The Results from Red Cell Shape Analyses of Blood Samples From Members of Myalgic Encephalomyelitis Organisations in Four Countries

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Abstract

Results from the red cell shape analyses of 5-drop samples of immediately-fixed venous blood from 620 male and 1558 female members of myalgic encephalomyelitis (ME) organisations in New Zealand, Australia, South Africa and England are reported. Both the age spectra and the male to female ratios of the participants are very similar. Increased percentages of flat cells are the most frequent change in both sexes in all four countries although the increases are much higher in the English results than in those from the southern hemisphere countries. Because the poor filtrability of ME blood which had been reported earlier could have been a consequence of red cell shape transformation, it is considered that the results reported here would support a proposal that ME is a hemorrheological disorder in which symptoms are manifestations of the consequences of impaired capillary blood flow. If this proposal can be confirmed by further investigations of blood filtrability and blood viscosity, then attention would be directed to the therapeutic potential of hemorrheological agents.

Introduction

Red cell shape analysis is a development from the observation that immediately-fixed red cells from healthy animals and humans could be classified into six different shape classes on the basis of simple, descriptive criteria. There are earlier reports of the scanning electron microscopic morphology of the cells in immediately-fixed blood samples. Qualitative studies of the blood in muscular dystrophy[2] and in spinocerebellar degeneration[3] have been reported and a quantitative study of the blood cells of patients with Huntington's Disease revealed increased percentages of stomatocytes.[4] The results from early studies of rapidly fixed blood samples[5,6,7] conflict with the textbook concept that all erythrocytes at rest are biconcave discocytes. However, textbook concepts are based upon the scanning electron microscopic morphology of red cells which had been collected into an anticoagulant and washed in a physiological solution before fixation. But it has been reported that the three most frequently used anticoagulants induced a time-related echinocytic transformation[8] and that four different physiological solutions caused different patterns of red cell shape change.[9] Those findings could have been anticipated as Miller et al[2] had noted that it was not possible to store unfixed red cells without them changing shape, and Yasuda et al [3] had commented that procedures prior to fixation greatly influenced red cell shape. Shape-changed, poorly-deformable red cells reduced the rate of capillary blood flow and resulted in cell trapping in different regions[10] and in increased vascular resistance.[11]

Echinocytic transformation has been shown to result in a reduced capacity to load and to release oxygen. Such observations support the proposal that change in red cell shape populations is not a benign event. The increase in stomatocytic cells, which are recognised as being poorly deformable in Huntington's Disease,[4] could be related causally to the reduced cerebral blood flow which has been reported to occur in Huntington's Disease patients.[13]

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Immediately-fixed blood samples from patients with type 1 diabetes had increased percentages of flat cells[14] and it is likely that the flat cells will contribute to the altered blood rheology which is a feature of the diabetic state.

Myalgic encephalomyelitis (ME) is a disorder of uncertain aetiology and with no generally accepted pathogenesis, which presents with a wide range of symptoms which may wax and wane in severity. While tiredness and easy exhaustability on exertion are very common symptoms, central nervous system dysfunction manifested as memory problems, brain fog and depression occur also. Because it is germane to the general discussion, it should be noted that Kobayashi et al[15] have observed that "satisfaction with life or mild depression influence regional cerebral blood flow, even in normal subjects." In patients with depression after stroke, mesial temporal cortical blood flow values were significantly lower in depressed patients than in nondepressed patients.[16] Earlier it had been reported that patients with depression had slightly but significantly reduced cerebral blood flow.[17]

None of those authors discussed the possible mechanisms of the reduced cerebral blood flow, but as a single blood sample from a patient with depression had a high percentage of flat cells it is possible that a hemorrheological factor is involved.

The basic philosophy behind the current international study is that if ME is a medical entity, then changes in red cell shape which have been observed in immediately-fixed blood samples from members of ME organisations in New Zealand should occur in the blood of ME patients wherever they reside. Because of the lack of resources it has not been possible to establish baseline values for the different cell shape classes in the blood of healthy subjects in each country.

Therefore the data presented here represent the percentages of cases where a result exceeded a cut off value which was the upper 95% confidence interval of data relating to healthy New Zealanders. The actual values are shown in **Figure 1** (p. 223). This method of summarising data for comparison is due to the known interdependence of cup forms and cells with altered margins. Environments which stimulate cup-transformation depress cells with altered margins, quite frequently to zero, and vice versa.

