Spuriously high MCV in a patient with diabetic ketoacidosis

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SUMMARY

We report a case of a falsely increased mean corpuscular volume (MCV) due to severe hyperglycaemia in a patient with diabetic ketoacidosis. This phenomenon results from in vitro swelling of hyperosmolar red blood cell size when diluted in an iso-osmolar buffer of a haematology analyser, and does not reflect a true macrocytosis in vivo. The magnitude of this effect is dependent on the glucose concentration of the sample and time of incubation prior to analysis. Haematology analysers from three different manufacturers were found equally sensitive to this phenomenon. Therefore, it is suggested to use reluctance when reporting and interpreting MCV results in the case of severe hyperglycaemia to avoid unnecessary additional investigation.

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CASE DESCRIPTION

A 55-year old man with insulin-requiring diabetes mellitus was admitted to the emergency department with a diagnosis of diabetic ketoacidosis and sepsis. The patient was found somnolent at home by his father who alerted the emergency services. He had not taken his diabetes medication for three days, resulting in at home glucose measurements around 500 mg/dL. Physical examination and anamnesis revealed polydipsia and a productive cough with limited dyspnoea. The patient had lost five kilograms in the past week. He had an extensive medical history among which diabetes mellitus type 1 since 1993 with major diabetic nephropathy (CKD stage III) complicated by multiple diabetic foot infections, HIV, syphilis, hepatitis C and various episodes of urinary retention for which the patient probes once a day.

Laboratory results are shown in *Table 1*. Admission values (D0) of point-of-care testing (POCT, ABL flex 90 blood gas analyser, Radiometer, Denmark) at the emergency department showed a glucose higher than the upper limit of measurement interval (>847 mg/dL), confirmed by the laboratory chemistry results which revealed a glucose of 2.083 mg/dL [ref: 74-106 mg/dL] (Cobas c702 analyser, Roche Diagnostics, Belgium). Laboratory haematology analysis (DxH 800 cell counter, Beckman Coulter, USA) revealed a macrocytic anaemia with an exceptionally high mean corpuscular volume (MCV) of 118 fL [ref: 81-101 fL], a haemoglobin of 10.9 g/L [ref: 13.1-17.2 g/dL], RBC of 3.3 x 10^{12}/L [ref: 4.5-6.5 x 10^{12}/L], a haematocrit of 0.39 [ref:0.39-0.50] and mean corpuscular haemoglobin concentration (MCHC) of 27.7 g/dL [ref: 32-36 g/dL). Vitamin B12 values, folate values and microscopic evaluation of the blood smear were within normal ranges, which made the diagnosis of a megaloblastic anaemia or a haematological malignancy such as myelodysplastic syndrome (MDS) unlikely.

The patient was treated vigorously with intravenous fluids (glucose 5% + 40 mEq KCl), insulin (actrapid six units per hour initially) and antibiotics for an urosepsis. Ten hours after admission (D+1) glucose decreased to 726 mg/dL with further decrease over the next few days to 69 mg/dL on D+5 (data not shown). Haematology values on D+1 were haemoglobin 11.2 g/dL, RBC 3.4 x 10^{12}/L; haematocrit 0.33, and MCHC 34.4 g/dL while MCV normalised to pre-admission values being around 95 fL (D-236) and remained stable in the following days.

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TABLE 1. Laboratory findings.

