Novel Assay of Metformin Levels in Patients With Type 2 Diabetes and Varying Levels of Renal Function

Clinical recommendations

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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 were measured once using liquid chromatography–tandem mass spectrometry (LCMSMS) in 137 type 2 diabetic patients with varying renal function (99 men) and followed repeatedly during 2 months in 20 patients (16 men) with estimated glomerular filtration rate (eGFR) <60 ml/min/1.73 m^2 body surface.

RESULTS — Patients with eGFR >60, 30–60, and <30 ml/min/1.73 m^2 had median trough metformin concentrations 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 intra-individual overall coefficient of variation 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 >20 μmol/l. Metformin measurement is less suitable for dose titration.

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Metformin is an insulin sensitizer used for treating type 2 diabetes, and the treatment is only rarely 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 (LCMSMS) 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 type 2 diabetic patients and to assess intra-individual variations in patients with renal impairment.

RESEARCH DESIGN AND METHODS

— Fasting venous blood samples were obtained in 137 type 2 diabetic patients (99 men, median age 60 years [range 31–83]), and 20 patients with glomerular filtration rate (GFR) <60 ml/min/1.73 m^2 (16 men, median age 68 years [range 48–83]) were studied at weeks 0, 2, 4, and 8. Serum metformin, cystatin C, and creatinine were analyzed in both groups. All participants provided informed consent, and the study was approved by the local ethics committee.

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. Then 5 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 min, respectively, from the HILIC column (Merck SeQuant, Umea, Sweden). 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 quadruple mass spectrometer (Sciex API 4000; Applied Biosystems, Carlsbad, CA). 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, MO) 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, and the results are linear between 0.05 and 125 μmol/l. At concentrations >125 μmol/l the sample is diluted. Coefficient of variation (CV) percentage during 20 months is 12% at the 3.6 μmol/l level and 6.3% at the 33 μmol/l level, with 56 samples at each level.

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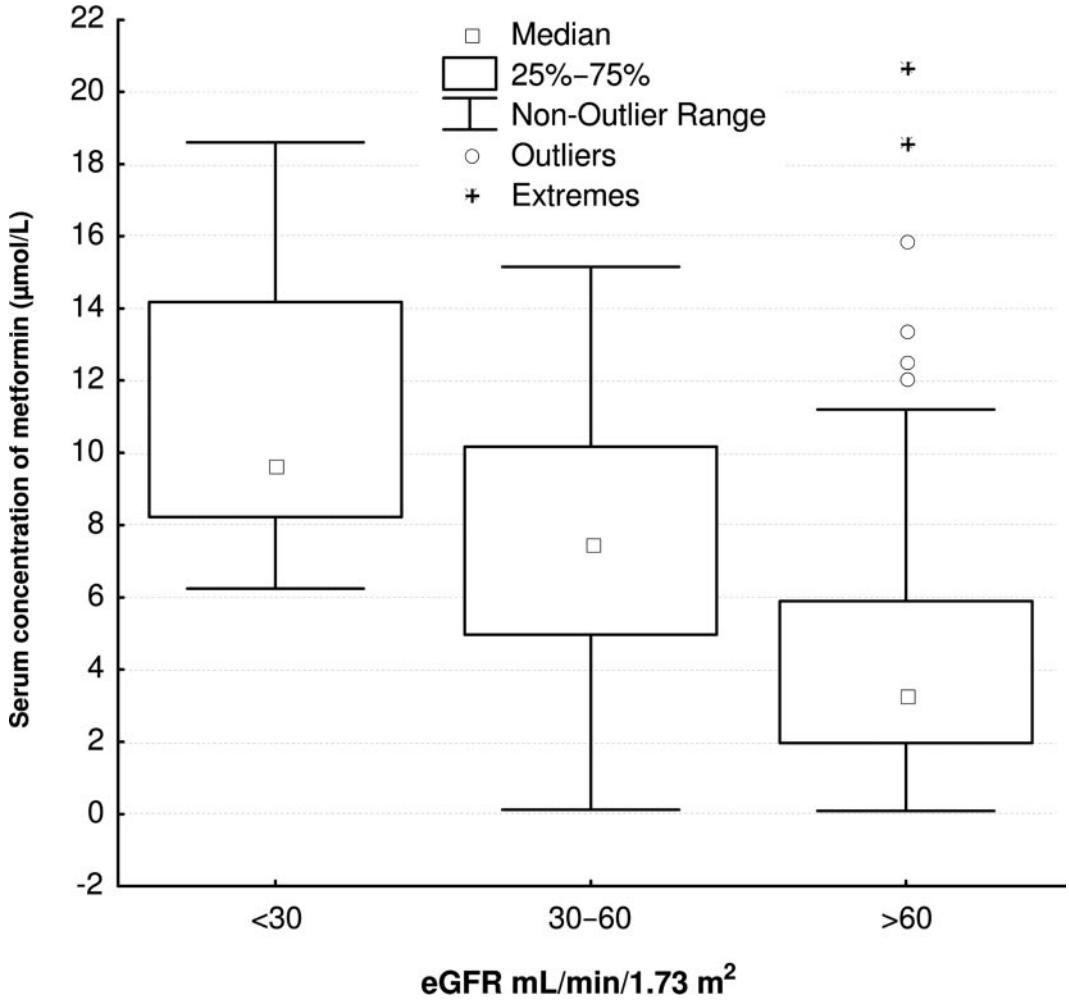

