Labile Hemoglobin A1c: Unexpected Indicator of Preanalytical Contraindications

To the Editor:

Labile hemoglobin A1c (Hb A1c), or pre–Hb A1c, is an intermediate in the synthesis of Hb A1c and is characterized by the reversible binding of glucose to Hb as a Schiff base (1). Its biological variation is related to recent fluctuations in glycemia and cannot provide suitable retrospective information on a patient’s long-term glycemic balance. Therefore, labile Hb A1c must be eliminated from the measured Hb A1c over the 6–8 weeks before sampling in order to estimate the quality of diabetic control (2, 3).

Most of the Hb A1c methods available on the market either are insensitive to labile Hb A1c (e.g., immunologic assays, affinity assays) or separate or even eliminate it during analysis (e.g., by HPLC or low-pressure liquid chromatography) (4). It might be of interest, however, to take labile Hb A1c values into account when interpreting Hb A1c results if an HPLC system capable of separating this fraction can be used. In some circumstances, measurements of labile Hb A1c might serve to detect preanalytical problems that could lead to inappropriate clinical decisions.

We report on 2 recent Hb A1c orders our laboratory received without any specific information from the clinical units. We used HPLC analysis to assay Hb A1c (Variant II analyzer equipped with the Variant II Hemoglobin A1C NU Kit; Bio-Rad Laboratories); the assay fulfilled analytical specifications. We noted discrepancies, however, in each sample between Hb A1c values that fell within the reference interval or were slightly increased and labile Hb A1c values that were substantially increased. Although no reference intervals for labile Hb A1c are currently available in the literature, we found the mean (SD) percentage of labile Hb A1c to be 0.95% (0.26%) of the total Hb in 100 diabetic patients with stable Hb A1c values between 4% and 6% (i.e., 20–42 mmol/mol).

We considered the level of labile Hb A1c to be high when it was >1.5% (i.e., mean + 2 SDs). Table 1 summarizes the initial information we obtained from the clinical units. The first patient had a history of diabetes mellitus with a mean Hb A1c value of 8.0% (64 mmol/mol). The Hb A1c value in this patient was 5.8% (40 mmol/mol), whereas the labile Hb A1c value was 3.1%. The second patient, whose diabetes developed subsequent to a pancreatic tumor, was admitted for a total pancreatectomy. The Hb A1c value was moderately high (6.7%, 50 mmol/mol), and the labile Hb A1c value was very high (3.6%). Both patients had high plasma glucose and fructosamine concentrations, indicating an imbalance in recent glycemic control. These results were consistent with the increase in labile Hb A1c. Considering the inconsistencies between the measured levels of Hb A1c and labile Hb A1c in these 2 patients, we requested additional information from the clinical unit, which indicated in both cases that a blood transfusion had been performed in the days preceding the Hb A1c assay.

It is well known that Hb A1c values can be difficult to interpret in specific clinical situations, such as the presence of certain Hb variants or any impairment of Hb metabolism. Blood transfusion impairs the interpretation of Hb A1c results because Hb A1c concentrations in the donor’s red blood cells are unrelated to the glycemic control of the recipient. Clinical units that may not be aware of this problem often routinely order Hb A1c assays for evaluating glycemic control, which can produce Hb A1c results that can lead to potential misinterpretation and incorrect medical decisions.

In both of the described cases, we detected the occurrence of a preanalytical error (i.e., ordering an Hb A1c assay after blood transfusion) from only the discrepant labile Hb A1c value in the absence of clinical information. Although labile Hb A1c is generally considered only an interfering substance

### Table 1. Clinical and biological data of the patients.

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Sex</th>
<th>Age, years</th>
<th>History</th>
<th>Hb A1c (mmol/mol),%</th>
<th>Labile Hb A1c, %</th>
<th>Mean Hb A1c (mmol/mol),%</th>
<th>Fructosamine, μmol/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Female</td>
<td>85</td>
<td>Road accident, diabetes mellitus</td>
<td>5.8 (40)</td>
<td>3.1</td>
<td>8.0 (64)</td>
<td>5.4</td>
</tr>
<tr>
<td>2</td>
<td>Male</td>
<td>65</td>
<td>Total pancreatectomy, diabetes mellitus</td>
<td>6.7 (50)</td>
<td>3.6</td>
<td>N/A&lt;sup&gt;d&lt;/sup&gt;</td>
<td>4.2</td>
</tr>
</tbody>
</table>

<sup>a</sup> Reference interval: 4%–6% of total Hb (20–42 mmol/mol).
<sup>b</sup> Previous 12 months.
<sup>c</sup> Reference interval: 2.8–3.9 μmol/g total protein.
<sup>d</sup> N/A, data not available.
in Hb A1c assays and to be useless for clinical diagnostic purposes (1–4), these 2 examples show that considering labile Hb A1c values when validating Hb A1c results has potential utility for monitoring conformity with preanalytical procedures and for ensuring the global quality of laboratory performance. We propose, however, that the estimated labile Hb A1c value be used only as a tool in interpreting Hb A1c results. We recommend that labile Hb A1c values should not be used separately, because the analyzers that separate labile Hb A1c from carbamylated Hb and Hb A1c are not calibrated for quantifying these subcomponents. Moreover, any problem that could alter the interpretation of labile Hb A1c must be taken into account. For example, extended storage of samples before analysis could cause red cells to consume glucose, which could lead to the dissociation of labile Hb A1c.

We believe that each Hb chromatogram must be studied carefully. Useful information may be obtained from all Hb fractions (5). Optimal use of Hb A1c values in clinical practice may be achieved only when both physicians (i.e., those ordering the test) and medical biologists (those performing the assays and interpreting the results) fully exert their respective responsibilities.

References

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Urinary Catalytic Iron in Patients with Type 2 Diabetes without Microalbuminuria—a Substudy of the ACCORD Trial

To the Editor:

The presence of microalbuminuria in patients with diabetes mellitus (DM)1 is associated with a 3- to 5-fold higher risk of cardiovascular mortality than in patients with DM without microalbuminuria and a risk of progression to end-stage kidney disease that is 10-fold higher. Predicting and/or preventing the development of albuminuria have substantial potential to improve outcomes and reduce costs in patients with DM.

Catalytic iron, also known as labile iron, consists of chemical forms that can participate in redox cycling. This property makes catalytic iron potentially hazardous because it can participate in the generation of powerful oxidant species, such as hydroxyl radicals and/or reactive iron–oxygen complexes such as the ferryl or perferryl ion (1,2). Animal models of glomerular disease exhibit increased urinary catalytic iron, and administration of an iron chelator reduces concentrations and protects against proteinuria (3). These findings suggest a pathogenetic role for catalytic iron. We postulated that the concentrations of catalytic iron may be higher in patients with DM, and if that is so, higher concentrations could precede the occurrence of proteinuria.

We conducted an ancillary study in a subgroup of 9 clinical centers (in 2 networks from the southeastern and western regions of the US) of the NIH-sponsored ACCORD (Action to Control Car-

1 Nonstandard abbreviations: DM, diabetes mellitus; ACCORD, Action to Control Cardiovascular Risk in Diabetes.