

High mean red cell volume: its incidence and significance in routine haematology

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SUMMARY With the advent of electronic particle counters of the Coulter S type the mean cell volume (MCV) has become an integral and useful feature of the red cell profile. Abnormally high values, often of minor degree, are particularly common but their precise clinical significance may be difficult to establish. This study defines the normal range and determines the incidence and distribution of the high MCV in routine hospital practice. Two hundred consecutive adult patients with an MCV of 100 fl or more were identified from the Coulter S analysis of 6542 blood samples and the underlying cause was established in 80%. Some of the clinical and economic implications of these findings are presented and briefly discussed.

Red cell size has for many years been used to classify anaemia (Wintrobe, 1930). Early studies employed diffraction (Pijper, 1919) or the painstaking measurement of projected images (Price-Jones, 1933) to detect changes in cell diameter in addition to the microscopic inspection of fixed and stained blood films with or without the aid of an eyepiece micrometer (Thorell, 1964). More recently cell size was related to mean cell volume (MCV), an index derived from the red cell count and packed cell volume. The adoption of this measurement, however, was restricted by the inaccuracies associated with visual red cell counting (Biggs and Macmillan, 1948) and the centrifuged haematocrit (England *et al.*, 1972).

A radical change followed the advent of electronic particle counters employing gating techniques (Mattern *et al.*, 1957) that facilitated the rapid measurement of red cell volumes and their subsequent computation as MCV. While the electronically derived MCV has been generally regarded as more accurate than that of earlier methods it harbours several sources of error (England and Down, 1976) and, in our view, has also created new clinical, laboratory, and economic problems.

The high capital cost, automation, and large work capacity of such instruments have all contributed towards the centralisation of routine

haematology. A large number of abnormal blood profiles including a raised MCV, many of which are of minor degree, are now encountered as an isolated finding without obvious or immediate clinical relevance.

This paper reports a study of the incidence and clinical associations of a high MCV in the routine work load of a teaching hospital laboratory with the object of defining the clinical and practical problems and thus to clarify not only the economic implications but also to formulate guidelines for investigating patients with a high MCV.

Patients and methods

The normal range of MCV for our laboratory was established from routine analysis of peripheral blood samples obtained from apparently normal subjects undergoing occupational health surveillance. One hundred males (Hb > 14 g/dl) and 100 females (Hb > 12 g/dl) were selected alphabetically to give equal cohorts of subjects between 15 and 69 years of age divided according to age and sex. Two hundred consecutive adult patients whose peripheral blood profile showed an MCV of 100 fl or more, a value significantly outwith the normal range, were then identified from the routine work load and formed the basis of this prospective study.

All subjects except 30 patients who were attending a leukaemia clinic and receiving cytotoxic therapy were investigated as follows: (1) a reticulocyte count was done and any neutrophil nuclear hyper-

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segmentation or erythroid macrocytosis seen in a stained blood film was noted by a medical member of the laboratory staff; (2) a request was made for assay of serum B₁₂ and folate activity, measurement of serum urea, and screening of liver function; (3) bone marrow examination was recommended only when indicated on clinical grounds; (4) detailed information was sought on alcohol consumption, smoking habit, previous gastric surgery, malignant disease, drug therapy, and the working or definitive diagnosis; (5) each patient's record was carefully reviewed at the end of the study by one of us (RJLD) to find, if possible, a satisfactory explanation for the raised MCV.

Routinely submitted samples of 0.5 dl venous blood with K₂ EDTA (1.5 g/l) as anticoagulant were in the main analysed within four hours of venepuncture by a Coulter Counter Model S (Coulter Electronics Inc, Harpenden, Herts, UK) operated by the technical staff. Calibration of the counter was checked twice weekly with 4C cell control (Coulter Electronics Inc) while day-to-day and within-day quality control was effected by whole blood samples and a 'cusum chart' technique (Cavill and Jacobs, 1973). Blood films were spread in the laboratory and examined after automatic fixation and staining by a Hema-Tek Slide Stainer (Ames Co, Slough, Bucks, UK). Reticulocyte counts were based on the counting of 500 cells. Serum B₁₂ and folate levels were assayed microbiologically by, respectively, the method of Spray (1955) with *Lactobacillus leichmanii* (NC1B 8118) as the test organism and the method of Waters and Mollin (1961) with *Lactobacillus casei* (NC1B 8010). Serum urea, bilirubin, aspartate aminotransferase, and alkaline phosphatase concentrations were routinely measured in the department of chemical pathology (Professor S. C. Frazer) by an automated Technicon system (Technicon Instruments, Basingstoke, Hants).

Results

The normal range of MCV for both sexes is shown in the Figure. No effect of age or sex was evident with 95% of subjects having an MCV within the range of 82-95 fl. The mean (\pm SD) for males was 88.22 fl (\pm 3.60) and for females 88.70 fl (\pm 3.75).

