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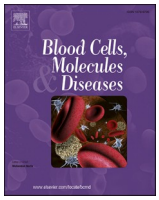

Erythrocyte deformability correlates with systemic inflammation

Carmen Jacob [a,b,*], Lakeesha Piyasundara [c], Maria Bonello [a,b], Michael Nathan [b], Stefania Kaninia [a,b], Aravinthan Varatharaj [a], Noémi Roy [c,1], Ian Galea [a,b,1]

[a] Clinical Neurosciences, Clinical and Experimental Sciences, Faculty of Medicine, University of Southampton, UK
[b] Wessex Neurological Centre, University Hospital Southampton NHS Foundation Trust, Southampton, UK
[c] Haematology Department, Oxford University Hospitals NHS Trust, Oxford, England, UK

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ABSTRACT

Recent evidence suggests that systemic conditions, particularly those associated with inflammation, can affect erythrocyte deformability in the absence of haematological conditions. In this exploratory study, we investigated the relationship between systemic inflammatory status and erythrocyte deformability (using osmotic gradient ektacytometry) in a heterogenous study population consisting of individuals with no medical concerns, chronic conditions, and acute illness, providing a wide range of systemic inflammation severity.

22 participants were included in a prospective observational study. Maximum Elongation Index (EI_{max}) in ektacytometry served as the readout for erythrocyte deformability. Inflammatory status was assessed using C-reactive protein (CRP) and self-reported symptoms associated with inflammatory activation (Sickness Questionnaire Scores, SicknessQ).

In a univariate linear regression, both CRP and SicknessQ scores significantly predicted EI_{max} (CRP: $F(1,20) = 7.751$, $p < 0.05$ (0.011), $R^2 = 0.279$; SicknessQ: $F(1,18) = 4.831$, $p < 0.05$ (0.041), $R^2 = 0.212$). Sensitivity analyses with multivariable linear regression correcting for age showed concordant findings.

Results suggest a linear relationship between erythrocyte deformability and biochemical and clinical markers of systemic inflammation. Replication of findings in a larger study, and mechanisms and clinical consequences need further in investigation.

1. Introduction

There is growing interest in understanding erythrocyte (mal-)function in non-haematological conditions. Erythrocyte deformability is considered a marker of both functional and structural erythrocyte integrity. Deformability is crucial for tissue perfusion, as it allows erythrocytes to travel through capillaries smaller than the size of their diameters. Whilst erythrocyte deformability has been extensively studied in the context of primary erythrocyte membrane disorders [1,2], recent evidence suggests systemic conditions, particularly those associated with inflammation, may affect erythrocyte deformability in the absence of haematological conditions. Examples include sepsis [3], COVID-19 [4], obesity [5], cardiovascular disease [6] and vitamin D status [7]. This is thought to be mediated by several factors, such as increased membrane rigidity as a consequence of oxidative stress [8,9], and direct and indirect effects of plasma proteins, hormones, gasotransmitters, damage associated molecular patterns and other metabolic and physicochemical factors [10,11].

In this explorative study, we investigated the relationship between systemic inflammatory status and erythrocyte deformability using ektacytometry in a heterogenous study population consisting of individuals with no medical concerns, chronic conditions, and acute illness. Two readouts of systemic inflammation were employed. C-reactive protein, a well-accepted biochemical readout, was the primary measure. We also assessed a clinical readout, namely symptoms associated with inflammatory activation or so-called “sickness behaviour” using the Sickness Questionnaire, or SicknessQ [12]. Based on previous research, we hypothesised that erythrocytes would exhibit reduced deformability in participants with higher levels of systemic inflammation.

* Corresponding author at: Clinical Neurosciences, Clinical & Experimental Sciences, Faculty of Medicine, University of Southampton, Mailpoint 806, Level D, Southampton General Hospital, Southampton SO16 6YD, UK.
E-mail address: c.jacob@soton.ac.uk (C. Jacob).
[1] Joint senior authors.

