Red Blood Cell Shape, Symptoms and Reportedly Helpful Treatments in Americans with Chronic Disorders.

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Abstract

This paper presents the results of analyses of the data and information provided by 632 Americans with chronic disorders in their applications for red blood cell shape analyses. Possibly as a manifestation of local usage, seven different diagnostic categories were represented while more than 100 cases were undiagnosed.

Irrespective of diagnostic category, red cell shape analyses showed the majority had high values for flat cells while about 4% had increased cup forms. As both cup forms and flat cells are poorly deformable cell types they would reduce the rate of blood flow in small capillaries thus reducing the rate of delivery of oxygen and nutrient substrates to the tissues.

Analyses of the symptoms recorded, in terms of tissue dysfunction revealed overall similarities in regions of dysfunction such as in muscles or the central nervous system.

Thus, the information provided by patients with differently named disorders was highly suggestive of a common causation. The common factor could be the altered blood rheology associated with changed red cell shape populations.

The findings indicate a need for an investigation of possible changes in blood rheology in chronic disorders as this could lead to the discovery of effective treatments.

Introduction

Red blood cells of healthy animals and humans can be classified into six different shape classes[1] on the basis of surface features. This simple approach had the aim of eliminating the need to identify cells in terms of classical nomenclature. For example, different classes of echinocytes are grouped with acanthocytes as "cells with altered margins" and it has been shown that altered red cell shape populations are present in several chronic disorders.[2] Such changes will be associated with a reduction in the percentage of biconcave discocytes. The importance of this is that the loss of discocytic form is associated with a reduction in red cell deformability and a reduced capacity to pass through capillaries smaller in diameter than the cell itself. So the adverse effect of poorly deformable red cells on blood flow rate in capillaries will be manifested as a reduced rate of delivery of oxygen and nutrient substrates to the tissues. Inadequately supplied tissues may not receive sufficient oxygen and nutrient substrates to sustain normal tissue function and they become dysfunctional. This change may have significant consequences if it involves any of those tissues which normally have a high rate of utilization of oxygen and nutrient substrates. Such tissues include muscle, nerve tissue and secreting glands, all of which are common sites of symptoms.

Increased percentages of flat cells are typical of both types of diabetes and the altered red cells probably play a key role in the well documented (but usually unrecognized) changes in blood rheology which are typical of the diabetic state.[3,4,5] A long-term study has shown the beneficial effects for diabetics of improving blood rheology by taking pentoxifylline (Trental).[6]

Many other chronic disorders show similar functional changes to those of the diabetic state (including the high percentage of flat cells) so it is reasonable to anticipate that such disorders will also exhibit altered blood rheology.

1. Red blood Cell Research Limited, 31 Bath Street, Dunedin, NZ 9001.

This report relates to information provided by Americans residing in at least nine states who had requested red cell shape analysis. Application forms for the blood test contain requests for information about the nature of their diagnosis; details of other ailments/diagnoses; assessment of their level of wellbeing; details of their four most worrisome symptoms and information about treatments and what they had found to be helpful. Unfortunately not all of the requested information was provided.

A total of 632 samples and questionnaires were received and their recorded diagnoses were as follows. Fibromyalgia (FM), 213 females; FM/CFS, 14 males, 161 females; CFS, 14 males, 71 females; CFIDS/ME, 4 males; 5 females; FM/CFIDS (ME), 29 females; Gulf War Syndrome, 7 males; Multiple sclerosis, 5 females, one male; undiagnosed, 15 males, 93 females, cases 70 years of age and older, 22. (Note that this age related category was separated from the main body of data as it is not possible to determine with confidence which symptoms relate to the aging progress and which relate to symptoms of FM, etc).