INCIDENCE AND DISTRIBUTION OF HIGH MCV

The 200 patients with an MCV \geq 100 fl were identified from the profiles of 6524 samples submitted during 26 whole working days in the spring of 1975. Because the investigation was repeated in some patients the total number of samples with a

high MCV was 256, giving an overall sample prevalence of 3.9%. When identified, 120 patients were attending a medical and 64 a surgical area of the hospital; the remaining 16 were attending the Maternity Hospital. One hundred and six were inpatients and 94 were outpatients. Their age and sex distributed according to the amount of the rise in MCV are shown in the Table (a). Twenty males (26%) and 61 females (50%) were under 50 years of age. The numerical importance of minor rises is emphasised by the fact that 145 (72.5%) of the 200 patients had an MCV of 100-104 fl.

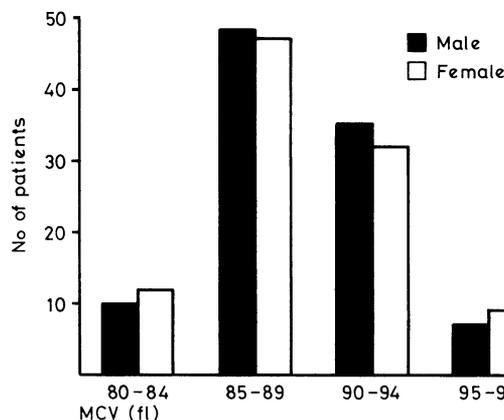


Figure Normal range of MCV.

RELATED BLOOD CHANGES

The prevalence and distribution of anaemia and the red and white cell morphology in the 170 patients without haematological malignancy are shown in the Table (b). Reticulocytosis was not regarded as the major factor contributing to the rise in MCV in any patient.

No patient had a Hb level < 7 g/dl. Anaemia, defined in this study as Hb level < 14 g/dl in men and < 13 g/dl in women, was found in only about one in three of patients with a minor (100-104 fl) or moderate (105-109 fl) rise in MCV but featured in 12 of the 15 patients with an MCV \geq 110 fl.

Macrocytosis was recognised microscopically in only 111 (65%) out of the 170 patients with a high MCV, and in these macro-ovalocytosis was detected infrequently (13.5%). On the other hand, round and target-shaped macrocytes were found in 86.5%. Neutrophil nuclear hypersegmentation was described in 11 of 15 patients with an MCV \geq 110 fl but in only 40 out of 155 with minor or moderate rises. Although neutrophil nuclear hypersegmentation is recognised as one of the features of the peripheral blood picture in uraemia (Jensson, 1958) we found

Table Distribution of high MCV in 78 males and 122 females according to age, sex, and haematological and biochemical values

	MCV (fl)					
	Males			Females		
	100-104	105-109	≥ 110	100-104	105-109	≥ 110
(a) No. of patients (200)	52	20	6	93	17	12
Mean age (years)	58.1 ± 17.1	55.9 ± 15.8	73.5 ± 11.3	50.4 ± 21.2	54.9 ± 19.6	51.5 ± 22.8
(b) No. of patients (170)	46	15	5	80	14	10
With anaemia	24	5	2	21	6	10
With macrocytosis:						
Oval	1	—	2	5	3	4
Round target	28	14	2	39	10	3
With neutrophil nuclear hypersegmentation	8	3	4	25	4	7
(c) No. of patients (21)						
With megaloblastosis	1	2	1	4	1	3
Without megaloblastosis	2	2	—	3	2	—
(d) No. of patients (76)	23	10	3	29	6	5
With low B ₁₂	2	2	3	5	1	3
With low folate	2	0	0	5	2	1
(e) No. of patients (107)	32	12	4	42	11	6
With abnormal liver function tests	12	7	1	6	3	2
(f) No. of patients (121)	35	11	4	56	10	5
With a high serum urea	12	3	3	11	0	3
(g) No. of patients (200)	52	20	6	93	17	12
With high MCV explained	40	16	6	70	16	12

no such relationship ($\chi^2 = 3.1$; $P > 0.05$) in patients with both a high MCV and high urea. While 52 of the patients, mainly those with primary haematological disorders including leukaemia, had diagnostic marrow aspirations done within a year of the beginning of this study, marrow examination was recommended and undertaken in a further 21 during the study. The latter are classified in the Table (c). Out of the 12 patients with megaloblastic haematopoiesis 10 (four males and six females) had Addisonian pernicious anaemia. Out of the nine patients without megaloblastosis, haematopoiesis was classified as megaloblastoid in one, macro-normoblastic in one, and normoblastic in the remaining seven.

