Red Blood Cell Shapes in Women with Fibromyalgia and the Implications for Capillary Blood Flow and Tissue Function

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Abstract

Results from red cell shape analyses of 623 blood samples from women residing in three countries are reported. Only 164 samples came from subjects with a confirmed physician's diagnosis of fibromyalgia (FM). Average ages of those with confirmed diagnoses were almost the same as those in the larger groups whose FM diagnostic situation is not known.

Mean percentages of the different cell types were remarkably similar between those with or without a known diagnosis and among the participants from the three countries. Increased flat cells was the most significant feature, and in the 5 groups the mean values ranged from 73.4% in the larger group of American women to 75.3% in the smaller group of English women. Because flat cells are poorly deformable and reduce the rate of capillary blood flow, this implies that the use of haemorheologic agents could be beneficial in those with FM.

Introduction

Although some physicians appear to be able to make a diagnosis of fibromyalgia (FM) with little difficulty, the potentially controversial nature of such a diagnosis is exemplified by the papers of Littlejohn[1] and Cohen & Quintner[2] which were published in the same issue of the Medical Journal of Australia in 1998.

On the one hand, Littlejohn[1] put forward the concept of a syndrome characterised by chronic pain and a number of other, apparently unrelated symptoms. It was claimed that in that model, fibromyalgia syndrome "represents a functional rather than a structural state (i.e. an amplification of normal pain processes." But Cohen & Quintner[2] considered that "Fibromyalgia syndrome is a bogus construct" which was supported by "...those favouring non-somatic causes of pain," but opposed by those "...favouring a primary musculoskeletal lesion." However, in terms of the findings which will be discussed in this paper there is a third possibility: namely, that the nature of FM pain is yet to be explained, while the other components of "fibromyalgia syndrome" are explicable in terms of inadequate rates of delivery of oxygen and nutrient substrates to sustain normal tissue function. There are a number of reports, which provide support for the claim of reduced rates of blood flow in different regions. Reduced cerebral blood flow in FM patients, as shown by SPECT scans, was reported by Mountz et al.[3] and Johansson et al.[4] while Bennett et al.[5] used xenon washout to show reduced rates of blood flow in muscles. The consequences of impaired capillary blood flow will include low levels of tissue oxygen, which was reported by Lund et al.[6] Jeschonneck et al.[7] stated, "Our hypothesis is that FM originates in muscular and microcirculatory disturbances," and carried out a study of skin blood flow above tender points. They reported, " a higher erythrocyte concentration, decreased erythrocyte speed and decreased flux of erythrocytes in the skin," and they noted, "This may result in an imbalance between oxygen supply and demand."

This evidence of impaired blood flow associated with low levels of tissue oxygen has special relevance for a hypothesis put forward by Bengston & Henrikson.[8] They stated " Our hypothesis is that any condition that could lead to constant tissue hy-

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poxia, e.g. through establishment of abnormal motor patterns might be a possible cause of fibromyalgia pain." Given the evidence obtained by neuroimaging techniques of reduced rates of blood flow in FM patients it is possible that this is a manifestation of the adverse effects of flat cells which will be reported in this study and which occur in abnormally high levels in a disorder with similar symptoms to FM, namely, myalgic encephalomyelitis.[9] Flat cells are one of the six shape classes of red cells that occur in the blood of healthy animals and human beings.[10] In a variety of chronic disorders there are changes in the red cell shape populations.[11]

Because a large part of the capillary network involves vessels which are smaller in diameter than that of the red blood cell, a major determinant of capillary blood flow is red cell deformability. It is the stiffness and reduced deformability of flat cells which reduces the rate of their passage through the microcirculation (as observed by Jeschonneck et al,[7]) and thus reduces the rate of delivery of oxygen and nutrient substrates to the tissues. An inadequate rate of delivery of the metabolic needs of tissues will have physiological significance for those tissues which normally have a high rate of utilisation of oxygen and nutrient substrates, such as muscles, nervous tissue and secreting glands. It is worth noting that most symptoms (apart from pain) relate to muscles and nervous tissue and could involve a functional hypoxic situation. While such observations might help to understand tissue dysfunction, it does not explain the cause of FM pain unless a hypoxic state acts as a stimulus for nociceptors.

This paper reports the results from red cell shape analyses of blood samples from two separate blocks of data. As submitted originally, the samples came from subjects with a diagnosis of FM residing in England (n=147), Canada (n=157) and the USA (n=155). Because the journal referees required evidence that the participants did have FM, which was not obtainable, the paper was withdrawn. During the following 18 months, further samples from England and America were assessed after completing a revised application form which included the question "Was this a physician's diagnosis. Yes/No."

