A case of discordant HbA_1c: a method-dependent error

Goce Dimeski, Carel J Pretorius, Anthony W Russell, Stephen P Miller, Robert J Bird and Jacobus P J Ungerer

Although glycated haemoglobin (HbA_1c) has become the key biochemical marker of long-term glycaemic control, analytical method-dependent differences in results can occur when haemoglobin variants are present or HbA_1c is reduced by decreased red cell survival. When the measured HbA_1c level is discordant with the patient's blood glucose measurements and clinical status, fructosamine is an alternative biochemical marker that can provide a more accurate estimate of the glycaemic control and enable clinicians to appropriately manage patients. (MJA 2009; 191: 347-349)

Clinical record

A 65-year-old, centrally obese man (body mass index, 32.7 kg/m^2) with a 23-year history of type 2 diabetes mellitus was referred to a diabetes clinic in January 2008 for stabilisation of his blood sugar levels. His diabetes was complicated by ischaemic heart disease, peripheral vascular disease, hypertension, dyslipidaemia, and stage 3 chronic kidney disease. His glycated haemoglobin (HbA_1c) level on referral was 11.0%, measured using an ion-exchange chromatography (IEC) method on a Bio-Rad Variant II analyser (Bio-Rad Laboratories, Sydney, NSW), and the laboratory fasting glucose measurement was 19.3 mmol/L (Box 1). Both these results were consistent with the patient's home blood glucose measurements (> 10 mmol/L).

On presentation, the patient's medication included human mixed insulin (Mixtard 30/70; Novo Nordisk, Sydney, NSW) (80 units before breakfast and 75 units before dinner), as well as simvastatin, aspirin, clopidogrel, diltiazem, ramipril, frusemide, omeprazole, bisoprolol, irbesartan and glycercyl trinitrate. The insulin regimen was changed to a basal-bolus regimen of insulin aspart and insulin glargine, which was titrated weekly on the basis of home blood glucose measurements.

After 6 weeks, the patient's HbA_1c level had decreased to 5.8%, which appeared to be inconsistent with the home blood glucose measurements and the laboratory fasting glucose measurement (15.1 mmol/L). This change in HbA_1c level coincided with the implementation of a modified method (“NU”) on the Bio-Rad Variant II analyser.

Haemoglobin (Hb) studies (electrophoresis and chromatography) demonstrated an abnormal Hb variant interfering with the HbA1c measurement. The patient's fructosamine level was tested and the result indicated improved glycaemic control, but not to the extent suggested by the HbA1c level. A week after the fructosamine measurement, the HbA_1c measurement was repeated with a Siemens DCA 2000 analyser (Siemens, Melbourne, Vic) using an immunoassay (IA) method, which is less likely to be influenced by Hb variants. The result was congruent with the fructosamine estimation of the glycaemic control (Box 1).

On subsequent visits, the patient's HbA_1c level was measured with a different IEC method on a Bio-Rad D-10 analyser (Bio-Rad Laboratories, Sydney, NSW), from which a substantially

1 Results from the various glycated haemoglobin (HbA_1c) analytical systems and other laboratory measurements

<table>
<thead>
<tr>
<th>Date</th>
<th>Analytical system</th>
<th>Analytical method</th>
<th>HbA_1c*</th>
<th>eAG (mmol/L)</th>
<th>Glucose (mmol/L)</th>
<th>Fructosamine (μmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>29 January 2008</td>
<td>Bio-Rad Variant II² (old method)</td>
<td>IEC</td>
<td>11.0%</td>
<td>14.9</td>
<td>19.3</td>
<td>—</td>
</tr>
<tr>
<td>11 March 2008</td>
<td>Bio-Rad Variant II (new method)</td>
<td>IEC</td>
<td>5.8%</td>
<td>6.6</td>
<td>15.1</td>
<td>—</td>
</tr>
<tr>
<td>1 April 2008</td>
<td>Bio-Rad Variant II (new method)</td>
<td>IEC</td>
<td>4.9%</td>
<td>4.9</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>3 May 2008</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>271</td>
</tr>
<tr>
<td>10 May 2008</td>
<td>Siemens DCA 2000§</td>
<td>IA</td>
<td>7.4%</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>28 November 2008</td>
<td>Bio-Rad D-10²</td>
<td>IEC</td>
<td>9.2%</td>
<td>12.0</td>
<td>7.8</td>
<td>280</td>
</tr>
<tr>
<td>9 December 2008</td>
<td>Bio-Rad Variant II (new method)</td>
<td>IEC</td>
<td>4.4%</td>
<td>4.4</td>
<td>—</td>
<td>234</td>
</tr>
<tr>
<td></td>
<td>Bio-Rad D-10</td>
<td>IEC</td>
<td>9.0%</td>
<td>11.7</td>
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<td>—</td>
</tr>
<tr>
<td></td>
<td>Siemens DCA Vantage⁵</td>
<td>IA</td>
<td>7.5%</td>
<td>9.3</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Roche Integra*</td>
<td>IA</td>
<td>7.7%</td>
<td>9.7</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Primus PDQ**</td>
<td>AC</td>
<td>7.9%</td>
<td>10.0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Bio-Rad in2it²</td>
<td>AC</td>
<td>6.8%</td>
<td>8.2</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>20 January 2009</td>
<td>Bio-Rad in2it</td>
<td>AC</td>
<td>7.3%</td>
<td>—</td>
<td>—</td>
<td>307</td>
</tr>
</tbody>
</table>