Although requested in all 170 patients, only 76 samples were received for assay of serum B₁₂ and folate activity. Out of the 68 patients with an MCV within the range 100-109 fl in whom assays were undertaken eight had a low serum B₁₂, seven a low serum folate, and two a deficiency of both. In contrast subnormal values were found in seven out of eight patients with an MCV ≥ 110 fl (see Table (d)).

LIVER FUNCTION TESTS

Liver function was screened in 107 (48 males, 59 females) of the 170 patients. The results (Table

(e)) show that 20/48 males (42%) had abnormal tests compared with 11/59 females (19%). This sex difference was statistically significant ($\chi^2 = 5.8$; $P < 0.02$). Patients with disordered liver function, particularly when associated with a biliary obstruction, commonly have target cells in their peripheral blood film. Such cells were found in 13/31 patients (42%) with abnormal liver function compared with 13/80 (16%) with normal liver function tests ($\chi^2 = 7.7$; $P < 0.01$).

CLINICOPATHOLOGICAL CORRELATES OF HIGH MCV

Malignant disease

Forty-one patients had malignant disease, which was haematological in 30 cases and non-haematological in 11. The spectrum of haematological malignancies comprised lymphoma (13), lymphocytic leukaemia (8), myeloma (5), granulocytic leukaemia (2), lymphoblastic leukaemia (1), and polycythaemia rubra vera (1). The patients (12 males, 18 females) were all receiving intermittent chemotherapy. The range of their MCV was 100-116 fl (mean 105).

Of the 11 patients with non-haematological malignant disease carcinoma was present in seven males and four females with a mean age of 76 years.

Four had hepatic metastases, two with obstructive jaundice.

Disordered erythropoiesis

The diagnoses in these 16 patients included Addisonian pernicious anaemia (10), sideroblastic anaemia (1), paroxysmal nocturnal haemoglobinuria (1), congenital erythroid hypoplasia (1), and autoimmune haemolytic anaemia (3). Two of the last group were receiving azathioprine.

Malabsorption

This group of 27 cases included patients who had had gastric surgery (19), coeliac disease (2), dermatitis herpetiformis (2), congenital duodenocolic fistula (1), and dietary deficiency alone (3). In 10 of these patients multiple factors contributed to the high MCV. Thus of the 19 (12 males, 7 females) who had had gastric surgery, and in whom the MCV ranged from 100-108 fl (mean 102), six were alcoholics, one had a grossly deficient diet, and three others had carcinoma.

Hepatobiliary disorders, including alcoholism

There were 57 patients in this group. The hepatobiliary disease found in 27 patients included cryptogenic cirrhosis (2), biliary tract obstruction (5), metastatic carcinoma (6), and congestive cardiac failure (14). Excessive alcohol consumption, defined as a regular intake of more than $\frac{1}{2}$ bottle of spirits or 6 pints (3.4 l) of beer per day, was found in 30 patients—24 males (mean age 54 years) and six females (mean age 49 years). In 21 patients the MCV was in the range 100-104 fl and in only one was the MCV > 110 fl. This latter patient, a 64-year-old man, had a duodenocolic fistula, malabsorption, and megaloblastic haemopoiesis due to B₁₂ deficiency. Serum folate was assayed in 19 patients with alcoholism and was low in only two. We cannot over-emphasise that in 26 out of the 30 alcoholic patients in whom liver function was screened biochemical abnormalities were detected in only 14 by the methods we used.

Drug therapy

In 59 patients drug therapy seemed to be the major contributing factor and involved cytotoxic chemotherapy in 29 instances; in a further 21 patients a variety of drug preparations were implicated and these included azathioprine (4), anticonvulsants (4), biguanides (4), sulphasalazine (6), dapsone (1), pyrimethamine (1), and co-trimoxazole (1).

Miscellaneous causes, including pregnancy

The 35 patients in this group comprised those with gastrointestinal bleeding (3), respiratory failure (5),

renal failure (4), cold haemagglutination (3), and aged samples (4). In most cases additional disease processes or factors were present. Surprisingly, this study identified 13 antenatal and three postnatal patients with a raised MCV (range 100-111 fl). In 13 the rise was minor (to < 104 fl) but consistent and was not associated with anaemia (Hb < 11 g/dl). Serum folate activity was assayed in only four patients and was low in one. While the cause of the high MCV was not obvious in most of these patients, a related folate deficiency was considered unlikely, and the degree of abnormality was considerably outwith physiological variation (Chanarin *et al.*, 1977). This unexpected finding has prompted us to investigate in more detail the MCV in pregnancy.

