Conjunctival sign in sickle cell anaemia: an in-vivo correlate of the extent of red cell heterogeneity

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SUMMARY A consecutive series of 22 stable adult inpatients with sickle cell anaemia were examined for the presence and severity of spontaneous 'comma' signs of the conjunctiva. Fifteen patients had severe conjunctival signs (more than 10 commas in the worse eye). The presence of severe conjunctival signs was associated with a broader distribution of intraerythrocytic haemoglobin concentrations ($p=0.0005$). The patient group with severe conjunctival signs was not found to be significantly different from the group without such signs for age, sex, haemoglobin value, reticulocyte count, α -globin gene number, percentage fetal haemoglobin, or the proportion of very dense cells (CHC >37 g/dl). Thus the singular heterogeneity of the erythrocytes in sickle cell disease may be indicative of the factor(s) responsible for the diagnostic comma sign.

The conjunctival sign in the sickle haemoglobinopathies has been termed the 'comma' sign because of its clinical appearance as short, truncated, isolated, dark vascular segments, and it is considered pathognomonic for these disorders.¹⁻³ It is of particular interest to clinicians not only because it can be readily detected and quantitated, but because its pathophysiology may reflect functionally and morphologically more deleterious microvascular events in other jeopardised tissues. The severity of the sign has been reported to be correlated with the number of irreversibly sickled cells (ISCs).⁴ However, the subjective quantification of the ISCs entails the limitation of interobserver variability. Recently, several cellular and molecular factors that may affect the severity of sickle cell disease have been identified.⁵ In particular, it is now possible to measure the distribution of intracellular haemoglobin concentrations,^{6,7} which appears to be the major factor that determines intracellular sickle haemoglobin polymerisation in peripheral blood. The purpose of the present investigation was to explore the relationship between the conjunctival

comma sign and several suspected modifying factors in patients with stable sickle cell anaemia.

Patients and methods

We studied a consecutive series of 22 patients with stable homozygous sickle cell disease who were admitted to the Clinical Hematology Branch of the National Institutes of Health for routine follow-up from April 1983 to April 1984. The patients were not in crisis during one month before or after the study date, had not received a blood transfusion in the prior four months, and were not receiving long-term medication other than folic acid. Their ages ranged from 19 to 51 years.

The diagnosis of sickle cell anaemia was made on the basis of haemoglobin electrophoresis on alkaline cellulose acetate and on acid citrate agar, DNA analysis of bone marrow aspirates, and peripheral blood examination; when possible it was confirmed by family studies.

Assessment of the conjunctival signs was performed by one of us (MSR) with the slit lamp biomicroscope. Each quadrant was examined in turn. The largest number of commas in any quadrant of either eye was the rating for that patient. The

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Fig. 1 Severe comma sign in sickle cell anaemia.

occurrence of 10 or fewer commas was graded as minimal, more than 10 commas as severe (Fig 1).

The haematological examination, performed by one of us (GPR) on the day of the conjunctival examination, included the total haemoglobin value, the reticulocyte count, the fraction of haemoglobin F (measured spectrophotometrically after alkaline denaturation), and the mean corpuscular haemoglobin concentration (MCHC), which was derived manually from the total haemoglobin and the packed cell volume. For each patient the number of α -globin genes was determined by restriction endonuclease analysis of DNA obtained from peripheral white blood cells by means of the α -globin specific plasmid JW-101.^{8,9} The red cell density profile for each patient was determined by the calibrated phthalate ester density method.⁷

Conjunctival and haematological assessments were done in a masked fashion and were repeated on a number of subjects, which confirmed the stability of these measurements. Statistical methods included the *t* test for variables with Gaussian distributions, the rank sum test for variables with non-Gaussian distributions, and χ^2 tests for categorical variables.¹⁰ Because several comparisons were tested, an association was considered significant if the calculated *p* value was less than 0.01.