The reasons for and the origins of the multiplicity of the terms for the diagnostic categories is unclear and should be a matter of concern as the multiple terms may undermine the legitimacy of a diagnosis which is a major problem for sufferers. While the presence of a sufficient number of tender points has general acceptance as a diagnostic criterion for FM, this is not so for the other diagnostic categories and they lack an accepted diagnostic marker. But because FM has an accepted diagnostic criterion, it is puzzling to understand what benefits arise from the use of terms such as FM/CFS and FM/CFIDS(ME). As a sizeable proportion of the patient population had physician's diagnoses for their condition, it is strange that there are no clear explanations for the use of such diagnostic categories.

Irrespective of the recorded diagnoses, there was a marked degree of uniformity in the words used to record their symptoms. Thus the terms used to describe symptoms provided a means of identifying regions of dysfunction. For example, terms such as brain fog, unable to think, balance and memory problems, depression, headaches, dizziness and cognitive dysfunction identified central nervous system dysfunction. Similarly, muscle dysfunction was indicated by the use of terms such as tiredness, weakness, exhaustion, exercise intolerance, lack of stamina fatigue and a lack of energy. Other terms related to dysfunction of the peripheral nervous system, digestive tract and sleep, while the frequency of pain was a marker for FM.

The information presented in this paper shows changed red cell shape populations in all diagnostic categories while of all symptoms recorded only pain was a defining symptom.

Material and Methods

1. Blood samples. A blood sample was 3-5 drops of venous blood obtained by syringe and fixed immediately in accordance with the instructions on the application form. The most important factor in the instructions was the requirement to release the tourniquet prior to drawing the blood to ensure the sample came from flowing rather than static blood. As quickly as possible after the needle was withdrawn from the vein 3-5 drops were dripped directly into 5ml of a 2.5% solution of glutaraldehyde in 0.1M-cacodylate buffer. The vial was re-capped, shaken vigorously to distribute the red cells and when convenient, mailed to New Zealand. As it has been shown that heat had no effect on the glutaraldehyde-induced cross-linking of the proteins in the red cell cytoskeleton, the sample did not require special packaging to prevent temperature change.

2. Preparatory techniques. When the blood cells had fixed for at least 24 hours, they were dehydrated in ascending concentrations of ethanol, to absolute, then trans-

ferred to pure, dry acetone for two washes. A drop of the acetone suspension was placed on a cover glass fixed to an aluminium pin stub by double-sided adhesive tape. When the preparation had dried at 37˚C, it was gold coated in a sputter coater.

3. Scanning electron microscopy. All samples were photographed in a Stereoscan 360 electron microscope operating at 10kV with a 20mm working distance at 1300 times magnification. The electron microscopist was instructed to photograph three randomly selected fields of well spread cells.

4. Red cell shape analysis. All samples were classified and counted by a single operator (LOS) and between 300 and 400 cells in each sample were assessed. The frequencies of the different red cell shapes was calculated from the results and expressed as a percentage of the total number of cells counted from that sample.

5. Symptom analysis. Applicants were requested to list their four most worrisome symptoms but 5.1% of the possible 2,432 symptoms were not recorded. The remaining 94.9% of possible symptoms were considered to be an adequate and representative sample. Symptoms were allocated to the following regions of dysfunction; muscular, central nervous system, sleep, peripheral nervous system, digestive tract and pain. A small proportion of symptoms, which did not relate to this classification were excluded from the analysis. In addition symptoms were separated into first, second, third and fourth records.

6. Treatment records. A large majority of applicants failed to provide any information concerning treatments they considered to be helpful. For example in applicants with an FM diagnosis only 29.6% provided any information.

Results.

1. Red blood cell shape analysis. Provided there were 4 or more records, the data were analysed according to the diagnostic category and state of residence. This showed that there were no geographical differences, i.e. the results from Alaska and Wisconsin were not different from those of Florida and Texas. All data are summarized in **Table 1** (p.4), which shows that irrespective of diagnostic category, those suffering from conditions in which there is a large degree of overlap in symptomatology had similar average values for the different red cell shape classes. The most frequent feature was a high value for flat cells although 4.3% had high values for cup forms. Details of the occurrence of cup forms are shown in **Table 2**(p.4). Of the 27 cases with high percentages of cup forms, 20 were from Texas with the remainder spread among 5 states. It is possible that the Texas figures indicate a viral infection in that state.

