Development of a reference MSMS method for plasma creatinine

Asian Pacific Conference of Chromatography & Mass Spectrometry 2010
14th - 16th January 2010

R Neil Dalton & Charles Turner
WellChild Laboratory
King’s College London
Evelina Children’s Hospital
Development of a reference MSMS method for plasma creatinine

Plasma creatinine and renal function

renal function and cardiovascular disease

Estimated glomerular filtration rate (eGFR)

Routine analytical measurement of plasma creatinine

Reference procedure
Development of a reference MSMS method for plasma creatinine

Plasma creatinine

A biomarker of glomerular filtration rate (GFR)

Kidney function
Development of a reference MSMS method for plasma creatinine
serum creatinine (MSMS) v formal GFR (DTPA clearance) in adults
(n=310), Prof Carlo Donadio

\[ y = 2956.7x^{-0.8345} \]
\[ R^2 = 0.856 \]
Development of a reference MSMS method for plasma creatinine ROC curve for MSMS serum creatinine in adults, GFR <80ml/min/1.73m² (n=310), Prof Carlo Donadio

Area = 0.89, CI 0.85-0.93
Development of a reference MSMS method for plasma creatinine

Plasma creatinine

early detection of renal disease

monitoring rate of progression of renal disease

classification for treatment/funding

cardiovascular risk

inappropriate use of normal range
Development of a reference MSMS method for plasma creatinine
The clinical problem


Prevalence 11% (19.2 million)
Stage 1: persistent albuminuria, normal GFR, 3.3% (5.9 million)
Stage 2: persistent albuminuria, GFR 60-89, 3.0% (5.2 million)
Stage 3: GFR 30-59, 4.3% (7.6 million)
Stage 4: GFR 15-29, 0.2% (0.4 million)
Stage 5: GFR <15, 0.2% (0.3 million)

11% of individuals >65y (without hypertension or diabetes) had stage 3 or worse
Development of a reference MSMS method for plasma creatinine

The clinical problem

Figure 1 | Kaplan-Meier analysis of cardiovascular outcomes according to the presence of chronic kidney disease. Composite outcome includes myocardial infarction, fatal coronary heart disease, stroke and all-cause mortality. Bars represent 95% CIs. Abbreviation: CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate. Permission obtained from American Society of Nephrology © Weiner, D.E. et al. J. Am. Soc. Nephrol. 15, 1307–1315 (2004).
Development of a reference MSMS method for plasma creatinine
serum creatinine v inulin clearance (adults)
Development of a reference MSMS method for plasma creatinine

Biological Variation of Serum Creatinine in Adults

Normal range misleading and dangerous

Precise analytical method
(inter assay imprecision <2%)

Individual patient tracking for early detection of kidney disease
Development of a reference MSMS method for plasma creatinine individual patient tracking for early detection of renal disease

Plasma creatinine v time

Plasma creatinine (µmol/l) vs Time (yr)
Development of a reference MSMS method for plasma creatinine

Attempt to compensate for between-individual differences

Emphasise plasma creatinine as a measure of GFR

GFR is a function of 1/plasma creatinine

GFR modelling – eGFR (ml/min/1.73m²)

\[170 \times [Pcr]^{-0.999} \times [Age]^{-0.176} \times [0.762 \text{ if patient is female}] \times [1.180 \text{ if patient is black}] \times [SUN]^{-0.170} \times [Alb]^{0.318}\]
Development of a reference MSMS method for plasma creatinine

90th centile %age absolute errors
12.9 ml/min/1.73m² (28.4%)

Reported eGFR
60 ml/min/1.73m²
90% confidence interval,
42-78 ml/min/1.73m²

All analyses in a single laboratory
AASK Core Biochemistry Lab,
Cleveland Clinic Foundation
Kinetic Jaffe assay on a Beckman Astra CX3, c.1984
Development of a reference MSMS method for plasma creatinine

Creatinine reacts with alkaline sodium picrate to produce an orange-red colour
Jaffe 1886

Folin used the reaction to measure creatinine in urine (1904) and then in plasma (1914)

Reaction product and mechanism still controversial
Development of a reference MSMS method for plasma creatinine
Susceptibility of glomerular filtration rate estimations to variations in creatinine methodology: a study in older patients. Ann Clin Biochem, 42, 11-18
Development of a reference MSMS method for plasma creatinine
UKNEQAS data, Finlay McKenzie

