Biomarkers of Progressive Renal Decline in Type 1 Diabetes

Andrzej S. Krolewski MD, PhD
Joslin Diabetes Center & Harvard Medical School
Boston, USA
Disclosures

I am a co-inventor of patent “Circulating TNFRs as predictors of renal decline in diabetes”, which was assigned to Joslin Diabetes Center, and is licensed to EKF Diagnostics.

I gave and was paid for lectures at:
Merck & Co., Inc; Boehringer Ingelheim GmbH; Eisai Co. Ltd; Concert Pharmaceuticals, Inc; Eli Lilly and Co.

I am recipient of research grant from Eli Lilly and Co and Pfizer, Inc to study “Markers of progressive renal decline in Type 2 diabetes”
Outline of Presentation

1) Progressive renal decline as a new paradigm of diabetic nephropathy in T1D

2) Circulating TNFRs as Biomarkers of progressive renal decline in T1D

3) Other biomarkers of progressive renal decline in T1D
Hemodynamic & Morphological Glomerular lesions

Traditional Model of Nephropathy in Type 1 Diabetes

Normo-albuminuria → Micro-albuminuria → Proteinuria → ESRD

Hemodynamic & Morphological Glomerular lesions
New Model of Nephropathy in Type 1 Diabetes
(Perkins et al. NEJM 2003)
New Model of Nephropathy in Type 1 Diabetes
(Perkins et al. NEJM 2003)
Early Progressive Renal Decline (eGFRcr-cys loss >3.3% ml/min/year) observed during 4-10 years of follow-up of 280 patients with T1D and Normoalbuminuria.
eGFRcys changes among 79 patients with new Onset of Microalbuminuria followed for 10-14 years

55 Pts. Had Stable Renal Function,

24 Pts. (30%) Had Progressive Renal Decline, eGFR loss between 3.3 - 23%/year

Perkins et al, JASN 2007,
Merchant et al. JASN 2009
GFRcys changes among 79 patients with new Onset of Microalbuminuria followed for 10-14 years

Perkins et al, JASN 2007, Merchant et al. JASN 2009
Progressive renal decline as a new paradigm of diabetic nephropathy

Perkins et al. NEJM 2003
Perkins et al. JASN 2007
Skupien et al. KI 2012
Krolewski et al. Diab Care 2013
Circulating TNF Receptors 1 and 2 Predict ESRD in Type 2 Diabetes


Circulating TNF Receptors 1 and 2 Predict Stage 3 CKD in Type 1 Diabetes

TNFR1 and TNFR2 are cell membrane bound receptors involved in key aspects of the immune response and other processes.

TNFR1 and TNFR2 can be measured in serum/plasma (R&D kits).

TNFR1 – is expressed by almost all cell types
- size of the intact form: 55kDa
- size of the cleaved form: 28kDa

TNFR2 – is expressed mainly by endothelial cells and immune cells
- size of the intact form: 75kDa
- size of the cleaved form: 30kDa
Mechanisms of generation of serum TNFR1

Adapted from Hawari, PNAS 2004,
Concentrations of circulating TNFRs were very stable during 4 year follow-up (N=78)

**Time of examination:**

<table>
<thead>
<tr>
<th>Markers</th>
<th>Baseline</th>
<th>4 -yr follow-up</th>
<th>Correlations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>X ± SD</td>
<td>X ± SD</td>
<td>Baseline vs.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Follow-up</td>
</tr>
<tr>
<td>TNFR1 (pg/ml)</td>
<td>1473 ± 446</td>
<td>1478 ± 606</td>
<td>0.77</td>
</tr>
<tr>
<td>TNFR2 (pg/ml)</td>
<td>2519 ± 725</td>
<td>2522 ± 1049</td>
<td>0.81</td>
</tr>
</tbody>
</table>

Gohda et al.  JASN 2012
Risk of Progressive GFRcre-cys loss (>3.3% ml/min/yr) during 4-10 years of follow-up by quartiles of baseline concentration of serum TNFR1 & TNFR2