2. Symptom analysis. The distribution of symptoms allocated according to region of dysfunction and separated into the different diagnostic categories are shown in **Table 3** (p.5)(pain); **Table 4** (p.5) (muscle dysfunction) and **Table 5** (p.6) (central nervous system dysfunction). Because such data will be sensitive to small numbers, only those diagnostic categories with 12 or more records were analysed. As sleep problems involved less than 10% of cases and peripheral nervous system and digestive tract problems were recorded in about 2% of cases, they will not be considered further.

3. Helpful treatments. An analysis of 175 randomly selected cases with FM and 75 cases with FM/CFS showed that only 29% of FM cases and 55% of FM/CFS cases had provided information about their experience with treatments. Because of the relatively small percentages of cases providing records it may be unwise to place too much credence on the information provided. There was a clear preference for homeopathic and naturopathic treatments although prescription medicines were commonly used for sleep problems and pain. Most cases took a variety of supplements with one respondent recording the daily use of 28 supplements and medications. While a number of cases recorded that they had failed to find any

Table 1. Percentage of cell types

			Percentages of cell types (s.d.)				
Diagnostic category	n	Average age (s.d)	Normal cells	Flat cells	Surface changes	Cup forms	Cells with altered margins
FM females	213	50.2(9.6)yrs	8.6(4.8)	73.5(6.7)	10.7(4.2)	4.7(4.4)	2.5(3.1)
FM/CFS females	161	50.1 (8.4)yrs	8.9(4.9)	73.3(6.5)	10.3(3.9)	4.8(4.4)	2.7(3.6)
male	14	50.0 (6.9)yrs	7.8(5.0)	76.2(6.2)	8.7(4.2)	3.3(3.3)	4.0(4.0)
CFS females	71	47.0 (10.6)yrs	8.1(4.3)	75.8(6.3)	9.0(3.6)	3.8(3.9)	3.1(2.9)
males	14	45.0 (11.0)yrs	6.4(3.5)	77.0(5.5)	8.9(3.7)	3.0(1.9)	4.7(5.1)
FM/CFIDS females	29	47.0 (11.4)yrs	8.1(4.0)	76.8(6.9)	9.0(4.0)	4.5(3.9)	1.6(1.7)
CFIDS/ME females	5	46.0 (7.5)yrs	7.6(4.4)	79.4(6.2)	8.4(2.5)	2.5(2.6)	2.1(2.3)
males	4	48.0 (9.6)yrs	9.0(5.3)	78.2(7.4)	6.3(2.3)	1.1(0.6)	5.8(3.7)
GWS males	7	46.0 (9.5)yrs	11.4(7.3)	71.5(6.6)	10.0(3.0)	5.2(5.0)	1.9(1.2)
ms	6	49.0 (7.8)yrs	7.5(3.8)	76.1(4.7)	9.9(1.9)	4.4(1.8)	2.1(2.2)
Undiag. females	93	46.0 (12.2)yrs	9.2(5.2)	75.4(6.9)	9.4(4.6)	3.6(3.2)	2.5(3.1)
males	15	48.0 (12.3)yrs	7.7(4.9)	74.6(6.2)	10.3(3.2)	3.4 (3.1)	4.0(3.3)

Table 2. Cases with increased cup forms

		Cases with increasedcup forms %				
Category	Age	Normal cells	Flat cells	Surface changes	Cup forms	Cells with altered margins
FM/females n=12	54.5% (6.7)yrs	9.9%(7.0)	61.6%(5.1)	11.4%(4.2)	16.5%(2.2)	0.6%(0.7)
FM/CFS females n=l0	47.1% (12.2)yrs	8.6%(4.0)	62.5%(4.8)	11.1%(5.0)	17.5%(2.3)	0.3%(0.4)
Mixed* n=5	47.2%(8.6)yrs	8.8%(5.3)	69.4%(3.7)	14.8%(4.4)	16.5%(2.2)	0.5%(0.5)

* FM/CFIDS/ME; ME; undiagnosed; CFS (2)

treatment that was helpful, beneficial effects were claimed for water therapy, yoga, acupuncture, chiropractic and massage. However some cases reported adverse responses to massage. Perhaps the most common observation was the need to obtain adequate refreshing sleep.