Unexplained rises in MCV

In 45 patients (22.5%) the high MCV could not be satisfactorily explained, though admittedly 14 of them had been incompletely investigated when the study ended. Most were outpatients, few were anaemic, and all but six had minor rises in MCV (100-104 fl). While a rise in MCV has been related to age, sex, and smoking habit (Helman and Rubenstein, 1975) we found no such associations in this group. Nevertheless, some interesting associations were found. Four patients had undergone radioactive thyroid ablation and one a partial thyroidectomy, but all seemed to be euthyroid and receiving adequate thyroxine replacement (Horton *et al.*, 1976); five had rheumatoid arthritis, and although three were being treated with mefenamic acid none showed evidence of the haemolysis that may be caused by this drug (Scott *et al.*, 1968); two had systemic lupus erythematosus; and five had hyperlipaemia.

Discussion

Rises in MCV as detected by particle counters of the Coulter Model S type cannot necessarily be equated with macrocytosis—a loosely defined term usually referring to the subjective assessment of red cell diameter in fixed and stained blood films. Thus, only 65% of blood samples with an MCV of 100 fl or more had macrocytes in the peripheral blood film. In the main, such a discrepancy reflects the greater sensitivity of electronic methods of red cell sizing over visual methods, particularly where increases in red cell diameter are uniform and small. On the other hand, some rises in MCV may be spurious (Young and Lawrence, 1975) and result from factors which interfere with the instrument counting and sizing processes. Recognising such spurious results can be of practical and diagnostic importance, such as drawing attention to haemagglutination (Hattersley *et al.*, 1971).

In 22.5% of our 200 patients no satisfactory explanation for the high MCV was evident, a finding comparable to that of Hattersley (1964). Although the rises in these cases were predominantly minor we believe they were real and not spurious. Certainly calibration and other instrument errors would seem unlikely, since in over 90% of these patients the MCV was monitored more than once during or outside the period of study and found to be consistently raised.

Historically, cell size has been used to classify anaemia and macrocytosis has all too commonly been equated with megaloblastosis. We found anaemia in less than half and megaloblastosis in only a few patients. We therefore emphasise that the MCV should be considered an important haematological measurement in its own right and the pathological significance of abnormal values assessed independently of the presence of anaemia. Furthermore, the pathological conditions associated with a raised MCV (McPhedran *et al.*, 1973; Chanarin *et al.*, 1973) are much more diverse than is often appreciated and a confusing plurality of disease processes not infrequently coexists in an individual patient. Our findings assert the importance of underlying dyserythropoiesis caused predominantly by deficiencies of B₁₂ or folic acid and the effects of drugs, particularly cytotoxic and immunosuppressant agents. The numerical importance of macronormoblastic erythropoiesis (Chanarin, 1976), however, is reaffirmed by our finding of a large number of patients with hepatobiliary disease and alcoholism (Wu *et al.*, 1975), the latter often occult and suspected only after finding the raised MCV.

Finally, some rises in MCV that are not megaloblastic or dyserythropoietic in origin may result from alterations in the physicochemical composition or disturbance of the homeostatic mechanisms controlling the dynamic equilibrium between the red cell, particularly its membrane, and plasma components. There is a linear relationship between erythrocyte lipid content and MCV (Brownlee *et al.*, 1974), and it is tempting to speculate that in some cases the macrocytosis associated with pregnancy, hepatobiliary disease, alcoholism, renal failure, and myxoedema may in part be related to changes in plasma lipids often found in these conditions.

In this study we have defined the magnitude of the problem posed by the high MCV in routine laboratory practice and indicated the potential investigative work load generated by this single finding. Before the introduction of the Coulter Model S to this region Davidson (1971), reporting a six-year survey of megaloblastosis, found that 1% of all blood samples submitted for routine

haematological examination had macrocytes evident on microscopic examination of the peripheral blood. This compares with an incidence of 3.9% for a raised MCV (less than half of which were associated with anaemia) in the present study. In the Grampian Area of Scotland, with an adult population of about 350 000 and a work load of 120 000 Coulter analyses annually, some 4000 patients with a raised MCV might be expected each year. The practical and economic implications of such an investigative work load must be realised, particularly when it is appreciated that the diversity and complexity of factors leading to rises in MCV preclude a single or uniform method of investigation. The investigative pattern must be tailored to the individual patient with a full appreciation of the factors known to contribute to rises in MCV, noting the clinical findings, haematological profile, and microscopic appearances of the peripheral blood film. Such a policy requires close co-operation and consultation between clinician and haematologist so that all relevant data may be collated to allow rational and definitive investigation.

In conclusion, we are very aware of how this study has exposed our limited understanding of the pathogenesis of increases in red cell volume, a frequent and everyday problem. At present one in five such increases remains unexplained. Improvements and simplification of the investigative procedure for satisfactory differentiation of the causes of a high MCV must await further elucidation of the basic mechanisms governing red cell size.

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