Individual reports had been submitted to the medical journals of New Zealand, Australia and South Africa but in all cases editors considered the results should go to a specialist journal so this combined report was submitted to the British Journal of Haematology. It was rejected on the basis that the paper was uncontrolled, despite this matter being dealt with in the text.

Methods

1. Participants. Most of the samples came from members of audiences who attended a lecture which provided the background observations leading up to the proposal that ME is a hemorrheological disorder. Individuals who requested red cell shape analysis were provided with a vial containing 5 ml of a 2.5% solution of glutaraldehyde in 0.1M cacodylate buffer at pH 7.4 and an instruction sheet which explained the procedure for the collection and immediate fixation of a blood sample. Samples consisted of five drops of venous blood which had been obtained by syringe and were mailed to Dunedin in packages labelled "Fixed human red cells, noninfective, nonperishable." Participants were requested to provide information about age, gender and their well being at the time of sample collection but only about 80% complied. When samples arrived in the laboratory they were registered and assigned a unique number.

2. Preparation for scanning electron microscopy. Samples which had fixed for

overnight at least, were prepared according to a published technique.[18] Cells were washed in buffer, dehydrated in ascending concentrations of ethanol to absolute, transferred to pure,dry acetone and two drops of the acetone suspension placed on a coverglass fixed to a pin stub with double-sided adhesive tape. After air-drying, cells were gold-coated in a sputter coater.

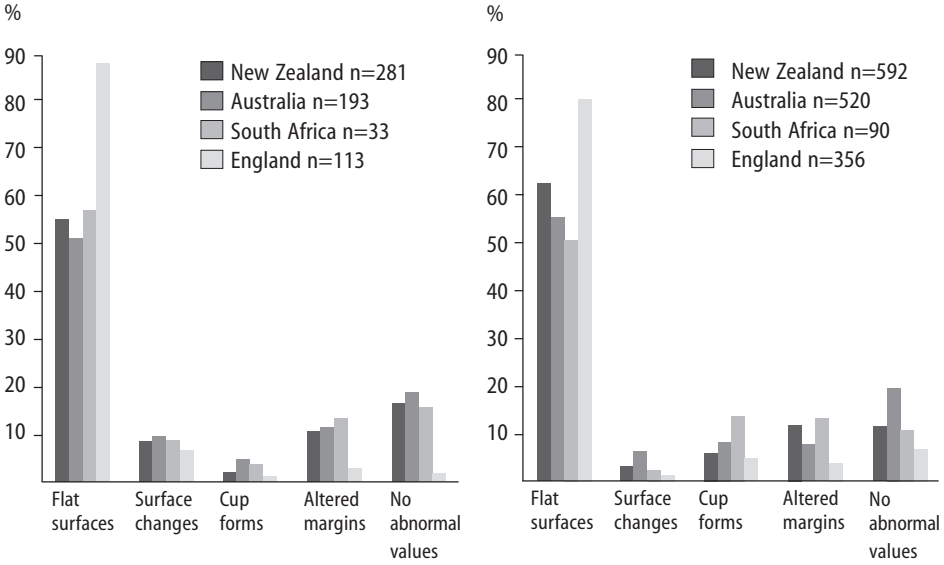
3. Scanning electron microscopy. All samples were photographed in a Stereo-scan 360 scanning electron microscope at 1300x, at lOkV, using a 20mm working distance. The electron microscopist was instructed to photograph three widely separated fields from randomly selected areas of well-spread cells. Micrographs identified only by their register number were recorded in a Mitsubishi video processing unit. Blinding was enhanced by photographing samples from different studies at the same session.

4. Red cell shape analysis. All samples were assessed by one individual (LOS) without knowledge of the identity of the sample. Cells were classified as biconcave discocytes, flat cells, cells with surface changes, cup forms (early and late) or cells with altered margins according to published criteria.[18] Micrographs showed between 90 and140 cells and approximately 320 cells were assessed from each sample. The proportions of the different cell types were expressed as percentages of the total number of cells counted from each sample.

Results

The red cell shape populations of 620 male and 1558 female blood samples were assessed. The overall male to female ratio was 1:2.5, but the value varied from 1:2.2 for New Zealand participants to 1:3.2 for English residents.

Figure 1. The proportions of cells of different types that exceed cut off values derived from blood samples from healthy New Zealanders. The values which have been established as the upper 95% confidence interval for normal in New Zealand are as follows. Flat cells: males 61.4%, females 60.4%. Cells with surface changes: males 23.4%, females 25.1%.Cup forms: males 11.7%, females 13.9%. Cells with altered margins: males 17.7%, females 16.8%.