Discussion

The results show that the blood of Americans with chronic disorders exhibit changes which probably alter blood rheology, have an adverse influence on capillary blood flow. and thus on symptomatology. Although there is a significant literature recording evidence that blood flow is reduced in the dysfunctional disorders, those reports do not consider altered blood rheology in a potentially causal role. However this is implied in the results from red cell shape analysis of blood samples from subjects with similar symptoms, a variety of diagnostic terms and altered red cell shape populations. Both types of red cell with the

Table 3. Symptoms separated into regions of dysfunction

	Symptoms separated into regions of dysfunction Percentages of recorded symptoms					
Diagnostic category	Pain	Muscles	CNS*	Sleep	PNS **	Digestive
FM female	31.2	29.0	24.1	11.0	1.4	3.3
FM/CFS females	30.0	29.4	30.0	8.5	0.6	1.6
males	31.8	22.7	31.8	4.5	4.5	4.5
CFS females	17.2	36.3	37.3	9.3	0.5	1.5
males	14.3	40.5	38.1	7.1	0.0	2.4
FM/CFIDS; females	26.8	27.8	30.9	7.2	2.1	5.2
CFIDS/ME females	27.8	38.9	22.2	11.1	0.0	0.0
males	29.4	23.5	23.5	11.8	0.0	11.8
GWS	15.3	26.9	50.0	3.8	3.8	0.0
MS	20.0	46.7	26.7	6.7	0.0	0.0
Undiagnosed females	29.6	32.3	29.4	5.6	3.0	3.0
males	20.5	33.3	35.9	15.4	2.6	0.0

CNS = Central nervous system
** PNS = Peripheral nervous system

Table 4. Pain as a symptom

	Pain as a symptom *			
	First symptom	Second symptom	Third symptom	Fourth symptom
FM females n = 199	61.8%	18.6%	16.6%	3.0%
FM/CFS females n = 149	43.6%	19.5%	24.2%	12.8%
FM/CFIDS (ME) females n = 29	40.0%	36.0%	24.0%	4.0%
CFS females n = 35	11.4%	37.1%	20.0%	31.4%
CFS males n = 14	16.6%	33.3%	16.6%	33.3%
Undiagnosed females n = 72	40.3%	23.6%	18.1%	18.1%
Undiagnosed males n = 15	25.0%	12.5%	37.5%	25.0%

* Limited to those diagnoses with 12 or more records

most prominent changes, i.e. flat cells and cup forms, share the common feature of being poorly deformable and for that reason will have an adverse influence on capillary blood flow. A reduction in capillary flow rate may lead to an inadequate rate of delivery of oxygen and nutrient substrates to sustain normal tissue function with the result that the tissues become dysfunctional. Therefore it seems not unreasonable to postulate that symptoms which arise from dysfunctional muscles and other

Table 5. Symptoms of Muscle dysfunction

Symptoms of muscle dysfunction*	First symptom	Second symptom	Third symptom	Fourth symptom
FM females n = 199	32.4%	29.7%	24.9%	13.0%
FM/CFS females n = 149	43.8%	23.3%	19.2%	13.7%
FM/CFIDS (ME) females n = 29	37.3%	29.6%	22.2%	11.1%
CFS females n = 36	56.8%	17.6%	13.5%	12.2%
CFS males n = 14	58.8%	11.7%	17.6%	11.7%
Undiagnosed females n = 72	40.2%	24.1%	27.6%	8.0%
Undiagnosed males n = 15	69.2%	15.4%	7.7%	7.7%