Ninety American and 74 English subjects confirmed that a physician had diagnosed them, but it is not known what diagnostic criteria were used in the two countries.

Control data relate to samples collected from healthy New Zealand blood donors in 1989 and 1990. The values were confirmed by samples obtained in 1993 from healthy subjects who were army personnel, members of the Police Force and Fire Service, nurses and teachers who acted as controls in a study published in 1993. Data relating the results from the two groups of healthy subjects are shown in **Table 1**, p.199.

Material and Methods

Participants. In the original report, the blood samples received for assessment came mainly from members of Fibromyalgia Support Groups, although in Canada, where such groups were uncommon, the majority were members of ME Support Groups. Most requests for red cell shape analysis came from individuals who had attended an illustrated lecture which described the origin of the test and reported the results from the test in cases with other chronic disorders. Those requesting the test were supplied with a vial of fixative and an information sheet which included a questionnaire and details of the procedure for obtaining a 5-drop sample of venous blood. The main features of the procedure were as follows. (a) A disposable, plastic, 1 ml syringe and a 20g needle would be used. (b) The tourniquet was to be released before the sample was drawn to ensure the sample came from flowing

Table 1. Details of the origin and confirmation of the New Zealand control data. Although the contrasted data relate to a variety of non-sedentary occupations and were separated in time by 4 years, they were not different from the control data.

Female control data

(a) Female blood donors, 1989, n=38. Average age 35.1 years, 95% confidence intervals 16.2-50.4 years

Normal red cells	Flat cells	Cells with surface changes	Total cup forms	Cells with altered margins
28.0%	43.6%	13.8%	5.9%	8.6%
14.9-43.1%	26.5-60.4%	2.5-25.1%	0.0-13.9%	0.4-16.8

(b) Female teachers, nurses, policewomen, etc, 1993, n=39. Average age 35.9 years, 95% confidence intervals 32.7-39.1 years

27.9%	43.9%	13.7%	5.7%	8.8%
12.7 - 43.1%	27.3 - 60.5%	2.6 - 24.8%	0.0 - 13.7%	0.2 - 17.4%

rather than from static blood. (c) The 5-drop blood sample was to be added to the 5 ml of fixative within seconds of the needle leaving the vein. (d) The recapped vial was to be shaken briefly but vigorously to distribute the red cells.

The fixative used is a 2.5% solution of glutaraldehyde in 0.1M-cacodylate buffer, which is based upon the recommendations of Hayatt.[12] Hayatt considered glutaraldehyde is unsurpassed in its ability to preserve cell ultrastructure. This achievement is due to the introduction of irreversible intra- and inter-molecular cross-links into cellular proteins by the dialdehyde." By this means it is very effective in stabilizing surface as well as intra-cellular structures. Thus the red cells and any organisms in the sample were fixed very rapidly and became non-infective and non-perishable. Because the samples had to be airmailed to New Zealand, Hayatt's comments,[12] (p.119) "Protein modification obtained through cross-linking by glutaraldehyde is irreversible and withstands treatment with acids, urea, semicarbazide and heat," are particularly relevant. The consequences of fixation meant that they could be airmailed to Dunedin, New Zealand for assessment without any need for temperature control in transit.

The average age for the 155 American subjects was 50.3 years, with 95% confidence intervals of 29.9 to 70.7 years. Comparable data relating to 157 Canadians was 49.4 years (19.8 to 79 years) and for 147 English women 47.9 years (24.6 to 71.2 years).

In the second set of samples from cases with a physician's diagnosis of FM, the average age of 90 Americans was 49.6 years (30.7 to 68.5 years) and for 74 English women 46.8 years (28.6 to 65 years).

Note that because it is not possible to differentiate age-related changes in blood rheology from the changes which occur in FM, a small number of cases who were 70 years and older were excluded from this analysis.

Sample Preparation. Samples were prepared for scanning electron microscopy according to a published technique[10] Briefly,

the cells were washed in buffer, dehydrated in ascending concentrations of ethanol to absolute then transferred to pure, dry acetone. One drop of cells suspended in acetone was placed upon a cover glass fixed by double-sided adhesive tape to an aluminium pin stub. After air-drying, the cells were coated with gold in a sputter coater.

Scanning Electron Microscopy. All samples were photographed in a Stereoscan 360 electron microscope. The electron microscopist was instructed to photograph 3 randomly selected areas of well-spread cells at 1300 x, 10 kV and with a 20mm working distance. The images were recorded on a Mitsubishi video recorder and identified only by a serial number.