[Graph showing data for different reagents and methods]
Development of a reference MSMS method for plasma creatinine
Routine laboratory performance

The importance of being “consistently wrong”

Good precision but awful accuracy

Huge differences between routine analytical methods

Not a problem for an individual laboratory, provided patients only have plasma creatinine measured by one method in one laboratory

Introduction of formula based (MDRD) eGFR for kidney disease staging and access to treatment

Standardisation/commutability of routine laboratory plasma creatinine measurements

The importance of being “consistently right”
Development of a reference MSMS method for plasma creatinine
NKF-K/DOQI guidelines

The serum creatinine concentration alone should not be used to assess the level of kidney function

Clinical laboratories should report an estimate of GFR using a prediction equation, in addition to reporting the serum creatinine measurement

Reduced/practical MDRD formula for GFR (ml/min/1.73m²):

$$186 \times [\frac{\text{Pcr}}{88.4}]^{-1.154} \times [\text{Age}]^{-0.203} \times [0.742 \text{ if patient is female}] \times [1.121 \text{ if patient is black}]$$

Treatment (and treatment costs) dependent on eGFR (K/DOQI stages 1-5)

Variations in creatinine methods imply no conformity in eGFR or K/DOQI staging
Development of a reference MSMS method for plasma creatinine
NKF-K/DOQI guidelines

Autoanalyzer manufacturers and clinical laboratories should calibrate serum creatinine assays using an international standard

Standard reference method
Cleveland Clinic Jaffe?
Stable isotope dilution mass spectrometry?

Stable isotope dilution mass spectrometry (IDMS) traceable analytical methods

New eGFR formula

Vickery S, Stevens PE, Dalton RN, van Lente F, Lamb EJ. (2006) Does the ID-MS traceable MDRD equation work and is it suitable for use with compensated Jaffe and enzymatic creatinine assays? Nephrology Dialysis Transplantation, 21, 2439-2445
Development of a reference MSMS method for plasma creatinine
IDMS traceable plasma creatinine methods for eGFR formula

Provide analytical method traceability

Standard reference material, NIST 912a

Matrix dependent analytical methods

Plasma based standard reference materials
(SRM 967 I, SRM 967 II)

GFR (ml/min/1.73m²):

$175\times[Pcr/88.4]^{-1.154}[Age]^{-0.203}[0.742 \text{ if patient is female}]\times[1.121 \text{ if patient is black}]$
Development of a reference MSMS method for plasma creatinine

Vickery S, Stevens PE, Dalton RN, van Lente F, Lamb EJ. (2006) Does the ID-MS traceable MDRD equation work and is it suitable for use with compensated Jaffe and enzymatic creatinine assays? Nephrology Dialysis Transplantation, 21, 2439-2445
Development of a reference MSMS method for plasma creatinine
IDMS traceable plasma creatinine methods

Interferences in clinical samples

Significant performance improvements

Highlighted manufacturers’
mathematical manipulation of results:
  Background subtraction
  Linearising factors

Despite significant efforts to make routine analytical methods for measuring plasma creatinine, Jaffe and enzymatic, traceable to stable isotope dilution MS/MSMS the problems of significant interferences in clinical samples still remain

NKDEP/IFCC study

Fully validated reference method, participation in RELA QA scheme
Development of a reference MSMS method for plasma creatinine
Generic assay criteria

Plasma creatinine reference stable isotope dilution LC-MSMS method

Sensitivity
Linearity
Sample volume
Carry over
Precision
Instrument
Development of a reference MSMS method for plasma creatinine product ion scan of creatinine, NIST-914a

Standard reference material

National Institute of Standards and Technology (NIST) standard reference material, creatinine 914a (Laboratory of the Government Chemist, Teddington, UK)
Development of a reference MSMS method for plasma creatinine
Stock standard and assay calibrators

Stock standard:

Stock aqueous creatinine standard, 10 mg/dL, was prepared by weighing 100mg of creatinine 914a, using a 4 decimal place analytical tare balance, and dissolving it in 1L (volumetric flask) of 0.1N HCl, prepared with >18.2 MΩ-cm deionised water. All performed at 22ºC.

