with the Schwartz formula (12% vs 19%; P = 0.054; Table 1).

This study is the first to evaluate intrapatient variability of CysC in children with stage 3 to 5 chronic kidney disease (CKD) (9). In contrast to the study in healthy adults, the intrapatient CV of CysC was significantly lower than that of SCr in children with CKD. The most probable explanation for this difference relates to the fact that muscle mass is changing in our pediatric patients. The mean (SD) height gain over the study period was 6.7 (4.4) cm. When we accounted for the variability in height, measurement of GFR with CysC appears beneficial. Because of the demonstrated benefit of CysC in children (11), CysC could be used for the longitudinal follow-up of patients with CKD, with improved intrapatient variability compared with SCr.

Table 1. Variability analysis for CysC and creatinine.

<table>
<thead>
<tr>
<th></th>
<th>CysC</th>
<th>Creatinine</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Intrapatient CV</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low concentration</td>
<td>3.1%</td>
<td>1.8%</td>
</tr>
<tr>
<td>Medium concentration</td>
<td>3.5%</td>
<td></td>
</tr>
<tr>
<td>High concentration</td>
<td>6.7%</td>
<td>1.8%</td>
</tr>
<tr>
<td>Controls [GFR, 90–135 mL·min⁻¹·(1.73 m²)⁻¹]</td>
<td>n = 38</td>
<td>n = 38</td>
</tr>
<tr>
<td>Mean concentration</td>
<td>0.80 mg/L</td>
<td>52.4 µmol/L</td>
</tr>
<tr>
<td>Inpatient CV</td>
<td>20%</td>
<td>36%</td>
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<tr>
<td>CKD patients</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stages 3–5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean concentration</td>
<td>2.81 mg/L</td>
<td>188.9 µmol/L</td>
</tr>
<tr>
<td>Inpatient CV</td>
<td>12%</td>
<td>13%*</td>
</tr>
<tr>
<td>Stages 3 and 4</td>
<td></td>
<td></td>
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<tr>
<td>Mean concentration</td>
<td>2.37 mg/L</td>
<td>155 µmol/L</td>
</tr>
<tr>
<td>Inpatient CV</td>
<td>12%</td>
<td>13%*</td>
</tr>
<tr>
<td>GFR estimate Inpatient CV</td>
<td>11%</td>
<td>11%*</td>
</tr>
<tr>
<td>Hemodialysis patients</td>
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<td></td>
</tr>
<tr>
<td>Inpatient CV</td>
<td>12%</td>
<td>19%*</td>
</tr>
<tr>
<td>GFR estimate Inpatient CV</td>
<td>17%</td>
<td>24%*</td>
</tr>
</tbody>
</table>

* Wilcoxon matched-pairs test: a P = 0.0012; b P = 0.0144; c P = 0.38; d P = 0.0234; e P = 0.0547.

References


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Autosampler Programming for Improved Sample Throughput in Liquid Chromatography/Mass Spectrometry

To the Editor:

Liquid chromatography/mass spectrometry (LC/MS) has rapidly assumed an important role in the clinical laboratory because of its speed and inherently superior sensitivity and selectivity. It has been applied extensively in clinical research in areas such as therapeutic drug monitoring, drug metabolism, pharmacokinetics, and clinical toxicology (1–
In recent years, considerable effort has been devoted to increasing the throughput of LC/MS analyses. Strategies include the use of short columns, “ballistic gradient” chromatography (4), and coupling HPLC systems in parallel to a single mass spectrometer (5, 6). Despite these improvements in sample throughput, the time required to complete an LC/MS analysis is generally limited by the speed of conventional autosamplers to a minimum of 3–5 min per sample. Here we describe how the software controlling injection of one commercially available LC/MS autosampler can be reprogrammed to markedly increase sample throughput. This was possible because the autosampler in question, the Agilent G1313A, is controlled by step commands, the sequence of which can be changed by the operator (7).

A chromatographic run can be described in terms of various times: e.g., idle time, dead time, chromatography time, and cycle time. The definitions of these times as they apply to a standard autosampler injection program are shown in Fig. 1. The idle time of the Agilent G1313A autosampler, which includes all preparation time before an injection, is ~1.0 min for an injection volume of 20 µL.

We have established that the cycle time of the Agilent G1313A autosampler can be reduced considerably by incorporating both the dead time and idle time into the chromatography time. This is done by changing the order of step commands from 1–draw sample, 2–needle into seat, and 3–inject in the standard program to 1–inject, 2–wait 1.5 min, 3–draw sample, 4–needle into seat, and 5–valve mainpass in the new program. By making the “inject” command come first followed by a wait of arbitrary length before the other commands, the function of the inject command becomes simply to trigger the mass spectrometer to acquire data, and the actual injection is carried out by the “valve mainpass” command. This means that the autosampler preparation steps occur after the inject command rather than before it. The next sample is injected on the column before analysis of the current sample is completed, and both the idle time and the dead time are incorporated into the chromatography time (Fig. 1). The only shortcoming of the new program is that the first injection is wasted because, during this time, only the mobile phase is in the sample loop.

As an illustration of the improved efficiency of the new program, we compared it with the standard program in a clinical pharmacokinetic study of metformin (8). We found that the new program reduced the cycle time from 4.0 min to 2.4 min and nearly doubled sample throughput without requiring modification of the LC/MS interface or compromising data quality. Experience with the new program suggests that it can increase sample throughput in almost any LC/MS assay except those in which matrix effects are present after the last peak and may interfere with the next sample. In this situation, the new program can still be used, but the chromatography time needs to be extended.

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Fig. 1. Representation of the cycles involved in the operation of an autosampler controlled by a standard program (top) and the new program (bottom). The syringe indicates the actual time of injection. The terms are defined as follows: Idle time, preparation time of the autosampler before injection (includes collecting a vial, drawing the sample, and injecting it); Dead time, time for an unretained solute to reach the detector; Chromatography time, time from when an unretained solute reaches the detector to when the chromatography returns to baseline after the last peak has eluted; Cycle time, sum of the idle time, dead time, and chromatography time.
References


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