Red Cell Shape Analysis. All analyses were performed blind without knowledge of the origin or identity of the sample, by one operator (LOS). Cells in the video prints were assessed under a 2x lens and classified into 6 different shape classes according to previously published criteria.[10] Cells could be normal biconcave discocytes; or pancake-like flat cells; or cells with surface changes such as bumps or ridges; or early cup forms which were cup, basin or dish shaped; or late cup forms which were swollen and dimpled; or cells with altered margins which in some cases were spiculed. Between 300 and 400 cells were assessed from each sample and the proportions of the different cell types expressed as percentages of the total number of cells counted.

Results

The results are summarized in **Table 2, p.201** and show (a) that in each group of results there are high mean percentages of flat cells which are illustrated in **Figure 1, p.202**; (b) that the results of those American and English women who had a physician's diagnosis for FM were virtually identical with those whose diagnostic status was not known; (c) the similarities of the grouped data are such that it can be assumed that they are from the same general population.

Discussion

The results show that in terms of the results of red cell shape analysis, samples obtained from subjects with a physician's diagnosis of FM are not different from those whose diagnosis was unconfirmed. Not only were the results relating to all classes of cell shapes very similar, but all geographical groups showed the same major feature of a similar, high, mean value for flat cells. Therefore it is essential that the pathogenic potential of flat cells is accorded the recognition needed to understand the effects of such cells on the rate of blood flow in capillaries.

During their passage through the microcirculation red cells must pass through vessels of less than 4 microns in diameter, which is about one half of the diameter of the red cell. This requires that red cells have the ability to change shape, to deform, so that they may traverse such small vessels. Red cell deformability is considered to be a consequence of the biconcave discocyte form and it is the "spare" membrane lining the concavities which make it possible for the haemoglobin to be redistributed as the red cell changes shape. Thus the loss of the discocytic form results in a reduction in the capacity of red cells to undergo deformation and for this reason flat cells are poorly deformable cells.

According to Whitmore[13] "if erythrocytes were made thinner or their membranes less flexible, there would be a rheological penalty of higher blood viscosity when in suspension." For this reason it is essential that the occurrence of high percentages of flat cells should not be considered as a benign event. In 1992 it was postulated that an increase in poorly deformable red cells would be manifested as reduced rates of blood flow in small capillaries so that the rate of delivery of oxygen and nutrient substrates may be inadequate

Table 2. The results from red cell shape analysis of 623 immediately fixed blood samples from patients in 3 countries, expressed as mean percentages with standard deviations (s.d) and 95% confidence intervals (95% C I). The results are grouped according to country of residence. Note the overall similarity of the grouped data.

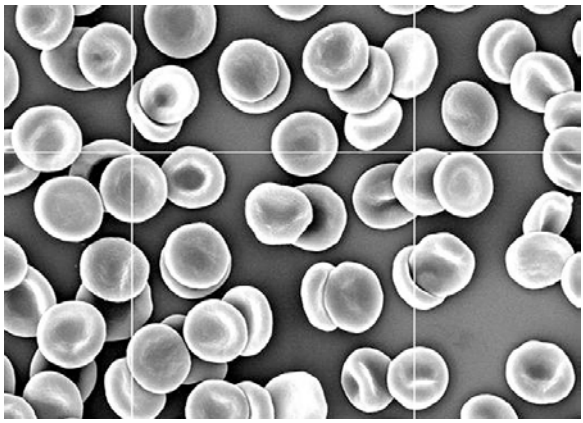
Country	Normal cells	Flat cells	Cell with surface changes	Cup forms	Cells with altered margins
American women n=155					
Mean (s.d)	9.5 (5.2)	73.4 (7.0)	10.0 (3.3)	4.7 (3.8)	2.5 (3.6)
95% C.I.	0.0-19.7	59.7-87.1	3.5-16.5	0.0-12.1	0.0-9.6
American women n=90**					
Mean (s.d)	7.5 (3.7)	73.6 (6.2)	11.7 (3.8)	4.0 (3.5)	3.1 (3.2)
95% C.I.	0.2-14.8	61.4-85.8	4.3-19.1	0.0-10.9	0.0-9.3
English women n=147					
Mean (s.d)	6.6 (4.7)	74.9 (6.7)	10.6 (4.4)	4.2 (4.3)	3.6 (4.5)
95% C.I.	0.0-15.8	61.8-88.0	4.3-19.1	0.0-12.6	0.0-12.4
English women n=74**					
Mean (s.d)	8.5 (3.8)	75.3 (6.3)	9.1 (3.6)	3.6 (2.9)	3.6 (6.3)
95% C.I.	1.1-15.9	63.3-87.3	2.0-16.2	0.0-9.3	0.0-15.9
Canadian women n=157					
Mean (s.d)	6.0 (3.1)	75.0 (5.4)	11.8 (2.7)	4.8 (2.9)	1.0 (1.3)
95% C.I.	0.0-12.1	64.4-85.6	6.5-17.1	0.0-10.5	0.0-4.4