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2. Methods

This prospective observational study recruited 22 individuals with no medical concerns, chronic conditions, and acute illness, providing a wide range of systemic inflammation severity. The only inclusion criterion was age >18 years. Past or ongoing haemolytic disorder was an exclusion criterion. The study had national research ethics committee (12/SC/0176) and institutional (ERGO5562) approval; participants were recruited after informed consent. On the study day, blood samples were collected, and participants completed the SicknessQ as a global measure of sickness behaviour. Blood samples were analysed for C-reactive protein (CRP) in the local laboratory using immunoturbidimetry on a Beckman Coulter AU5800/680 analyser (lower limit of quantification: 1 mg/L). Erythrocyte deformability was assessed using osmotic gradient ektacytometry on a laser optical rotational red cell analyser [13] (RR Mechatronics, Netherlands). Erythrocyte deformability was measured over a range of osmotic pressures from 50 mOsmol/kg to 500 mOsmol/kg in steps of 10 mOsmol/kg under constant sheer stress of 30 Pa at 37 °C and expressed as the maximum elongation index (EI_{max}). The method is described in detail elsewhere [14]. Primary outcome measures were maximum deformability (EI_{max}) and CRP. The SicknessQ score was included as a clinical readout for self-rated inflammation-associated sickness behaviour and was a secondary outcome measure. Possible scores range from 0 to 30, with higher scores indicating greater inflammation-associated symptoms. CRP values of <1 mg/L were coded as 0. CRP and SicknessQ scores were log-transformed (ln(x + 1)) prior to analysis with univariate regression analyses. Sensitivity analyses were performed correcting for age, as a degree of variation in erythrocyte deformability with age is reported in the literature [15]. Partial correlations were used to quantify the amount of variance contributed by individual variables. Statistical analyses were performed using SPSS v28 (IBM).

3. Results

Out of 22 participants, 5 participants were healthy, 7 had chronic medical conditions (e.g., obesity, diabetes, asthma), and 10 were ill inpatients at a tertiary hospital being treated for various acute conditions (e.g., stroke, infection, complications of autoimmune or oncological conditions). Mean age was 55.2 years (SD 14.63, range 25–81 years) and 12 participants were female. Mean CRP was 18.14 mg/L (SD 42.37, range 0–157.00 mg/L, median 1.50) and mean SicknessQ score was 5.70 (SD 5.877, range 0–21, median 3.50); 2 participants were unable to complete the questionnaire due to their underlying disorder causing fluctuating cognitive capacity.

CRP significantly predicted EI_{max} in a univariable linear regression ($F(1,20) = 7.751$, $p < 0.05$ (0.011), $R^2 = 0.279$, see Table 1A); as did SicknessQ ($F(1,18) = 4.831$, $p < 0.05$ (0.041), $R^2 = 0.212$, see Table 1A). Age, but not gender, may potentially affect erythrocyte deformability [15]. Therefore, a sensitivity analysis was conducted adjusting for age. CRP significantly predicted EI_{max} in this multivariable linear regression (overall model $F(2,19) = 3.699$, $p < 0.05$ (0.044), Fig. 1A, Table 1B). The model explained 28 % of observed variance in erythrocyte deformability, and most of the variance was explained by CRP (22.2 %), not age. Similarly, SicknessQ scores significantly predicted EI_{max} in a multivariable linear regression adjusting for age (overall model $F(2,17) = 4.905$, $p < 0.05$ (0.021), $R^2 = 0.366$, see Fig. 1B, Table 1B). The model explained 36.6 % of observed variance in erythrocyte deformability, and most of the variance was explained by SicknessQ (29.1 %), not age.

4. Discussion and conclusions

The findings of this pilot study support the hypothesis that reduced erythrocyte deformability associates with systemic inflammation. To our knowledge, this is the first study to report a relationship between erythrocyte deformability and both biochemical and clinical markers (CRP, SicknessQ) of systemic inflammation. Sickness behaviour is a coordinated set of responses which occur during systemic inflammation, and it is mediated by cytokines [16]. The SicknessQ questionnaire has previously been shown to be sensitive to transient immune activation, and several cytokines (interleukin-6, interleukin-8, and tumour-necrosis-factor-alpha) have been shown to mediate SicknessQ scores in an experimental immune challenge [12]. It is likely that CRP elevation, sickness behaviour and erythrocyte deformability are separate manifestations that result from systemic inflammation. However, it is possible that there is an interplay between these factors; for example, reduced erythrocyte deformability may conceivably affect cerebral microcirculation and oxygen delivery which in turn may contribute to sickness behaviour. Erythrocyte deformability is one of a number of factors determining blood viscosity during various clinical conditions [17].

The results of this study support the use of osmotic gradient ektacytometry, in particular maximum deformability, or EI_{max}, as a reliable and sensitive method to investigate erythrocyte deformability in normal physiology and medical conditions other than haemolytic anaemias. Other ektacytometry parameters apart from EI_{max}, such as the osmotic pressures when the elongation index is minimal (O_{min}; i.e. osmotic fragility), and when it is 50 % of EI_{max} (O_{hyper}; i.e. intracellular viscosity) [2] should be investigated in this context. Exploratory analyses in this sample did not show a correlation between markers of inflammation and O_{min} or O_{hyper}, but we have previously shown an increase in erythrocyte osmotic fragility with inflammation using traditional osmotic fragility testing [18]. Contributing reasons to the lack of sensitivity of O_{min} or O_{hyper} in this sample could include technical factors and the small sample size. Findings here should be replicated in a larger sample

Table 1
Results of linear regression models examining the association between inflammation readouts (C-reactive protein and self-reported sickness behaviour, as predictors) and erythrocyte deformability (maximum elongation index, as measured by osmotic gradient ektacytometry, as independent variable). A: univariate analyses. B: sensitivity analyses correcting for age. CRP: C-reactive protein, SicknessQ: Sickness Questionnaire Score.