- **Normoalbuminuria**
  - N=284

- **Microalbuminuria**
  - N=246

<table>
<thead>
<tr>
<th>Quartile</th>
<th>TNFR-1 (%)</th>
<th>TNFR-2 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q1</td>
<td>5%</td>
<td>14%</td>
</tr>
<tr>
<td>Q2</td>
<td>9%</td>
<td>6%</td>
</tr>
<tr>
<td>Q3</td>
<td>7%</td>
<td>12%</td>
</tr>
<tr>
<td>Q4</td>
<td>28%</td>
<td>24%</td>
</tr>
</tbody>
</table>

Risk of Early Progressive GFR loss in %

- **Normoalbuminuria**
  - p<0.001

- **Microalbuminuria**
  - p<0.001

Krolewski et al.
Diabetes Care 2013
All had Proteinuria at baseline

<table>
<thead>
<tr>
<th>Characteristics at baseline CKD 1/2/3</th>
<th>N=442</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at T1D Dx (y)</td>
<td>14 ± 9</td>
</tr>
<tr>
<td>Duration of T1D (y)</td>
<td>25 ± 9</td>
</tr>
<tr>
<td>Duration of care at Joslin (y)</td>
<td>19 ± 8</td>
</tr>
<tr>
<td>Lifetime HgbA1c (%)</td>
<td>9.3 ± 1.5</td>
</tr>
<tr>
<td>eGFRcr (ml/min)</td>
<td>81 ± 30</td>
</tr>
<tr>
<td>ACR</td>
<td>718 (420 – 1337)</td>
</tr>
<tr>
<td>Serum TNFR1 pg/ml</td>
<td>2494 (1997 – 3251)</td>
</tr>
<tr>
<td>Serum TNFR2 pg/ml</td>
<td>4419 (3023 – 5854)</td>
</tr>
</tbody>
</table>

During 7-20 years of follow-up (95 % completion):

- Median # of serum creatinines to evaluate eGFR trajectories: 10 (4-20)
- Number of ESRD: 159

Skupien et al. KI 2012
Example of renal function changes during follow-up
Trajectory is linear and well represented by eGFR slope based
on serial serum creatinines.

All serum concentrations were used to estimate GFR with EPI-CKD formula and
linear regression was used to determine eGFR slope, in this case -4.2 ml/min/year
Risk of renal decline in T1D pts. with proteinuria by quartiles of baseline serum concentration of TNFR2

Follow-up outcomes

- Slope of renal decline (ml/min/1.73m²/year)
  - Quartile of serum TNFR2 at baseline
  - p<0.001

- Incidence of ESRD (per 100 p-y)
  - Quartile of serum TNFR2 at baseline
  - p=0.012

- Cumulative risk of ESRD (%)
  - Quartile of TNFR2
  - p<0.001

Skupien et al. Diabetes Care 2014
Mean eGFR loss according to medians of baseline serum TNFR2 and other relevant markers in CKD 1−2 and CKD 3 sub-groups.

<table>
<thead>
<tr>
<th>Baseline markers</th>
<th>Serum TNFR2 levels</th>
<th>CKD 1&amp;2</th>
<th>CKD 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>HgbA1c Below M.</td>
<td>-3.0</td>
<td>-3.9</td>
<td></td>
</tr>
<tr>
<td>HgbA1c Above M.</td>
<td>-5.0</td>
<td>-6.4</td>
<td></td>
</tr>
<tr>
<td>ACR Below M.</td>
<td>-3.4</td>
<td>-3.6</td>
<td></td>
</tr>
<tr>
<td>ACR Above M.</td>
<td>-4.5</td>
<td>-7.1</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Below M.</th>
<th>Above M.</th>
<th>Below M.</th>
<th>Above M.</th>
</tr>
</thead>
<tbody>
<tr>
<td>HgbA1c</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACR</td>
<td></td>
<td></td>
<td></td>
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</table>

p<0.001
Outline of Presentation

1) Progressive renal decline as a new paradigm of diabetic nephropathy in T1D

2) Circulating TNFRs as Biomarkers of progressive renal decline in T1D

3) Other biomarkers of progressive renal decline in T1D
Distribution of eGFRcr slopes in patients with T1D & proteinuria