**Cases with a physician's diagnosis of FM

to sustain normal tissue function. Furthermore, the consequences of this could be serious if it involved any of those tissues which normally have a high metabolic requirement for oxygen and substrates.[14]

The claim that the rate of capillary blood flow is reduced when red cell shape populations are altered is supported by reports of studies in which neuroimaging techniques showed impaired cerebral blood flow in conditions with altered red cell shape populations. In 1977, Markesbery and Butterfield[15] reported that there were increased populations of stomatocytes in the blood of patients with Huntington's Disease and in 1985 Tanahashi et al. showed by means of 133 xenon that cerebral blood flow was reduced in such patients.[16] Some New Zealand children with Down Syndrome were found to have values of stomatocytes similar to those reported in Huntington's Disease (unpublished) and xenon inhalation has shown that regional cerebral blood flow was reduced in patients with Down Syndrome.[17] New Zealand patients with multiple sclerosis were shown to have increased levels of cells with surface changes[18] and Swank et al.[19] reported that in multiple sclerosis patients "...there occurred a progressive, generalised decrease in cerebral blood flow." Costa et al.[20] reported brainstem hypoperfusion in all of

Figure 1. The red blood cells of a woman with a physician's diagnosis of FMS. Flat cells dominate the sample and seventy two percent flat cells were revealed by red cell shape analysis. Note the normal biconcave discocyte left of centre. A cell with surface changes (a ridge) is at bottom right. Some cells present a concave (dished) surface but are not normal cells and are possibly intermediate forms between biconcave disks and flat cells. Original magnification 1100X.



67 English ME/CFS patients and noted the occurrence of a generalized reduction in brain perfusion. It is of some significance that in blood samples from more than 2000 ME patients residing in four countries, the most frequent change was an increase in flat cells.[10] An early study of ME blood found reduced filterability of red cells[21] so the anticipated consequence of poorly deformable flat cells will be reduced rates of delivery of oxygen and nutrient substrates to the tissues. So it is very likely that the poor muscle blood flow in fibrositis patients reported by Bennet et al;[5] the low levels of oxygen in the muscles of FM patients recorded by Lund et al;[6] the reduced rates of cerebral blood flow recorded by neuroimaging techniques[3,4] and the "decreased erythrocyte speed and decreased flux of erythrocytes in the skin,"[7] could be related directly to the adverse effects of flat cells on microcirculatory blood flow.

The marked similarity of the results from red cell shape analysis from patients residing in three countries implies that the patients were a segment of a uniform population and stimulate the thought that it is impaired capillary blood flow which is responsible for the tiredness, exercise intolerance and muscle dysfunction as well as the frequently reported cognitive problems. However the possible role of impaired blood flow in the pathogenesis of FM pain remains unclear. In a scholarly treatise on the topic Bennett[22] pointed out that "...chronic pain, including fibromyalgia, often has a non-nociceptive component related to the phenomenon of central sensitisation" and emphasized that chronic pain is not persistent acute pain. From a patient's perspective such concepts will not be easy to grasp and it might be helpful to consider pain as being physiological (when it is the consequence of tissue damage) or non-physiological (when tissue damage cannot be demonstrated). Sorensen et al.[23]

compared the effects of different analgesics and noted that patients with FM according to the ACF criteria included some with different pain processing mechanisms. They concluded that both spinal and/or supraspinal nociception was involved in the pathogenesis of pain in FM. So at this time, there is still no complete explanation of the non-physiological pain which is the main burden of the FM patient.

Arachnoiditis is another whole body pain disorder which is not uncommonly misdiagnosed as FM. In an unpublished study it was found that the high percentages of flat cells typical of FM occurred also in patients with arachnoiditis. However arachnoiditis pain is considered to be a "hypoxic "pain, although the basis of this belief is not known. But when arachnoiditis patients took sufficient oil of evening primrose to increase their blood levels of prostaglandin E1 (PGE1), they reported pain relief presumably as a consequence of the PGE 1-induced improvement in red blood cell deformability. If those observations can be confirmed in a properly controlled trial, then the possibility that FM patients might benefit from the effects of haemorheologic agents such as evening primrose oil needs to be explored.

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