Letter to the Editor
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# Hemoglobin Camperdown [β104Arg→Ser] Detection During Hemoglobin $A_{1c}$ Measurement via Capillary Electrophoresis

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Dear Editor,

Reports on the analytical evaluation of capillary electrophoresis (CE) for glycated hemoglobin ($HbA_{1c}$) measurement have already been published [1]. There have been several recent reports concerning the impact of the presence of hemoglobin variants on $HbA_{1c}$ measurement with the CE method. One study focused on the analytical interference of some hemoglobin variants [2], demonstrating that hemoglobin variants S, C, D, and E do not interfere either with $HbA_{1c}$ measurement or the glycation rate of hemoglobin variants, which is equal to the glycation rate of HbA. In a second report, analytical evaluation of CE showed that hemoglobin variant E and β-thalassemia do not affect $HbA_{1c}$ results. Moreover, most hemoglobin variants common to China can be detected by using a CE analyzer, and $HbA_{1c}$ values do not seem to be affected [3]. We report a case of hemoglobin Camperdown (HbCa) detected by using a Capillarys 2 Flex Piercing instrument (C2FP, Sebia, Lisses, France) during $HbA_{1c}$ measurement.

A 76-yr-old man receiving follow-up care for type 2 insulin-dependent diabetes was admitted to our hospital for a necrotic wound on the heel. To manage the diabetes, HbA1c was measured on a C2FP instrument in our clinical biology laboratory at Rouen University Hospital, France. The CE software flagged an alarm stating "*Atypical profile*" with an $HbA_{1c}$ value of 4.2% (22 mmol/mol). We observed an abnormality on the electropherogram (Fig. 1), with a peak of unknown hemoglobin quantified at 3.0% and named fraction 1 by the CE software. There was no other abnormality on the electropherogram. The sample was also tested with a Bio-Rad Variant II analyzer (Bio-Rad, Marnes-la-Coquette, France) in the clinical biology laboratory at Le Havre General Hospital, France, which measured the $HbA_{1c}$ value at 52.2% (547 mmol/mol) because of migration of a probable hemoglobin variant in the $HbA_{1c}$ zone. Concentration of fructosamine (261 μmol/L; 205-260 μmol/L) was just above the reference values, which was not consistent with the two $HbA_{1c}$ values obtained. The patient's last $HbA_{1c}$ measurement was performed in our laboratory 10 yr previously. At that time, we observed a peak of unknown hemoglobin on HPLC (Variant, Bio-Rad), and we were not able to quantify $HbA_{1c}$. The unknown hemoglobin was identified in the National Hemoglobinopathy Reference Laboratory (Créteil, France) as HbCa [β104Arg→Ser]. Ten years ago, the percentage of HbCa was estimated at 51.5%, which was consistent with a heterozygote patient. We supposed

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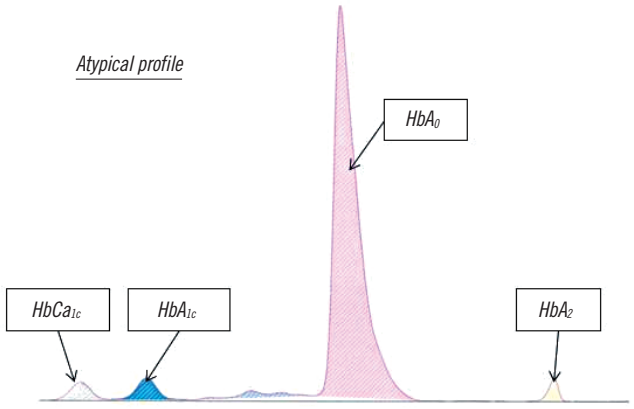


**Fig. 1.** Abnormal electropherogram obtained by Capillarys 2 Flex Piercing instrument. Abnormal electropherogram with software alarm "atypical profile" showing a peak of unknown hemoglobin ($HbCa_{1c}$), which is the glycated form of hemoglobin Camperdown (HbCa). HbCa is not visible owing to co-eluation with $HbA_0$.

that the unknown fraction 1 on the electropherogram was the glycated fraction of HbCa. We observed that both glycated fractions were clearly separate, unlike HbCa and $HbA_0$, which exactly coeluate, explaining the abnormal value of $HbA_{1c}$ calculated by the CE software. A manual off-line recalculation of the global glycated fractions percentage was performed, by integrating the sum of both peaks instead of $HbA_{1c}$ with the formula for percentage of glycated fractions= $(HbA_{1c}+HbCa_{1c})/(\text{Total } HbA_0 \text{ peak})\times 100$. We then applied the calibration equation of the software, thereby obtaining a value of 6.3% (45 mmol/mol) for global glycated fractions.

HbCa is a rare variant, first described in 1974 [4], which is not associated with hematological abnormalities. Hemoglobin variants can affect the accuracy of $HbA_{1c}$ measurement, resulting in interference. This interference depends on the type of hemoglobin variant and the method used. Recently, Gaborit *et al*. [5] published the effects of this variant on $HbA_{1c}$ measurement using HPLC and immunoassay methods. They concluded that it was not possible to measure the exact $HbA_{1c}$ value with the affinity chromatography method. Moreover, immunoassay methods are blind to detecting such variants and probably underestimate $HbA_{1c}$ values. Herein we describe for the first time a case of HbCa interfering with CE $HbA_{1c}$ measurement. The C2FP $HbA_{1c}$ method is not able to differentiate $HbA_0$ from HbCa. The presence of an unknown peak must alert the pathologist. The CE software value is affected by negative bias due to the co-elution of HbCa and $HbA_0$. Manual recalculation seems to yield good results for global glycated fractions, consistent with fructosamine. However, the threshold for $HbA_{1c}$ values must be applied with great care. In fact, the glycated rate of this rare variant has not yet been validated by any publication in the literature. Fructosamine, glycated albumin, or daily capillary blood glucose remain good markers to manage such patients.

## Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

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## REFERENCES

1. Jaisson S, Leroy N, Meurice J, Guillard E, Gillery P. First evaluation of Capillarys 2 Flex Piercing® (Sebia) as a new analyzer for $HbA_{1c}$ assay by capillary electrophoresis. Clin Chem Lab Med 2012;50:1769-75.
2. Weykamp C, Kemna E, Leppink S, Siebelder C. Glycation rate of haemoglobins S, C, D, E, J and G, and analytical interference on the measurement of HbA1c with affinity chromatography and capillary electrophoresis. Clin Chem Lab Med 2015;53:e207-10.
3. Ji L, Yu J, Zhou Y, Xia Y, Xu A, Li W, et al. Erroneous HbA1c measurements in the presence of β-thalassemia and common Chinese hemoglobin variants. Clin Chem Lab Med 2015;53:1451-8.
4. Wilkinson T, Gough P, Owen MC, Carrell RW, Kronenberg H. Detection of variants of haemoglobin D in Australia: haemoglobin D Camperdown alpha 2 A beta 2 121 Glu leads to Val and haemoglobin D Punjab alpha 2 A beta 2 121 Glu leads to Gin. Med J Aust 1974;26:636-7.
5. Gaborit B, Nicolay A, Valéro R, Bégu A, Badens C, Bellanné-Chantelot C, et al. Comparison of performances of various HbA1c methods in Haemoglobin Camperdown variant detection: consequences in diabetes management. Clin Chim Acta 2009;403:262-3.


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