Raised Coulter mean corpuscular volume in diabetic ketoacidosis, and its underlying association with marked plasma hyperosmolarity

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SUMMARY   An abnormal and transient increase of the Coulter mean red cell volume (MCV) was observed in four of 10 patients with diabetic decompensation. A sequential study of haematological and biochemical measurements in these patients revealed that this phenomenon was associated with the hyperosmolar state. The proposed mechanism and the clinical implications of this change are briefly discussed.

Recently we have reported a marked but transient increase of the Coulter mean red cell volume (MCV) in diabetic ketoacidosis[1] which was reversed after correction of the hyperglycaemia and ketoacidotic state. To substantiate this observation and to explore possible mechanisms underlying the change, we have undertaken a detailed study of a further 10 diabetic patients, eight with ketoacidosis and two with hyperosmolar non-ketoacidosis syndrome. We report and discuss our main findings and the mechansim for the observed Coulter MCV change.

Material and methods

The 10 patients, comprising two males and eight females (age range: 8–78 yr) were admitted over a 13-month period to Aberdeen Royal Infirmary with decompensated diabetes mellitus. The majority were established insulin-dependent diabetics but two were studied at presentation of their diabetes and one was a maturity onset diabetic who had omitted oral medication.

The investigations undertaken on and serially during the hospital admission included: peripheral blood profile (Coulter S calibrated with 4C; venous blood with K_2EDTA, 1 mg/ml, as anticoagulant); venous Microhaematocrit (Hawksley, England) obtained by centrifuging for 3 min at 12000 g; microscopy of stained blood film; biochemical profile (Sequential Multichannel Analyser with Computer, Technicon Ltd, USA); blood glucose measured by a

glucose-oxidase method and a glucose analyser (Beckman, Glenrothes, Scotland); serum osmolarity measured by depression of freezing point (Osmette A, Precision Systems Inc, USA); and pH of arterial blood only if clinically warranted using a blood gas analyser (IL model C13, Instrumentation Laboratories, Dugnano, Italy). The conscious level of all patients on admission was clinically graded as follows:

Grade 1—alert and responds coherently to questions.
Grade 2—drowsy but responds to minimal stimulation.
Grade 3—miminal response to maximal stimulation.
Grade 4—responds to deep pain only.

Results

Throughout this section and the subsequent discussion, the patients are considered in three groups (Table) according to blood pH and plasma osmolarity. Group A (patients 1 and 2) were markedly hyperosmolar and acidotic. Group B (patients 3 and 4) were markedly hyperosmolar but not acidotic. Group C (patients 5–10) were not hyperosmolar but were acidotic.

As seen in the Table, the only patients with an abnormally raised Coulter MCV are those in Groups A and B (patients 1–4) where hyperosmolarity is the single common factor. Further it is to be noted that it is only in these same patients that there is a disproportionate increase in both the Coulter MCV and Coulter PCV compared to the manually derived values shown in parentheses.

Factors other than hyperosmolarity and which are

Haematological and biochemical data of the 10 patients on hospital admission

Patient			Osmolarity (mosmol/l)	MCV (fl) Coulter (Manual)	PCV l/l Coulter (Manual)	Arterial pH	Plasma glucose (mmol/l)	Serum electrolytes (mmol/l)				Conscious level (Grade 1–4)
No	Sex	Age (yr)						Na	K	HCO₃	Urea	
Group A												
1	F	19	381	100 (92)	0·62 (0·54)	6·7	36·0	129	5·7	2·0	13·8	4
2	F	40	419	105 (81)	0·55 (0·43)	6·9	53·0	131	6·0	2·5	12·8	3
Group B												
3	F	66	395	98 (82)	0·49 (0·41)	7·38	53·5	146	3·2	17	22·6	3
4	F	78	415	104 (89)	0·49 (0·42)	7·3	49·2	158	4·2	13	15·0	3
Group C												
5	M	8	305	86 (84)	0·48 (0·46)	7·3	18·3	134	4·5	7	7·6	2
6	M	16	297	97 (100)	0·51 (0·53)	—	15·4	135	5·1	10	5·9	2
7	F	19	289	89 (89)	0·49 (0·50)	7·2	23·9	129	5·0	8	9·2	2
8	F	78	313	91 (91)	0·41 (0·41)	—	40·5	125	4·7	26	16·7	1
9	F	18	295	85 (82)	0·48 (0·47)	7·08	21·0	142	6·0	3	10·0	2
10	F	25	287	86 (84)	0·50 (0·49)	7·16	34·6	136	6·6	8	17·6	2
Reference values												
Male				83–96	0·41-0·52	7·35-7·42	3·5-5·6	137–145	3·6-4·8	21–25	3·6-7·6	
			281–297									
Female					0·34-0·44						3·0-7·8	