*Limited to those diagnoses with 12 or more records

Table 6. Symptoms of CNS dysfunction

Symptoms of CNS dysfunction*	First symptom	Second symptom	Third symptom	Fourth symptom
FM females n = 199	6.9%	23.0%	27.6%	51.7%
FM/CFS females n = 149	12.1%	30.2%	22.1%	35.6%
FM/CFIDS (ME) females n = 29	20.0%	23.3%	13.3%	43.3%
CFS females n = 35	23.7%	30.3%	28.9%	17.1%
CFS males n = 14	12.5%	37.5%	25.0%	25.0%
Undiagnosed females n = 72	19.0%	24.1%	26.6%	30.4%
Undiagnosed males n = 15	7.1%	35.7%	28.6%	28.6%

* Limited to those diagnosis with 12 or more records

tissues (central and peripheral nervous system dysfunction; the digestive tract and sleep problems) are the consequences of impaired capillary blood flow. Such a mechanism could account for the great measure of symptom overlap, which occurs in those conditions with similar alterations in red cell shape populations.

As the largest diagnostic group is FM it may be informative to review those parts of the literature, which report problems of blood flow as shown by different techniques. Lund et al[7] used MDO oxygen electrodes to show abnormally low levels of oxygen in the skin over tender point. Xenon washout was used to show that muscle blood flow was least in fibrositis patients, 80% of whom were below the average level of aerobic fitness. As cigarette smoking stiffens red cells it was surprising that tobacco use showed no correlation with muscle blood flow.[8] Single photon emission computed tomography was used to assess cerebral blood flow in women with FM[9] and the authors claimed "We have provided the first evidence that FM patients, compared with normal controls, are characterized by

significantly lower rCBF in the hemithalamus and the left and right heads of the caudate nucleus." Another study of cerebral dysfunction in FM[10] used xenon inhalation to assess regional cerebral blood flow and it was noted that "The main finding was that the fibromyalgia group had lower values in the dorsolateral frontal areas, bilaterally." Such studies document the evidence of impaired blood flow, which would be expected because of the adverse effects of poorly deformable flat cells revealed by red cell shape analysis. However Sietsema et al[11] found "..no abnormality in the overall rate and pattern of utilization of oxygen during muscular exercise." A similar conclusion was reached in a study using electron resonance spectroscopy to assess muscle energy and metabolism[12] and the authors noted that "…muscle energy metabolism in FMS patients is not different from that in sedentary controls." It is unclear why these studies did not confirm the results obtained in neuroimaging studies. In the latter study, patients were exercised to volitional exhaustion, so it is worthwhile noting that red cell shape changes have been found with much lower levels of physical activity[13,14] and those effects would be additive to any pre-existing changes.

As the postulated mechanism responsible for lowering the rate of capillary blood flow is the reduced deformability of flat cells it is of some significance that Jeschonneck et al[15] have reported the assessment of blood flow in the skin above tender points, using laser Doppler flowmetry. Just as would have been expected, the effects of poorly deformable red cells were reflected in their results. They reported "Our study showed a higher erythrocyte concentration, decreased erythrocyte speed and decreased flux of erythrocytes in the skin and lower skin temperatures above the tender points in FM patients than in healthy controls." They noted their findings were in agreement with Lund's results[7] with the MDO oxygen electrode.

On a more general note it should be emphasized that in other disorders with altered red cell shape populations Neuro imaging techniques have demonstrated reduced cerebral blood flow. In 1977, Markesbery and Butterfield reported increased cup forms in the blood of patients with Huntington's disease.[16] Tanahashi et al used Xenon washout to report impaired cerebral blood flow in Huntington's patients.[17] Unpublished results of red cell shape analysis in children with Down Syndrome showed that Down children in Dunedin and Christchurch in New Zealand had cup-transformed cells in their blood, but in Toowoomba, Australia, Down children had increased flat cells. In Durban, South Africa, one half of Down children had flat cells while the other half had cup forms. As both cup forms and flat cells are poorly deformable it is of relevance that Melamed et al reported that Down children had impaired cerebral blood flow.[18] Multiple sclerosis patients in this study had high values for flat cells and Swank et al[19] reported that MS people had a progressive, generalized reduction in brain perfusion. A report of more than 2000 cases of ME[20] showed increased flat cells as the most common change and Costa et al[21] reported hypoperfusion of the brain stem in all of 67 English cases of ME/CFS and a generalized reduction in brain perfusion. It should be noted that none of these authors discussed what mechanisms might be operating to cause the blood flow problems they had demonstrated.