Assay calibrators:

Assay calibrators at 0.25, 0.50, 0.75, 1.0, 2.0, 5.0, and 10.0mg/dl were prepared, at 22ºC, by weighed dilution with >18.2 MΩ-cm deionised water. Multiple 0.5ml aliquots of each calibrator were stored in screw top polypropylene vials at -80ºC.
Development of a reference MSMS method for plasma creatinine
Stable isotope internal standard

MRM m/z 114.2/44.2

Stable isotope – deuterium labelled, $^2\text{H}_3$

(D-3689) CDN Isotopes, Qmx Laboratories, Thaxted, UK

MRM m/z 117.2/47.2

Stock 10mM in 0.1N HCl, stored in 1ml aliquots at -80°C
Development of a reference MSMS method for plasma creatinine
Certified reference material (CRM)

EU Community Bureau of Reference certified reference material sera
(Report EUR 17115 EN)

Three pooled, spiked, and lyophilised sera with assigned values – primarily ID GCMS (Laboratory of the Government Chemist, Teddington, UK).

Each prepared by weighed addition of 1ml of >18.2 MΩ-cm deionised water and mixing according to the recommended procedure. Multiple 100µl aliquots of each CRM were stored in screw top polypropylene vials at -80°C.

Assigned value (limits of uncertainty)

<table>
<thead>
<tr>
<th>CRM</th>
<th>Value (uncertainty)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRM 573</td>
<td>68.7 (1.4) µmol/l</td>
</tr>
<tr>
<td>CRM 574</td>
<td>105.0 (1.3) µmol/l</td>
</tr>
<tr>
<td>CRM 575</td>
<td>404.1 (7.1) µmol/l</td>
</tr>
</tbody>
</table>
Development of a reference MSMS method for plasma creatinine
Standard reference material (SRM)
External QC material (RELA)

SRM: For each assay, 2*1ml aliquots of frozen human sera (stored and shipped at -80°C) with assigned values – NIST ID GCMS and IDMS (NKDEP/IFCC study organisers)

Assigned value (limits of uncertainty)

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>SRM 967 I</td>
<td>0.75 (0.021) mg/dl</td>
</tr>
<tr>
<td>SRM 967 II</td>
<td>3.92 (0.083) mg/dl</td>
</tr>
</tbody>
</table>

RELA: 5*2 5ml aliquots of lyophilised sera, RELA 2008 KSA and RELA 2008 KSB (DGKL-RfB, Bonn, Germany)

Each prepared by weighed addition of 5ml of >18.2 MΩ-cm deionised water and mixing according to the recommended procedure. Multiple 500μl aliquots of each RELA were stored in screw top polypropylene vials at -80°C

Results submitted as obtained value (limits of uncertainty)
Development of a reference MSMS method for plasma creatinine
Sample preparation

Standards, CRMs, SRMs and plasma samples (10µl, manual Eppendorf pipette) diluted with 50µl deionised water containing 2.5 nmol $^2$H$_3$-creatinine (Eppendorf multipette)

Proteins precipitated with 200µl acetonitrile (Rathburn Chemicals Ltd, Walkerburn, UK) (Eppendorf multipette)

Following mixing and centrifugation, supernatants transferred to a 96 deep well plate, a further 200µl of acetonitrile added and 2µl automatically injected into a mobile phase stream (200µl/min) of acetonitrile: water (1:1) with 0.025% formic acid
Development of a reference MSMS method for plasma creatinine
Chromatography and MSMS parameters

Chromatography performed on a Chirobiotic T 100 x 2.1mm column with a 2cm x 4.0mm guard column (Advanced Separation Technologies, Congleton, UK)

Precursor/product ion pairs (m/z 114.2/44.2, 117.2/47.2) were acquired in positive ion multiple reaction monitoring mode using a Sciex API4000 Q-trap (Applied Biosystems, Warrington, UK)

Results calculated using Analyst 1.4.2
Development of a reference MSMS method for plasma creatinine Chromatography
Development of a reference MSMS method for plasma creatinine

Linearity

IFCC plasma creatinine LFR 2008-October-09 rdb (creatinine): "Linear" Regression ("1/x" weighting): y = 0.00544 x + 5.75e-009 (r = 0.9998)
## Development of a reference MSMS method for plasma creatinine

