetes

Extreme Plumbers
"All Clear!"
Tested if different PE-conjugated antibodies (CXCR4, CD164, CD117 and TrkA) have an effect on the enumeration of three commonly-used progenitor cell markers, KDR-FITC, CD133-APC and CD34-PECY7

Cui et al PlosOne 2012
Titration of each individual antibody is essential before combination for cell enumeration.
The effect of diabetes on BM-PCs was assessed in three nPOD iliac crest BM cases:
1) case 6126, not diabetic (ND) male (M), age 25 years
2) case 6161, Type 1 diabetic (T1D), M, age 19 years
3) case 6132, T2D, female, age 52 years.

Frozen specimens were thawed following nPOD instructions in DMEM+10% FBS. In addition, cells were left 2 hours at 37°C, 5% CO₂ to recover from thawing before FACS analysis.
Recovery after thawing out of shipped frozen nPOD BM-MNC

A) number of tripan blue positive BM-MNCs. B) Percentage of 7ADDneg-alive BM-MNCs after thawing.
<table>
<thead>
<tr>
<th>Definition</th>
<th>Antigenic profile</th>
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<tbody>
<tr>
<td>Hematopoietic PCs</td>
<td>CD34&lt;sup&gt;pos&lt;/sup&gt;, CD133&lt;sup&gt;pos&lt;/sup&gt;, and c-kit&lt;sup&gt;pos&lt;/sup&gt;</td>
</tr>
<tr>
<td>T-lymphocytes</td>
<td>CD45&lt;sup&gt;pos&lt;/sup&gt;/CD3&lt;sup&gt;pos&lt;/sup&gt;</td>
</tr>
<tr>
<td>B-lymphocytes</td>
<td>CD45&lt;sup&gt;pos&lt;/sup&gt;/CD19&lt;sup&gt;pos&lt;/sup&gt;</td>
</tr>
<tr>
<td>Natural Killer (NKs)</td>
<td>CD3&lt;sup&gt;neg&lt;/sup&gt;/CD56&lt;sup&gt;pos&lt;/sup&gt;/CD16&lt;sup&gt;pos&lt;/sup&gt;</td>
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<tr>
<td>Mesenchymal Cells (MSCs)</td>
<td>CD73&lt;sup&gt;pos&lt;/sup&gt;/CD105&lt;sup&gt;pos&lt;/sup&gt;/CD90&lt;sup&gt;pos&lt;/sup&gt;/CD34&lt;sup&gt;neg&lt;/sup&gt;/CD45&lt;sup&gt;neg&lt;/sup&gt;</td>
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<tr>
<td>Endothelial cells (ECs)</td>
<td>CD45&lt;sup&gt;neg&lt;/sup&gt;/CD31&lt;sup&gt;pos&lt;/sup&gt;/CD144&lt;sup&gt;pos&lt;/sup&gt;</td>
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<tr>
<td>Early endothelial PCs</td>
<td>CD34&lt;sup&gt;pos&lt;/sup&gt;/CD14&lt;sup&gt;pos&lt;/sup&gt;/CD45&lt;sup&gt;dim&lt;/sup&gt;/KDR&lt;sup&gt;pos&lt;/sup&gt;/CXCR4&lt;sup&gt;pos&lt;/sup&gt;</td>
</tr>
<tr>
<td>Late EPCs (IEPCs)</td>
<td>CD34&lt;sup&gt;pos&lt;/sup&gt;/CD14&lt;sup&gt;neg&lt;/sup&gt;/CD45&lt;sup&gt;neg&lt;/sup&gt;/KDR&lt;sup&gt;pos&lt;/sup&gt;/CXCR4&lt;sup&gt;pos&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
Hematopoietic stem cells

Population
- 7AAD<sup>neg</sup>
- CD45
- CD34
- CD133
- cKit

% indicated population on tot BM-MNCs

- ND
- T1D
- T2D

Hematopoietic progenitors

CD45
CD133
cKit
Lymphocytes

7AAD<sup>neg</sup> CD45

T Lymphos CD3

B Lymphos CD19

NKs CD56/CD16

% indicated population on tot BM-MNCs

ND T1D T2D

Lymphocytes

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Donors with Diabetes
Mesenchymal stem cells

Population
- All Events
- 7AADneg
- CD45neg
- CD34neg
- CD105
- CD73
- CD90

Mesenchymal Cells

% indicated population on tot BM-MNCs

- ND
- T1D
- T2D

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Endothelial cells

- 7AAD\textsuperscript{-}neg
- CD45\textsuperscript{-}neg
- CD31
- CD144
- ECs

Endothelial Cells

% indicated population on tot BM-MNCs

Population
- All Events
- 7AAD\textsuperscript{-}neg
- CD45\textsuperscript{-}neg
- CD31
- CD144

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Early EPCs

CD34<sup>neg</sup> CD14<sup>neg</sup> CD45<sup>dim</sup> KDR/CXCR4<sup>neg</sup> 7AAD<sup>neg</sup> CD45<sup>neg</sup> KDR/CXCR4<sup>pos</sup>

Late EPCs

Early EPCs

Endothelial Progenitor Cells

Population

- All Events
- 7AAD<sup>neg</sup>
- CD45<sup>dim</sup>
- CD34<sup>pos</sup>
- CD14<sup>neg</sup>
- KDR/CXCR4<sup>neg</sup>
- KDR/CXCR4<sup>pos</sup>

% indicated population on tot BM-MNCs

- ND
- T1D
- T2D

early EPCs

late EPCs

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nPOD
We successfully verified a SOP to thaw nPOD BM specimens for cytometric analysis.

Samples were adequate to measure the percentages of different populations of BM cells.

However, the proportion of cells can be skewed because of distinctive cell loss during freezing/thawing.
Perspectives

**BM**
- Collect info on clinical correlates
- Compare with fresh samples
- Focus on certain populations (CD34+, CD34+KDR, lineage) to study cell specification

**CVD**
- Extend nPOD to cardiovascular complications (heart, large vessels, limb muscle)
- Focus on similar molecular mechanisms

**Links**
- Connection with other thematic areas