Novel assay of metformin levels in patients with type 2 diabetes mellitus and varying levels of renal function – clinical recommendations

Running title: Serum metformin levels in impaired renal function

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Objective: To study trough levels of metformin in serum and its intra individual variation in patients using a newly developed assay.

Research Design and Methods: Trough serum levels of metformin was measured once using Liquid Chromatography Tandem Mass Spectrometry (LcMSMS) in 137 type 2 diabetes patients with varying renal function (99 men) and followed repeatedly during two months in 20 patients (16 men) with estimated GFR (eGFR) below 60 ml/min/1.73 m² body surface.

Results: Patients with eGFR >60, 30-60, and <30 ml/min/1.73 m² had a median trough metformin concentration of 4.5 µmol/l (range 0.1-20.7, n=107), 7.71 µmol/l (0.12-15.15, n=21), and 8.88 µmol/l (5.99-18.60, n=9), respectively. The median intraindividual overall coefficient of variation (CV) was 29.4 % (range 9.8-74.2).

Conclusions: Determination of serum metformin with the LCMSMS technique is useful in patients on metformin treatment. Few patients had values over 20 µmol/L. Metformin measurement is less suitable for dose titration.
Metformin is an insulin-sensitizer used for treating type 2 diabetes (T2DM) and treatment is on rare occasions complicated by lactic acidosis. The substance is cleared from the blood through the kidneys (1) and impaired renal function may lead to accumulation.

We have combined new technologies, Liquid Chromatography tandem Mass Spectrometry (LC-MSMS) and Hydrophilic Interaction Liquid Chromatography (2) in the development of a novel method for determination of metformin in serum.

The aim was to study trough levels of metformin in T2DM patients and to assess intraindividual variations in patients with renal impairment.

RESEARCH DESIGN AND METHODS

Patients – 1. Fasting venous blood samples were obtained in 137 (99 men, age 60 (31-83) years) T2DM patients. 2. Twenty patients (16 men, median age 68 (48-83) years) with GFR <60 mL/min/1.73 m² were studied at week 0, 2, 4 and 8.

Serum metformin, cystatin C and creatinine were analyzed in both groups. All participants provided informed consent, the study was approved by the local Ethics Committee (341/2008). Clinicaltrials.gov NCT00767351.

Method for analysis of metformin: One part serum was mixed with 10 parts internal standard fortified acetonitril. After centrifugation one part of the supernatant was diluted with 20 parts of the mobile phase. Five µL of the diluted supernatant was injected into the liquid chromatograph.

The mobile phase is a mixture of water, acetonitril, formic acid and ammonium acetate (pH 2-3). It elutes fenformin and metformin after 2 and 3 minutes respectively from the HILIC (Merck SeQuant, Umea, Sweden) column. The mobile phase enters the mass spectrometer and positively ionized molecules of the eluted compounds are formed by electro spray ionization (3). The positively ionized molecules are fragmented by collision with nitrogen (3) to form fragments with the mass/charge of 70.8 and 105.2 for metformin and fenformin respectively. The intensity of these fragments are measured and calculated to represent concentration data of metformin.

We used a standard high performance liquid chromatograph combined with a triple quadrupole mass spectrometer, Sciex API 4000, (Applied Biosystems Inc., Carlsbad, CA, USA) The chromatographic separation of metformin and its internal standard fenformin was performed with isocratic HILIC-elution.

1,1-dimethylbiguanide hydrochloride and fenformin hydrochloride (Sigma-Aldrich, St. Louis, USA) were used as a reference substance for metformin and internal standard in the assay, respectively.

The lower threshold for detection is 0.05 µmol/L, the results are linear between 0.05 and 125 µmol/L. At concentrations >125 µmol/L the sample is diluted. CV% during 20 months is 12% at 3.6 µmol/L level and 6.3% at 33 µmol/L level, 56 samples at each level.

Estimation of GFR: Estimation of GFR was based on cystatin C (4,5) determined by an immunoturbidimetric method on a Hitachi Modular P analysis system.

Use of creatinine for estimating GFR did not change the results of the study (data not presented).

Statistical analysis: Results are given as median values and range or interquartile range.

SPSS (15.0, Chicago, USA) was used. Wilcoxon rank-sum test (p<0.05) and Spearman’s non-parametric test (p<0.05) were used when appropriate

RESULTS
Trough levels in relation to renal function: The nine of the 137 patients that had an eGFR <30 ml/min/1.73 m² showed a median trough value of S-metformin at 8.88 μmol/l (5.99-18.60). Twentyone patients had an eGFR of 30-60 ml/min/1.73 m², median S-metformin of 7.71 μmol/l (0.12-15.15). 107 patients had an eGFR of >60 ml/min/1.73m², median S-metformin of 4.5 μmol/l (0.1-20.7). The median doses of metformin were 1500 mg (1000-3000), 1500 mg (500-3000) and 1500 mg (500-3000) respectively (figure 1).

Intraindividual variance of metformin concentrations: The median intra individual variation of the S-metformin level in the 20 patients during the 8 week period with repeated measurements was 29.4 % (CV) (range 9.8-74.2). Six of the patients had an eGFR <30 mL/min/1.73 m² and 14 had an eGFR between 30 and 63 mL/min/1.73 m². Median S-metformin was 10 μmol/L (interquartile range 5.3-16). There was no correlation between CV of S-metformin and GFR (r=0.3131, p=0.156).

CV of the four eGFR values were 7.5 % (range 5.9-12.5). Median eGFR at week 0, 2, 4 and 8 was 37, 34, 36 and 33 respectively. There was a significant difference between first (week 0) and last (week 8) median value of eGFR (p=0.023).

CONCLUSIONS
It is widely acknowledged that metformin therapy is beneficial in treating diabetes type 2 and should be made available to as many patients as possible. One obstacle to this has been the possible risk of lactic acidosis in patients with impaired renal function. We have had seven cases of patients on treatment with metformin admitted with lactic acidosis with metformin levels ranging from 256 to 682 (median 330) μmol/L. These data suggest that high levels of serum metformin are needed to cause lactic acidosis.

Prevailing clinical experience has led to recommendations that metformin may be used at eGFR above 30 ml/min/1.73m² (6, 7). Our study supports these guidelines showing that patients above this GFR limit rarely had metformin levels above 20 μmol/L which seems to be a safe level.

If the current guidelines gain general recognition it becomes even more important to advocate cessation of metformin therapy when renal failure develops abruptly or in our opinion in any severe disease, especially when there is risk of dehydration.

Results from this study show a considerable intraindividual CV, 29.4% of metformin concentrations in 20 patients with impaired renal function.

There was a wide range (10-74 %) of variability, with only four participants having CVs below 20 %. This variability probably reflects the heterogeneity of the study population. The wide intraindividual variation seen in this study probably also exists in daily clinical practice.

Based on these findings we propose that

• eGFR should be used to estimate renal function is patients using metformin.
• LCMSMS can be used as a routine method to evaluate trough serum concentrations of metformin. 20 μmol/L may be used as preliminary upper therapeutic limit.
• Intraindividual CV is high, the technique is less suitable for dose titration.
• The LCMSMS method may help to differ between metformin-associated lactic acidosis and other causes.
REFERENCES

LEGEND
Figure 1: Box-plot of trough metformin levels in 137 patients grouped in patients with eGFR<30 mL/min/1.73 m², (n=9), 30-60 mL/min/1.73 m² and >60 mL/min/1.73 m² (n=107). The outliers marked in the group with eGFR > 60 mL/min/1.73 m² are most probably due to patients accidentally taking their medication before the blood test.