### Results

<table>
<thead>
<tr>
<th>Sample</th>
<th>Assigned (measured) value (limits of uncertainty)</th>
<th>Mean value, n=19 (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRM 573</td>
<td>0.78 (0.016) mg/dl</td>
<td>0.79 (0.012) mg/dl</td>
</tr>
<tr>
<td>CRM 574</td>
<td>1.19 (0.015) mg/dl</td>
<td>1.20 (0.014) mg/dl</td>
</tr>
<tr>
<td>CRM 575</td>
<td>4.57 (0.080) mg/dl</td>
<td>4.56 (0.038) mg/dl</td>
</tr>
<tr>
<td>SRM 967 I</td>
<td>0.75 (0.021) mg/dl</td>
<td>0.75 (0.015) mg/dl</td>
</tr>
<tr>
<td>SRM 967 II</td>
<td>3.92 (0.083) mg/dl</td>
<td>3.85 (0.040) mg/dl</td>
</tr>
<tr>
<td>RELA 2008 KS A</td>
<td>1.32 (0.021) mg/dl</td>
<td></td>
</tr>
<tr>
<td>RELA 2008 KS B</td>
<td>3.11 (0.026) mg/dl</td>
<td></td>
</tr>
</tbody>
</table>

Study patient samples: average 1.2% CV

Certificate subsequently issued
Development of a reference MSMS method for plasma creatinine

RELA results

<table>
<thead>
<tr>
<th>LabCode</th>
<th>A (e.u.)</th>
<th>B (e.u.)</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>1,338</td>
<td>3,213</td>
<td>0,031 ID/LO/MS</td>
</tr>
<tr>
<td>12</td>
<td>1,288</td>
<td>3,142</td>
<td>0,036 spectrophotometry</td>
</tr>
<tr>
<td>18</td>
<td>1,348</td>
<td>3,23</td>
<td>0,018 ID/LO/MS/MS</td>
</tr>
<tr>
<td>19</td>
<td>1,316</td>
<td>3,145</td>
<td>0,034 ID/LO/MS</td>
</tr>
<tr>
<td>76</td>
<td>1,321</td>
<td>3,111</td>
<td>0,026 ID/LO/MS/MS</td>
</tr>
</tbody>
</table>

REL2008
25.08.09
Development of a reference MSMS method for plasma creatinine NKDEP/IFCC study – preliminary observations

Despite significant efforts to make routine analytical methods for measuring plasma creatinine, Jaffe and enzymatic, traceable to stable isotope dilution MS/MSMS the problems of significant interferences in clinical samples still remain

Clinical extremes

Method/manufacturer dependent differences
  Jaffe v Jaffe, Jaffe v Enzymatic, Enzymatic v Enzymatic

Problems: manufacturer dependent manipulation of analytical data, low values

Systems designed to divert samples from specific patient groups to a particular method

Routine MSMS? Research studies and clinical trials
Development of a reference MSMS method for plasma creatinine
Conclusion and observations

A simple, low sample volume, NIST traceable, certified reference LC-MSMS method for plasma creatinine has been developed

Fully compliant with the MDRD ID-MS method traceable eGFR formula

GFR (ml/min/1.73m²):
175*[Pcr/88.4] -1.154*[Age] -0.203*[0.742 if patient is female]*[1.121 if patient is black]

Not valid if GFR>60ml/min/1.73m² (CKD-EPI formula)

Not valid for hospital in-patients with unstable renal function
Development of a reference MSMS method for plasma creatinine ROC curves for MSMS serum creatinine and MDRD eGFR in adults, GFR <80ml/min/1.73m² (n=310), Prof Carlo Donadio

![ROC curve diagram](image-url)

- True positive rate (Sensitivity)
- False positive rate (1 - Specificity)
- Area 0.92 for eGFR MDRD
- Area 0.89 for MSMS creatinine (µmol/l)
Development of a reference MSMS method for plasma creatinine
Acknowledgements

Charles Turner
Edmund Lamb and colleagues
Finlay McKenzie
Frederick van Lente
Carlo Donadio

The WellChild Trust
Guy’s & St Thomas’ Charity
Guy’s & St Thomas’ NHS Foundation Trust
Development of a reference MSMS method for plasma creatinine ROC curve for MSMS MDRD eGFR in adults, GFR <80ml/min/1.73m² (n=310), Prof Carlo Donadio

Area = 0.92, CI 0.89-0.95