	D-236	D0 (admission)	D+1	D+2	D+3	Reference interval
POCT (arterial blood)						
pH	–	6.97	–	–	–	7.35-7.45
pO_2, mmHg	–	46.8	–	–	–	83-108
pCO_2, mmHg	–	22.4	–	–	–	35-48
Glucose, mg/dL	–	>847	–	–	–	74-106
Lactate, mmol/L	–	3.0	–	–	–	0.5-1.6
Sodium, mmol/L	–	124	–	–	–	136-145
Potassium, mmol/L	–	4.3	–	–	–	3.4-4.5
Chemistry						
CRP, mg/L	12	227	224	212	118	<5
Vitamin B12, ng/L	279	639	ND	ND	ND	197-771
Folate, µg/L	2.4	4.5	ND	ND	ND	2.4-17.7
Sodium, mmol/L	142	122	144	145	142	136-145
Potassium, mmol/L	4.4	4.4	4.2	4.3	4.1	3.4-4.5
Glucose, mg/dl	**155**	2083	**726**	**178**	**124**	**74-106**
Creatinine, mg/dL	2.64	5.20	4.36	3.06	2.54	0.67-1.17
Haematology						
Haemoglobin, g/dL	11.8	10.9	11.2	10.6	11.2	13.1-17.2
Haematocrit, ratio	0.33	0.39	0.33	0.31	0.32	0.39-0.50
RBC, x 10^{12}/L	3.5	3.3	3.4	3.3	3.3	4.5-6.5
MCV, fL	**96**	118	**96**	**95**	**96**	**81-101**
MCH, pg	34.1	32.6	33.2	32.6	33.5	27-35
MCHC, g/dL	36	28	34	34	35	32-36
Platelets, x 10^9/L	179	163	ND	120	131	150-450
Leukocytes, x 10^9/L	6.1	12.6	9.4	8.2	6.9	4.5-11.0

**ND (not determined)*

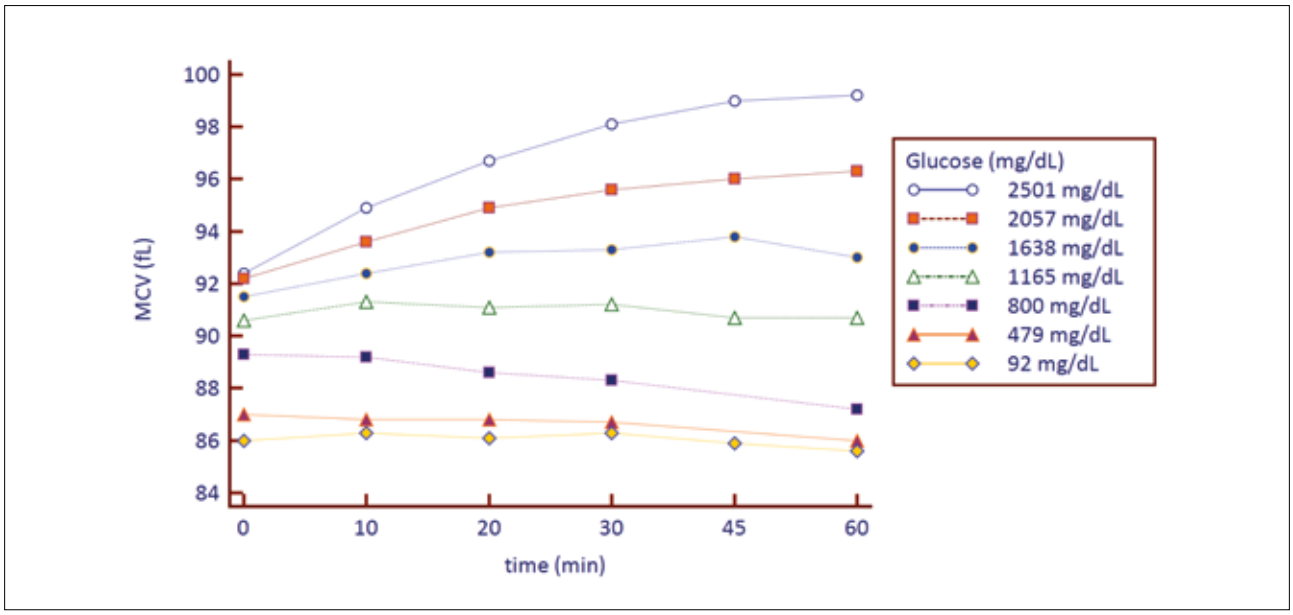

FIGURE 1. Changes of MCV in function of glucose concentration and incubation time at room temperature.