also altered in diabetic decompensation, are unlikely to have contributed to the increased Coulter MCV, as they obtain equally in the three patient groups.
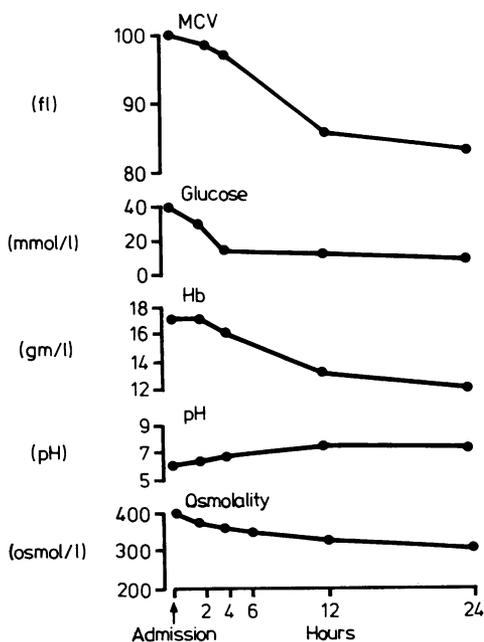
To demonstrate that the abnormally high Coulter MCV was spurious rather than real, Romanowsky stained peripheral blood films were examined. There was no red cell macrocytosis but echinocytosis was noted. The pattern, including the typical time relation of the alteration in Coulter MCV and sev-

Time scale of the MCV change and its relation to some other laboratory parameters (patient 1)

eral biochemical parameters is illustrated in the Figure. The magnitude of the change in MCV is especially striking and in this instance (patient 1) amounted to 18 fl over an interval of 24 h. The true Coulter value for this particular patient was known to be 82 fl as recorded on several previous occasions when her diabetes was stable and under satisfactory control. It is also seen that the return of the MCV to normal coincides most closely with the correction of serum osmolarity, in contrast to that of the other biochemical parameters.

Discussion

The Coulter MCV is normally a stable and highly reproducible parameter in healthy adult subjects[2] but osmotic errors in electronic particle counter MCV determinations have previously been recognised.[3-5] In this study, we demonstrated a marked rise in the Coulter MCV above the reference range in the hyperosmolar group of patients and that following clinical recovery this reverted to the normal resting value for the individual. Our results suggest that this transient increase of the Coulter MCV is not only spurious but additionally is related to the principles involved in cell counting and sizing in the Coulter system which employs a non-isotonic solution (Isoton II with a measured osmolality of 339 mosmol/l) in the diluting process.

The first of these propositions is supported by the miscroscopic appearance of the red cells in stained blood films prepared at the bedside from native blood of patients with a high Coulter MCV. The red cells of such patients were not macrocytic. The second conclusion arises from the marked disparity

between the Coulter MCV and its calculated counterpart. The Coulter MCV in the four hyperosmolar patients averaged some 15·4% higher than the calculated

$$MCV \left(= \frac{Microhaematocrit}{Coulter\ RCC} \right) \text{ whereas normally the}$$

Coulter MCV would be expected to be about 3% lower than the calculated value. This represents an important paradoxical finding in diagnosis of hyperosmolar patients.

The explanation for this abnormal and transient increase in the Coulter MCV in only the hyperosmolar patients would appear to concern the osmotic equilibrium that normally exists in vivo between red cells and their surrounding plasma. In diabetic decompensation when plasma osmolarity is increased, the main contributory factors are the high molar concentration of glucose and the net body water loss. In such a plasma environment, the erythrocytes must also acquire a hypertonic intracellular content. If these erythrocytes are then introduced into a relatively hypotonic diluent as in the process of Coulter counting, water will enter the cell, induce swelling and thereby lead to an erroneously high Coulter MCV value.

Other factors are commonly present in decompensated diabetics and might have been responsible for the observed increase in the Coulter MCV, thus acidosis may induce red cell swelling with entry of chloride ions subsequent to ionisation of intracellular haemoglobin.[6] This is a true swelling of the red cell and as such would be manifest as an increase in both the centrifuged and the Coulter haematocrits. however, we have demonstrated that it is the Coulter haematocrit only which is disproportionately increased in the hyperosmolar patients. Other red cell factors which may be altered in diabetic ketoacidosis and interfere with the Coulter cell sizing process include aggregation,[7] deformability,[8] membrane lipid and cholesterol content,[9] and electrical resistivity and surface membrane charge.[10] However their effects would have been manifest as a raised Coulter MCV in the non-hyperosmolar ketoacidotic group C. We therefore conclude that the increase in Coulter MCV is primarily an osmotic phenomenon.

The underlying mechanism however is not simply an increase in plasma osmolarity but also an accumulation of glucose or its metabolites within the red cell.

These propositions are supported by the experimental studies and observations reported by Allen *et al*[11] who suggested a similar mechanism may account for the high Coulter MCV recorded in some patients receiving excessive intravenous carbohydrate feeding.

In conclusion, an abnormally high Coulter MCV in diabetic decompensation appears to be commonly associated with plasma hyperosmolarity and represents an osmotic imbalance. It is proposed that a similar osmotic imbalance may occur in other non-insulin requiring tissue such as brain, and may have a role in the pathogenesis of cerebral oedema occurring during treatment of these patients. Finally, from a clinical viewpoint the recognition of a markedly raised MCV may alert the clinician to the hyperosmolar state, and the need to consider modification of the fluid replacement and insulin therapy. Pathologists involved with the provision of a clinical laboratory service should also be aware of this considerable inaccuracy in the measurement of the mean corpuscular volume by Coulter counters in such patients.

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