As the most frequent change in this study was high values for flat cells, and this occurred irrespective of diagnostic category, it can be expected that at least in some part of the body, capillary blood flow rate will be impaired. In 1992[22] it was postulated that the reason people responded differently to an infection by the same virus was a consequence of differences in mean capillary diameter. On the basis of a hypothetical normal distribution curve it was suggested that individuals whose mean capillary diameter fell into the first quartile of the distribution would be at risk of chronic ill-

ness after exposure to some agent which triggered red cell shape change. It has been suggested[23] that "Capillary diameter may also explain why women are afflicted not only more frequently but often more severely than men, since capillary diameter may be larger in men." While a proposal to test this concept by the measurement of conjunctival capillaries was formulated, the proposal was not funded.

However the idea of focal distribution of clusters of small capillaries may have relevance to the fact that 42 cases (6.6%) recorded depression as a symptom, particularly so as Johansson et al[10] reported a reduction in regional cerebral blood flow in the dorsolateral parts of the frontal lobes of the brain of FM patients. Positron emission tomography was used to study prefrontal cortex metabolism in patients with four types of depression and controls.[24] The authors noted that cerebral blood flow was highly correlated with glucose metabolism in normal subjects and they concluded that "the results of the data evaluations presented here provide compelling evidence that abnormal glucose metabolism in the anterior left prefrontal cortex is common to the expression of major depression in the subject groups examined." Another study also used positron emission tomography to investigate cerebral blood flow in patients with major depression[25] and the authors noted "In the depressed group as a whole rCBF was decreased in the left cingulate and the left dorsolateral prefrontal cortex." Such observations are highly suggestive of a role for impaired cerebral blood flow in the pathogenesis of depression so it could be relevant that high values for flat cells were present in blood samples from six cases of bipolar disorder. For this reason the reported benefits of fish oils rich in omega -3 fatty acids in bipolar disorder are explicable[26] and are consistent with the expected improvements in red cell flexibility which had been reported previously.[27] While studies of cerebral blood flow in depression have produced variable results, none of the authors who report reductions in regional cerebral blood flow discuss what mechanisms might be involved. Yunus et al[28] reported a 32% frequency of depression in 31 FM patients aged more than 60 years and a 40% frequency in 63 FM patients aged 59 years or younger. It is not clear why the incidence of depression should be so much higher than the 6.6% of 213 FM patients in this study. In a study of cerebral blood flow in more than 600 subjects decreased cerebral blood flow occurred with increasing age in the prefrontal, inferior temporal motor and frontal temporal areas, with the greatest changes in the prefrontal and parietal areas.[29] However there was no indication that depression was a problem in the participants

Conclusion

If it were asked, "Has this analysis provided any useful information?" the answer would have to be "Yes." The number of diagnostic categories needs to be reduced and rationalised by the use of specific criteria. The fact that all categories shared the common feature of changed red cell shape populations makes it highly likely that altered blood rheology is involved causally in symptom development. To reject the proposal that impaired capillary blood flow plays an important role in these chronic disorders would imply that normal tissue function can occur in the absence of normal blood flow. As similar red cell shape changes were present in different disorders shown to have impaired cerebral blood flow, it is not surprising that depressive illness should be associated with altered red cell shape populations. It is of considerable significance that bipolar disorder has been shown to respond to daily supplements of fish oil rich in omega-3 fatty acids which have been shown to improve red cell membrane fluidity[27] as fish oil is one of the recognized hemorrheologic agents. This observation raises the possibility that other hemorrheologic agents (evening primrose oil, gingko extract, Trental) might improve patient wellbeing by their beneficial influence upon red cell deformability.

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