1. Age. The data are summarised in **Figure 1** (p. 223) and **Table 1** (below). There is considerable inter-country similarities. Overall ages ranged from 5-69 years for males and 4-69 years for females. As it is not possible to separate with certainty the red cell shape changes due to ME from those associated with aging, the results from 63 cases (New Zealand 24; Australia 29; England 10) who were 70 years or older were excluded from the study.

2. Red cell shape analysis. The percentages of the different cell shapes summarised in **Table 1** indicate that there are marked similarities between the male and female data. Increased percentages of flat cells was the most frequent change in both sexes although the percentages of this cell type were much higher in the English results. About 12% of cases had no abnormal values which is consistent with the fluctuating nature of the symptoms.

Discussion

Results from the red cell shape analysis of a substantial number of blood samples from members of ME organisations with similar age spectra and male to female ratios, but living in four countries showed the common feature of having high percentages of flat cells in their blood. English samples had higher percentages of cases with increased flat cells than occurred in the samples from other countries.

Although Mukherjee et al[19] reported unusual red cell morphology in a small number of ME blood samples they did not study immediately fixed red cells and for that reason their findings are not relevant to this study.

It is possible that the enucleate state of the mature red cell places it at the mercy of its environment and it seems likely that agents or events which give rise to red cell shape transformation perturb the red cell environment. Triggering events may be vi-

Table 1. Age and gender data relating to the participants from the four countries.

	Males	Females
New Zealand		
mean (S.D.)	32.6 (13.7) years	37.7 (13.6) years
95% confidence intervals	5.7-59.5 years	11.0-64.3 years
range	5-69 years	5-69 years
n	226	517
Australia		
mean (S.D.)	45.3 (11.1) years	44.2 (13.3) years
95% confidence intervals	23.5-67.1 years	18.1-70.3 years
range	5-69 years	4-69 years
n	145	472
South Africa		
mean (S.D.)	43.6 (12.0) years	32.6 (13.7) years
95% confidence intervals	20.1-67.1 years	5.7-59.5 years
range	16-64 years	5-67 years
n	33	90
England		
mean (S.D.)	39.7 (13.6) years	43.2 (12.8) years
95% confidence intervals	13.0-66.4 years	18.1-68.3 years
range	7-68 years	10-69 years
n	113	356

ral or bacterial infections or an exposure to a toxic stimulus. The effects of hormonal change may be manifested as change in the red cell shape populations. It is not known what *in vivo* events are responsible for a change in a particular cell type but it has been observed that the same virus initiated both cup transformation and increased cells with altered margins in different individuals. ME patients whose automated blood screens are normal, may show very abnormal shape populations.

As high percentages of flat cells can be expected to affect blood rheology and therefore capillary blood flow adversely, these results are in agreement with the report of the poor filtrability of ME blood.[20] These findings support the idea that ME is a haemorheological disorder and imply that capillary dimension will play an important role in determining the distribution and severity of symptoms. It has been proposed that on the basis of the normal distribution of mean capillary diameters, there will be a segment of the population whose values fall in the first quartile of the distribution.[21] It is envisaged that those who develop the symptoms of ME have the anatomical feature of smaller than usual capillaries which puts them at risk of becoming symptomatic when exposure to some triggering event causes red cell shape transformation. This concept provides a basis for understanding why many ME patients report loss of symptoms while on dietary supplementation with evening primrose oil.

Seed oil of evening primrose contains the essential fatty acid substrates which lead to the synthesis of prostaglandin E 1 (PGE1). Manku et al[22] reported that 4 x 500mg capsules of evening primrose oil had no effects on the blood levels of PGE 1 although a significant increase in PGE 1 levels occurred with an intake of 8 x 500mg capsules of Efamol oil of evening primrose. The effects of PGE, are to increase the fluidity of the lipid bilayer of the red cell membrane[23] which increased red cell deformability as assessed by filtrability. The results of those studies increase the likelihood that the benefits of evening primrose oil for ME patients will relate to the consequences of improved microcirculatory blood flow. However not all ME patients respond to evening primrose oil and other haemorheological agents may prove helpful. But there will be no great advance in this form of treatment until the intracellular mechanisms responsible for changing red cell shape are fully investigated.

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