			Biochemical readout (CRP)	Clinical readout (Sickness behaviour)	
A. Univariate linear regression models					
Model	F statistic		7.751	4.831	
	Degrees of freedom (between groups, within groups)		1,20	1,18	
	Significance		$p < 0.05$	$p < 0.05$	
Variables	Standardised beta coefficients		−0.528	−0.460	
	B coefficients (95 % confidence interval)		−0.002 (−0.003, 0.000)	−0.003 (−0.006, 0.000)	
	Significance		$p < 0.05$	$p < 0.05$	
B. Sensitivity analyses adjusting for age					
Model	F statistic		3.699	4.905	
	Degrees of freedom (between groups, within groups)		2,19	2,17	
	Significance		$p < 0.05$	$p < 0.05$	
Variables	Standardised beta coefficients	Age	−0.035	Age	−0.396
		CRP	−0.513	SicknessQ	−0.5134
	B coefficients (95 % confidence interval)	Age	−1.375E-5 (−0.000196, 0.000169)	Age	−0.000164 (−0.000335, 0.000006)
		CRP	−0.001876 (−0.003557, −0.000195)	SicknessQ	−0.003525 (−0.006345, −0.000704)
	Significance	Age	$p = 0.877$	Age	$p = 0.058$
		CRP	$p < 0.05$	SicknessQ	$p < 0.05$

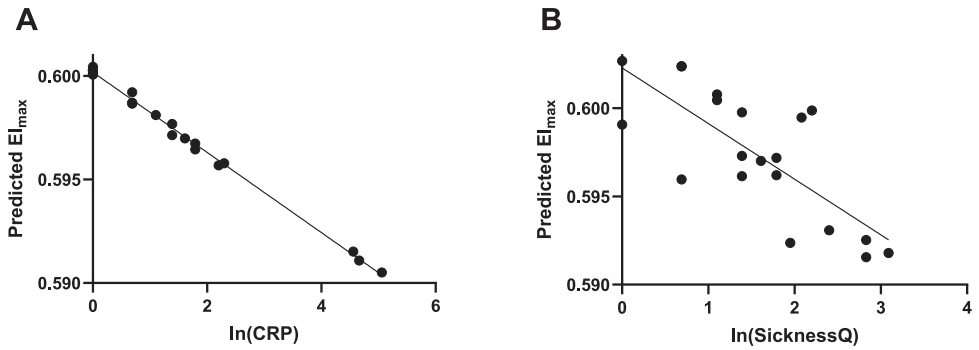

Fig. 1. Erythrocyte deformability as a function of biochemical and clinical readouts of systemic inflammation. A: Model-predicted maximum elongation index (EI_{max}) as a function of the observed C-reactive protein, corrected for age as detailed in Table 1B. B: Model-predicted EI_{max} based on self-reported sickness behaviour (SicknessQ) and corrected for age as detailed in Table 1B. Straight line indicates model fit. CRP: C-reactive protein. SicknessQ: Sickness Questionnaire Score.

alongside measurement of oxidative stress markers, nitric oxide metabolites, prostaglandins, and leukotrienes as putative mediators of inflammation-associated reduction in erythrocyte deformability [10]. Further studies are needed to identify the underlying molecular mechanisms contributing to reduced erythrocyte deformability during inflammation, and possible clinical consequences.

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CRediT authorship contribution statement

Carmen Jacob: Writing – original draft, Visualization, Project administration, Investigation, Formal analysis, Conceptualization. **Lakeesha Piyasundara:** Writing – review & editing, Investigation. **Maria Bonello:** Writing – review & editing, Investigation. **Michael Nathan:** Writing – original draft, Investigation. **Stefania Kaninia:** Writing – review & editing, Investigation. **Aravinthan Varatharaj:** Writing – review & editing, Investigation, Funding acquisition. **Noémi Roy:** Writing – review & editing, Resources, Investigation, Funding acquisition, Conceptualization. **Ian Galea:** Writing – original draft, Supervision, Resources, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare no potential conflicts of interest relevant to this work.

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