Although the patient showed a quick recovery, a discrepancy in admission laboratory values versus repeated laboratory results remained unexplained: what was the cause of the major macrocytosis in this sample? An inconsistency in MCV values over days within the same patient can indicate erroneous sample identification or sample mix-up, but stability in other measured parameters ruled out this option. Did the measured MCV value reflect true macrocytosis in vivo, then?

ANSWER

In normal conditions, the MCV reflects the size of the red blood cells (RBC). In the presented case, the MCV measured in vitro does not reflect the true in vivo RBC size. Measured MCV values may be spuriously high in hyperosmotic conditions as a result of specific measurement conditions: most laboratory haematology analysers dilute samples with an iso-osmotic diluent for measurement of RBC and platelets and the corresponding indices such as MCV or haematocrit (HCT). However, in case of hyperglycaemia, the intracellular osmolality of the erythrocyte is higher than the osmolality of the diluent, causing diluent to rapidly cross the erythrocyte membrane during analysis to establish a new equilibrium thereby expanding the volume of the red cells in vitro. As certain RBC indices reported by cell counters are derived of or dependent on MCV values, such as HCT and MCHC, these latter parameters might by affected as well.[1]

EFFECT OF GLUCOSE CONCENTRATIONS ON MCV MEASUREMENTS

To investigate the effect of hyperglycaemia on MCV measurements, venous EDTA blood (K3EDTA, Sarstedt, Germany) obtained from a healthy volunteer was spiked using a 600 mg/mL glucose solution to obtain five aliquots with plasma glucose concentrations up to 2.500 mg/dL. RBC indices of the five aliquots and an untreated aliquot were determined with the Beckman Coulter DxH 800 cell counter within five minutes from spiking and subsequently approximately every ten minutes. Glucose was measured simultaneously on separate aliquots with a Cobas c702 analyser (Roche Diagnostics, Belgium).

Figure 1 shows the Beckman Coulter DxH 800 results for MCV from a healthy volunteer sample to which various amounts of glucose were added. The increase in MCV at five minutes incubation at room temperature for the highest glucose concentration was 7 fL in comparison to a sample to which no glucose was added. Interestingly, at higher glucose concentrations (1.638 mg/dL and up) the MCV increased in a time-dependent way, reaching a plateau after +/- 45 minutes with a maximal increase of 14 fL after 60 minutes of incubation at room temperature.

COMPARISON BETWEEN HAEMATOLOGY ANALYSERS

In a second experiment the effect of hyperglycaemia on MCV measured by three different haematology analysers was compared. EDTA blood aliquots were spiked at two glucose concentration levels. After one hour of incubation, both samples, together with an unspiked sample, were analysed with a Beckman Coulter DxH 800 cell counter, the ADVIA 2120i analyser (Siemens Healthcare Diagnostics, USA) and

TABLE 2. Comparison of MCV measured by three haematology analysers at different glucose concentrations.

	Beckman Coulter DxH 800	Advia 2110i	Sysmex XN1000
2269 mg/dL glucose	103 fL	102 fL	102 fL
1179 mg/dL glucose	92 fL	92 fL	90 fL
56 mg/dL	87 fL	84 fL	86 fL

the Sysmex XN1000 analyser (Sysmex Corporation, Japan). The Beckman Coulter DxH 800 is an impedance-based analyser that dilutes an aliquot of the blood sample isoosmotically, generating a flow of red blood cells that pass through a small aperture. Passage of cells generates an electrical resistance, and the number and height of electric pulses allow accurate determination of both the RBC and the MCV.[2] The Advia 2120i haematology analyser uses optical flow cytometry for the analysis of cells. Scattering at least at two angles allows the determination of RBC and MCV. The diluent contains sodium dodecyl sulphate (SDS) and glutaraldehyde that causes isovolumetrically sphering of the red blood cells to eliminate shape as a variability factor.[2]

The Sysmex XN1000 analyser dilutes an aliquot of the blood sample and measures the RBC via a DC electrical impedance method, similar to the Coulter principle.[2] The HCT reflects the cumulative pulse height of the RBC, i.e. it is a summation from individual pulses in de RBC channel. The MCV is then calculated using the RBC and HCT (MCV=HCT/RBC*10).