eAG = estimated average glucose. IEC = ion-exchange chromatography. IA = immunoassay. AC = affinity chromatography. * All HbA_1c results are aligned to the National Glycohemoglobin Standardization Program (http://www.ngsp.org). † Reference range, 190–285 μmol/L. ‡ Bio-Rad Laboratories, Sydney, NSW. § Siemens, Melbourne, Vic. DCA Vantage is an upgraded model of the DCA 2000. ¶ Roche Diagnostics, Sydney, NSW. ** Primus Diagnostics, Kansas City, Mo, USA.
higher result was obtained (Box 1). A further fructosamine measurement (280 μmol/L) suggested the glycaemic control was better than the HbA1c results now indicated.

To investigate the effect of the Hb variant on the different HbA1c assays, the patient’s 9 December 2008 sample was analysed using the different analytical systems available locally (Box 1). The highest (9.0%) and lowest (4.4%) HbA1c results were obtained from IEC methods on different analysers from the same supplier.

Before the modification of the Bio-Rad Variant II method, there was incomplete separation of the Hb variant and HbA1c peaks (Box 2, A). After implementation of the modified method, the separation of the Hb variant and HbA1c peaks was much more distinct (Box 2, B and C).

The instrument software integrates only the one HbA1c fraction or peak and therefore underestimates the HbA1c level by about 50%. The Bio-Rad D-10 chromatogram (Box 2, D) is similar in appearance to the chromatogram from the original Bio-Rad Variant II method (Box 2, A). The range of HbA1c values obtained with the affinity chromatography (AC) and IA methods spanned a range from 6.8% to 7.9% (Box 1); this variation is within the anticipated interlaboratory performance. To avoid such variation, one analytical method should consistently be used for monitoring longitudinal changes.

The patient’s Hb variant was characterised as a heterozygote for the Hb Athens-Georgia mutation by a reference laboratory (Southern Cross Pathology Australia, Melbourne, Vic). This is a very rare and clinically silent variant detected in Caucasians where only one Hb variant is affected,1,2 and, depending on the separation method, it can produce two peaks on the chromatogram, as was the case with the modified Bio-Rad Variant II NU method (Box 2, B).

**Discussion**

This case illustrates some of the caveats associated with measuring glycaemic control. A multilaboratory approach is required to achieve optimal glycaemic control, as failure to recognise the true state of control might result in inappropriate clinical management decisions and less than optimal patient care.

The rate of HbA1c formation is proportional to the average blood glucose concentration over the life span of red blood cells (about 120 days). Methods used to measure HbA1c are divided into the three categories of IEC, AC and IA. In the 2008 Royal College of Pathologists of Australasia Quality Assurance Program (http://www.rcpa.qap.com.au), 59% of participants used IA, 30% used IEC and 11% used AC methods, performed on 29 different commercial platforms. About 40% of all HbA1c measurements were performed with point-of-care instruments that use either IA or AC methods.

Interference from abnormal Hb variants can lead to falsely elevated or lowered HbA1c results with IEC methods. AC methods are the least affected by Hb variants, as the method separates all glucose-modified Hb molecules from non-modified molecules, and calculates the HbA1c value from the total glycated Hb result.3,5 More than 1300 Hb variants have been identified,3 and about half of these are clinically silent.4 An HbA1c level < 6% or > 15% in patients with diabetes can often be due to Hb variant interference.3,6

The prevalence of Hb variants is highest in Mediterranean regions, Africa and Asia, particularly India and Pakistan. IA methods are particularly used in these regions. However, unlike with HbA1c, to date there are no published accepted treatment targets for fructosamine, nor are the different fructosamine measurement methods standardised.

The most recent consensus statement on the standardisation of HbA1c measurement recommends that laboratories should report an HbA1c-derived average glucose or estimated average glucose (eAG) value alongside the HbA1c level.10 As the eAG is calculated from the HbA1c level, it will not assist in those
patients whose HbA_{1c} measurement is not accurate because of Hb variants or reduced red cell survival.

The HbA_{1c} result and the eAG should correlate with the patient’s glucose measurements and clinical status. If they are discordant, the HbA_{1c} result should be rechecked using an alternative analytical method that is less susceptible to interference from Hb variants. Alternatively, fructosamine measurement might be a useful adjunct in these cases. It may be prudent to rely more on glucose tests when there is uncertainty about long-term markers of glycaemic control, although the long-term markers may still be valuable for trend analysis.

Considering current migration patterns, with more than half of the nearly 150 000 settlers arriving in Australia in the 2007–08 financial year coming from Africa, the Middle East and Asia, patients with Hb variants and decreased red cell survival will become more prevalent in the Australian population. This case emphasises the limitations of HbA_{1c} assays in such patients and the importance of communication between clinicians and the laboratory when unexpected results occur.

Competing interests
None identified.

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References

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