Biomarkers of Progressive Renal Decline in Type 1 Diabetes

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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
Traditional Model of Nephropathy in Type 1 Diabetes

New Model of Nephropathy in Type 1 Diabetes
(Perkins et al. NEJM 2003)
Baseline eGFR-cr-cys < 105 ml/min
N=14 (120) 9%

Baseline eGFR-cr-cys ≥ 105 ml/min
N=14 (165) 12%

Follow-up in Years
Early Progressive Renal Decline (eGFR-cr-cys loss > 3.3% ml/min/year) observed during 4-10 years of follow-up of 280 patients with T1D and Normoalbuminuria

E Krolewski et al. Diabetes Care, 2013

Baseline eGFR-cr-cys
2005 ml/min
N=14 (120)
Baseline eGFR-cr-cys
≥ 105 ml/min
N=14 (165)

Follow-up in Years

E GFRcys changes among 79 patients with new Onset of Microalbuminuria followed for 10-14 years

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

20 Pts. (25%) Had Progressive Renal Decline, eGFR loss between 3.3 - 17%/year
55 Pts. Had Stable Renal Function,
Perkins et al, JASN 2007,
Merchant et al. JASN 2009
Progressive renal decline as a new paradigm of diabetic nephropathy

Circulating TNF Receptors 1 and 2 Predict ESRD in Type 2 Diabetes

Tumor Necrosis Factor Receptors - TNFRs

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)

<table>
<thead>
<tr>
<th>Time of examination:</th>
<th>Baseline</th>
<th>4-yr follow-up</th>
<th>Correlations Baseline vs. Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>X ± SD</td>
<td>X ± SD</td>
<td>r</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

Krolewski et al. Diabetes Care 2013
Study on Progressive Renal Decline in T1D Pts. with Proteinuria: Pts. developed Proteinuria while attending the Joslin Clinic between 1991-2004 and were followed until 2011

All had Proteinuria at baseline
Characteristics at baseline CKD 1/2/3 N=442

| Age at T1D Dx (y) | 14 ± 9 |
| Duration of T1D (y) | 25 ± 9 |
| Duration of care at Joslin (y) | 19 ± 8 |
| Lifetime HgbA1c (%) | 9.3 ± 1.5 |
| eGFR (ml/min) | 81 ± 30 |
| ACR | 718 (420 – 1337) |
| Serum TNFR1 pg/ml | 2494 (1997 – 3251) |
| Serum TNFR2 pg/ml | 4419 (3023 – 5854) |

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

- Median # of serum creatinines: 10 (4–20)
- Number of ESRD: 159

Example of renal function changes during follow-up

Trajectory is linear and well represented by eGFR slope based on serial serum creatinines.

Risk of renal decline in T1D pts. with proteinuria by quartiles of baseline serum concentration of TNFR2

Follow-up outcomes

- Decrease in glomerular filtration rate (GFR) significant (p=0.02)
- Increase in serum TNFR1 at baseline
- Increase in serum TNFR2 at baseline

N=159

Risk of renal decline by quartiles of baseline serum concentration of TNFR2

Follow-up outcomes

- Change in eGFR (ml/min)
- Percentage change in serum creatinine

N=159

Skupien et al. KI 2012

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>Average eGFR ml/min/loss/year according to:</th>
<th>CKD 1&amp;2</th>
<th>CKD 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Serum TNFR2 levels</td>
<td>Below M.</td>
<td>Above M.</td>
</tr>
<tr>
<td>HgbA1c</td>
<td>Below M.</td>
<td>-3.0</td>
<td>-4.6</td>
</tr>
<tr>
<td></td>
<td>Above M.</td>
<td>-5.0</td>
<td>-7.7</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACR</td>
<td>Below M.</td>
<td>-3.4</td>
<td>-4.0</td>
</tr>
<tr>
<td></td>
<td>Above M.</td>
<td>-4.5</td>
<td>-8.0</td>
</tr>
<tr>
<td></td>
<td>p=0.001</td>
<td></td>
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</tbody>
</table>

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Distribution of eGFRc 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

<table>
<thead>
<tr>
<th>Urinary Biomarkers by groups:</th>
<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>
<td></td>
</tr>
<tr>
<td>IgG3 –0.52</td>
<td>NGAL –0.42</td>
<td>IL6 –0.49</td>
<td>MMP2 –0.42</td>
<td></td>
</tr>
<tr>
<td>IgG1 –0.47</td>
<td>TFF3 –0.34</td>
<td>gp130 –0.33</td>
<td>PAI-1 –0.41</td>
<td></td>
</tr>
<tr>
<td>IgG2 –0.42</td>
<td>TFF3 –0.34</td>
<td>gp130 –0.33</td>
<td>TIMP1 –0.38</td>
<td></td>
</tr>
<tr>
<td>IgG3A –0.37</td>
<td>TIMP2 –0.38</td>
<td></td>
<td>TIMP2 –0.38</td>
<td></td>
</tr>
</tbody>
</table>

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

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>TNFα2 (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.
Diagnostic Decision Tree to identify non-progressors, progressors and rapid progressors

Album. < 750

MCP-1 < 1000

TNFR2 < 4500

MCP-1 ≥ 1000

TNFR2 ≥ 4500

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. Pennathur & 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