As shown in *Table 2*, the effect of blood glucose concentrations on MCV were comparable between the different automated haematology analysers.

DISCUSSION

Haematology analysers allow quick and accurate complete blood count (CBC) analysis. Red blood cell indices (MCV, MCH, and MCHC) provide information concerning size and haemoglobin content of red blood cells. The MCV receives considerable attention from clinicians as it is used for morphological classification of anaemia (microcytic, normocytic and macrocytic), thereby contributing to a diagnostic orientation in anaemic patients.[3] In addition, the MCV has a valuable role in monitoring the pre-analytical conditions of the sample, since it is one of the most stable parameters (i.e. controlled very tightly physiologically) in the CBC with a variability less than 6% over time, thereby indicating possible sample mix-up in case of large variation for a given patient.[4] Spuriously high MCV values in samples containing high levels of glucose is a known interference of automated haematology analysers and was first reported in 1963.[1,5,6] However, this is probably an under-recognised phenomenon. This in vitro effect on the MCV has been observed both in hyperglycaemic diabetic patients and when a sample is drawn near a glucose infusion line, the latter also diluting the sample resulting in decreased RBC and haemoglobin values.[1]

Not only glucose but also other solutes such as sodium and urea can cause changes in blood osmolality. Hence, hyper- or hyponatremia contributes to changes of MCV in vitro as seen with high levels of glucose. As a result, the osmolality of samples from hyperglycaemic patients with concurrent hyponatremia may be normal.[1,7] However, the effect on MCV measurements is not solely dependent on the osmolality as such, but also on the speed of transport of the solute over the RBC membrane: increased urea concentrations lead to a rise in osmolality but the high RBC membrane permeability to urea induces a very rapid equilibrium during incubation of the sample with dilution buffer in vitro before analysis, thereby not affecting the MCV.[6,8]

In accordance to the literature, we found a time- and concentration-dependent increase of MCV after addition of glucose to an EDTA sample. Furthermore, we found similar sensitivities of the MCV to hyperglycaemia using three different analysers. In contrast, Van Duijnhoven and Treskes (1996) demonstrated different sensitivities between two analysers, as a result of differences in composition of the diluent and measuring interval.[6] However, one of the analysers (Technicon H2) evaluated in the latter study is no longer commercially available.

One way to monitor errors is the use of a MCV delta check. A delta check compares current results to previous results; if the difference exceeds predetermined limits, the laboratory information system (LIS) flags the result so the discrepancy can be investigated. For the MCV, a value >5% within one week in the same patient suggests pre-analytical errors such as a patient identification error or sample mix-up. However, as shown in this case, also analytical interferences may contribute to a positive delta check, such as falsely high in vitro determined MCV value due to hyperglycaemia.

KEY MESSAGES FOR CLINICAL PRACTICE

1. **Mean corpuscular volume (MCV) is an indicator of red blood cell size and is physiologically tightly regulated and stable over time.**
2. **Large intra-individual variations in MCV should raise the suspicion of erroneous patient ID or sample mix-up.**
3. **Several conditions such as vitamin B12 or folate deficiency and myelodysplasia (MDS) can cause macrocytosis with elevated MCV.**
4. **Hyperosmotic conditions such as hyperglycaemia can induce a spuriously elevated MCV as a result of technical measurement principles on haematology analysers.**

CONCLUSION

Measurements of mean corpuscular volume (MCV) in hyperglycaemic samples using automated blood cell counters may result in spuriously high MCV values and also false results for erythrocyte indices calculated based on MCV results. Only when osmolality inside the erythrocyte is similar to the diluents osmolality, the erythrocyte volume is measured correctly by haematology analysers. Three commonly used haematology analysers, Beckman Coulter DxH 800, Siemens ADVIA 2120i and Sysmex XN1000 were found equally susceptible to this interference. Thus, in the case of severe hyperglycaemia, one should be reluctant in reporting and interpreting MCV results. This would prevent unnecessary additional tests to find the cause of the spurious macrocytosis.

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