Figure 1—*Box-plot of trough metformin levels in 137 patients grouped in patients with eGFR <30 ml/min/1.73 m^2* (n = *9), 30–60 ml/min/1.73 m^2, and >60 ml/min/1.73 m^2* (n = *107). The outliers marked in the group with eGFR >60 ml/min/1.73 m^2 are most likely due to patients accidentally taking their medication before the blood test.*

Estimation of GFR

Estimation of GFR was based on cystatin C (4–6) 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 shown).

Statistical analysis

Results are given as median values and range or interquartile range. SPSS 15.0 was used. Wilcoxon rank-sum ($P < 0.05$) and Spearman nonparametric tests ($P < 0.05$) were used when appropriate.

RESULTS

Trough levels in relation to renal function

Of the 137 patients, 9 had an eGFR <30 ml/min/1.73 m^2 and a median trough value of S-metformin at 8.88 µmol/l (range 5.99–18.60); 21 patients had an eGFR of 30–60 ml/min/1.73 m^2 and a median S-metformin of 7.71 µmol/l (range 0.12–15.15); and 107 patients had an eGFR of >60 ml/min/1.73m^2 and a median S-metformin of 4.5 µmol/l (range 0.1–20.7). The median doses of metformin were 1,500 mg (1,000–3,000), 1,500 mg (500–3,000), and 1,500 mg (500–3,000) for each group, respectively (Fig. 1).

Intra-individual 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 CV 29.4% (range 9.8–74.2). Of the 20 patients, 6 had an eGFR <30 ml/min/1.73 m^2, and 14 had an eGFR 30–63 ml/min/1.73 m^2. Median S-metformin was 10 µmol/l (interquartile range 5.3–16). There was no correlation between CV of S-metformin and GFR ($r = 0.3131$, $P = 0.156$).

The CV of the four eGFR values was 7.5% (range 5.9–12.5). Median eGFR at weeks 0, 2, 4, and 8 was 37, 34, 36, and 33, respectively. There was a significant difference between the 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 type 2 diabetes 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 µmol/l (median 330). These data suggest that high levels of se-

rum 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/1.73 m^2 (7,8). 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 with any severe disease, especially when there is risk of dehydration.

Results from this study show a considerable intra-individual CV of 29.4% for 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 intra-individual 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 in patients using metformin.
- LCMSMS can be used as a routine method to evaluate trough serum concentrations of metformin and that 20 μmol/l may be used as preliminary upper therapeutic limit.
- when intra-individual CV is high, the technique is less suitable for dose titration.
- LCMSMS may help to differ between metformin-associated lactic acidosis and other causes.

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