N = 140
Median = -4.1 ml/min/yr

Progressed to ESRD
Correlation (Spearman) between eGFR slopes and urinary biomarkers

**Urinary Biomarkers by groups:**

<table>
<thead>
<tr>
<th>Glomerular</th>
<th>Tubular</th>
<th>Inflammation</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACR -0.59</td>
<td>KIM1 -0.57</td>
<td>MCP1 -0.59</td>
<td>Fibronectin -0.47</td>
</tr>
<tr>
<td>IgG3 -0.52</td>
<td>NGAL -0.42</td>
<td>IL6 -0.49</td>
<td>MMP2 -0.42</td>
</tr>
<tr>
<td>IgG1 -0.47</td>
<td>TFF3 -0.34</td>
<td>gp130 -0.33</td>
<td>PAI-1 -0.41</td>
</tr>
<tr>
<td>IgG2 -0.42</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgGA -0.37</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In total 36 biomarkers were examined and 17 with r>0.300 and p<0.001 are presented.

Niewczas et al. unpublished data
Biomarkers associated with time to onset of ESRD according to Cox regression analysis.

<table>
<thead>
<tr>
<th>Biomarker at baseline</th>
<th>Univariable models</th>
<th>Multiplevariable model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR</td>
<td>p-value</td>
</tr>
<tr>
<td>HbA1c</td>
<td>1.32</td>
<td>0.0005</td>
</tr>
<tr>
<td>TNFR2 (serum)</td>
<td>2.04</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Albumin/cr</td>
<td>2.45</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>KIM-1/cr</td>
<td>1.88</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>NGAL/cr</td>
<td>1.75</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>MCP-1/cr</td>
<td>2.41</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>IL-6/cr</td>
<td>1.51</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

HRs indicate the effect of a 1 quartile change.
Using Diagnostic Decision Tree, we developed Diagnostic Algorithm to predict time to onset of ESRD.

Diagnostic Decision Tree is a statistical procedure that searches among all candidate predictors (*n*=45) for the ones that result in the best discrimination of the outcome, i.e. time to ESRD.

Skupien et al. unpublished data
Diagnostic Decision Tree to identify non-progressors, progressors and rapid progressors

- MCP-1 < 1000
  - Album. < 750
    - MCP-1 ≥ 1000
      - TNFR2 < 4500
        - n=28
          - 5-yr CI = 0 %
      - TNFR2 ≥ 4500
        - n=44
          - 5-yr CI = 18 %
  - Album. ≥ 750
    - MCP-1 ≥ 1000
      - TNFR2 < 4500
        - n=30
          - 5-yr CI = 30 %
      - TNFR2 ≥ 4500
        - n=22
          - 5-yr CI = 61 %

- MCP-1 ≥ 1000
  - TNFR2 < 4500
    - n=42
      - 5-yr CI = 80 %
  - TNFR2 ≥ 4500
    - n=47
      - 5-yr CI = 26 %
Biomarkers of Progressive Renal Decline in Type 1 diabetes

- Progressive renal decline is the major feature of diabetic nephropathy
- Serum concentration of TNFRs can identify patients at risk of renal decline disregarding albuminuria abnormalities
- Multiple urinary biomarkers are predictors of renal decline but they provide redundant information
- Sensitivity and specificity of these biomarkers is increasing with increasing CKD stages
- In CKD 3 a one-time measurement of serum TNFR2, urinary albumin and MCP-1 allowed us to identify patients whose progression to ESRD would be rapid, moderate, slow or beyond 8 years of follow-up.
- Currently we are trying to confirm our findings in other populations of Type 1 and Type 2 diabetes.
Department of Genetics and Epidemiology

Collaborators:
Drs. R. Stanton & J. Roshan, Joslin Clinic
Drs. J.V. Bonventre & V. Sabbisetti, Harvard Medical School
Drs. S. Pennatur & A. Galecki, Michigan University
Distribution of eGFRcr slopes in patients with T1D & proteinuria

CKD 1&2, N = 302
Median = -2.7 ml/min/yr

CKD 3, N = 140
Median = -4.1 ml/min/yr