Assessing albuminuria

Methodological considerations with clinical impact

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Introduction

Albuminuria is a urinary biomarker that has been shown to be a predictor of renal and CV events.

As such albuminuria has a place in clinical practice: kDOQI stages 1 and 2 are defined by presence of micro-albuminuria.

There is strong lobby for standardisation of measurement of serum creatinine (Cleveland Clinic / IDMS traceable) to obtain the most reliable GFR estimate.

Until recently little attention has been paid to standardisation of albuminuria (exception Miller et al, Clin Chem 2009;55:24-38)
# Micro-albuminuria

## Definition and classification

<table>
<thead>
<tr>
<th></th>
<th>Spot urines (first morning void, or random)</th>
<th>24h urine</th>
<th>Overnight (timed)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Albumin Concentration (mg/l)</td>
<td>Alb/creat ratio (mg/gram)</td>
<td>Albumin Excretion (mg/24h)</td>
</tr>
<tr>
<td>Normal</td>
<td>&lt; 20</td>
<td>M F</td>
<td>&lt; 30</td>
</tr>
<tr>
<td>Micro-albuminuria</td>
<td>20 – 200</td>
<td>M F</td>
<td>30 – 300</td>
</tr>
<tr>
<td>Macro-albuminuria</td>
<td>&gt; 200</td>
<td>M F</td>
<td>&gt; 300</td>
</tr>
</tbody>
</table>
Assessment of albuminuria
Questions to address

1. What assay to use? (answer: immunochemistry polyclonal)
2. What urine sample to use?
   24hr urine collection, first morning void or a spot sample?
3. Which albuminuria measure to use: urinary albumin concentration, albumin/creatinine ratio, or 24hr albumin excretion?
4. Does it matter whether we use fresh urine samples or stored samples?
5. If we are going to use frozen urine samples, what is important?
   pre-storage handling, storage temperature, sample handling
Assessment of albuminuria
Which assay to use?

Type of assay
- Colorimetric test strips
- Immunochemistry based
  - Monoclonal AB
  - Polyclonal AB
- Size exclusion (HPLC)

Monomer → Dimerization → Polymerization

Monomer → Fragmentation

Loss of immunoreactivity ??

Monoclonal AB, Polyclonal AB

What urine samples to use?
Median urinary albumin concentration

- Median 24-hour [IQ-range]: 7.6 [4.8-12.7]
- Median Overnight [IQ-range]: 7.2 [4.5-12.0]
- Median Spot (morning) [IQ-range]: 11.9 [7.8-25.8]

N=250

Witte et al, JASN 2009;20:436-43
What urine samples to use?
Coefficient of variation

Intra-subject coefficient of Variation (%)

Urinary Albumin Concentration

P = < 0.01
P = < 0.01
P = 0.08

24-hour  FMV  Spot (morning)

N=250

Witte et al, JASN 09;20:436-43
What urine samples to use? Median albumin:creatinine ratio

- Median 24-hour [IQ-range] 1.00 [0.65-1.54]
- Median Overnight [IQ-range] 0.67 [0.50-1.17]
- Median Spot (morning) [IQ-range] 1.21 [0.68-2.37]

P = 0.023
P = < 0.001
P < 0.001

N=250

Witte et al, JASN 09;20:436-43
What urine samples to use?
Coefficient of variation

Intra-subject coefficient of Variation (

<table>
<thead>
<tr>
<th>Albumin:creatinine ratio</th>
<th>24-hour</th>
<th>FMV</th>
<th>Spot (morning)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P = &lt; 0.01</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P = &lt; 0.01</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>P = 0.58</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

N=250

Witte et al, JASN 09;20:436-43
What urine samples to use?  
Prevalence of microalbuminuria

Witte et al, JASN 09;20:436-43
**Which albuminuria measure to use?**

**Predicting CV outcome**

<table>
<thead>
<tr>
<th></th>
<th>24 hr urine</th>
<th>First morning void</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UAE (mg/24hr)</td>
<td>UAC (mmol/L)</td>
</tr>
<tr>
<td>Overall</td>
<td>0.65</td>
<td>0.62</td>
</tr>
<tr>
<td>Subgroups</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>0.64</td>
<td>0.62</td>
</tr>
<tr>
<td>Female</td>
<td>0.66</td>
<td>0.59#</td>
</tr>
<tr>
<td>&lt;47 yr</td>
<td>0.58</td>
<td>0.52</td>
</tr>
<tr>
<td>&gt;47yr</td>
<td>0.65</td>
<td>0.64</td>
</tr>
</tbody>
</table>

* p < 0.05 vs UAC, # p < 0.05 vs UAE

**AUC ROC curve**

**N=3432**

Random sample of the general population

*Lambers-Heerspink et al, Am J Epidemiol 08;168:897-905*
Which albuminuria measure to use?
Predicting renal outcome

- UAE 24hr; AUC = 0.78
- UPE 24hr; AUC = 0.78
- ACR FMV; AUC = 0.82

p<0.001

N=701

Lambers Heerspink et al, submitted
RENAAL: DM2 nephropathy
Which albuminuria measure to use? ACR “incorporates” the influence of age

PREVEND

Lambers-Heerspink, Gansevoort et al, Am J Epidemiol
2008;168:897-905
Frozen storage (-20 C) of urine samples
Influence of duration of storage and sample handling

* Not significantly different from zero

Percentage change in UAC, %

Predictive value of albuminuria

Does it matter when urine has been stored frozen?


P<0.01
Frozen storage of urine samples
Does urinary pH matter?

Screening for albuminuria
The past (1892)

ALBUMINURIA

IN

HEALTH AND DISEASE

WITH TWO APPENDICES

(1) A CONTRIBUTION TO THE THEORY OF URINARY SECRETION.
(2) THE HYGIENIC TREATMENT OF ALBUMINURIA.

BY

DR. H. SENATOR,

PROFESSOR EXTRAORDINARY OF MEDICINE, PHYSICIAN TO THE ROYAL CHARITÉ HOSPITAL, AND TO THE AUGUSTA HOSPITAL OF BERLIN.

WITH ONE LITHOGRAPHED ILLUSTRATION.

TRANSLATED BY

DR. T. P. SMITH.

Gansevoort and Ritz, Nephrol Dial Transplant 2008
Conclusions

When assessing the clinical impact of urinary biomarkers it is essential to take into consideration methodological issues:

1. Which assay was used? Polyclonal? Intra- and interassay CV?

2. What urine samples were used? Preferably 24hr collections or first morning voids.

3. In case first morning void samples are used, normalise for creatine concentration.

4. Fresh or frozen? Preferably use fresh urine samples.

5. If frozen, what were storage conditions and how was sample handling? Frozen at -80°Celsius, pH adjustment (or protease inhibitors?), vortexing?