Results

Among the 22 patients with sickle cell anaemia 7 had

minimal and 15 had severe conjunctival signs (Table 1). Although there were no significant differences between these two groups with respect to age, sex, haemoglobin value, MCHC, reticulocyte count, or the percentage of fetal haemoglobin, there was a suggestion that the presence of severe conjunctival signs was associated with evidence of an increased

Table 1 Haematological indices and comma sign in sickle cell anaemia

	Comma sign		Significance level (2-tail <i>p</i> -value)
	Minimal (<i>n</i> =7)	Severe (<i>n</i> =15)	
Age (years)*	32.3 (9.5)	30.9 (7.3)	0.75
Sex (% women)	42.9	26.7	0.79
Hb (g/dl)†	9.15 (0.32)	8.69 (1.08)	0.07
MCHC (g/dl)*	33.08 (1.83)	34.74 (2.07)	0.08
Reticulocyte (%)*	8.2 (2.8)	11.6 (5.6)	0.07
HbF (%)†	2.0 (3.0)	1.4 (0.6)	0.40
Dense Cells (%)†	4.66 (4.18)	8.00 (8.34)	0.31
D ₅₀ (SG units)*	1.100 (0.006)	1.102 (0.004)	0.41
R60 (SG units) × 10 ⁻³ *	10.04 (2.96)	17.80 (5.80)	0.0005

Hb=haemoglobin. MCHC=mean corpuscular haemoglobin concentration. HbF=fetal haemoglobin. Dense cells=erythrocytes with specific gravity >1.120 or corpuscular haemoglobin concentration >37 g/dl. D₅₀=median red cell density. R60=specific gravity units, range over which the middle 60% of red cell densities are distributed (see Rodgers *et al*).
*Mean (standard deviation).

†Median (interquartile range/2). Note: the interquartile range contains half the total frequency.

haemolytic rate (i.e., a lower haemoglobin value, $p=0.07$, and a higher reticulocyte count, $p=0.07$). Our group included two patients with sickle cell disease (SS) and coexisting homozygous α -thalassaemia (α/α -) and five patients with SS and heterozygous α -thalassaemia ($\alpha/\alpha\alpha$), which did not significantly alter the expression of the conjunctival sign ($p=0.13$ for trend analysis). The red cell density profiles were not significantly different between the groups for the fraction of very dense red cells (>1.120 specific gravity or corpuscular haemoglobin concentration (CHC) >37 g/dl) or for the median density value (D_{50}) of red cells. On the other hand the density range over which the middle 60% of red cell densities (or intracellular haemoglobin concentration) were distributed (R60 value) was markedly greater in the group with severe conjunctival signs ($p=0.0005$). A multiple logistic analysis showed that, once the R60 value was accounted for, the two groups did not differ for any other variable.

Discussion

The comma sign in SS, although easy to assess clinically, is labile and subject to known variations.¹² In this study all the patients had stable sickle cell anaemia and had not been recently transfused, and prolonged exposure of the conjunctiva to the heat of the slit lamp was avoided during the examinations.

Although this microvascular abnormality is pathognomonic of sickle cell disease, its pathogenesis remains obscure. Equally uncertain is why this ocular manifestation is less pronounced in genetic variants of SS. Previous investigators have found a significant correlation between the severity of the sign and the number of ISCs.⁴ Some have interpreted these phenomena as due to in-situ sickling; other groups have pointed out possible contributions of vasomotor tone and sludging of flow^{11,12} to the comma sign. Though ISC counts were not assessed systematically, data available for nine (35%) of our patients confirmed the previously reported association. The four patients in the minimally affected group had a mean ISC count of 5.90 (standard deviation 2.41), and the five patients in the severely affected group had a mean ISC count of 17.84 (standard deviation 8.91) ($p<0.05$ by the t test and rank sum test data not shown). This is consistent with the highly significant relationship found in supplementary experiments on 10 SS patients between the ISC count and the degree of red cell heterogeneity, as reflected in the R60 value ($p<0.001$).¹³

The lack of an association of high levels of haemoglobin F or α -thalassaemia with the severity of the comma sign, which are both thought to reduce the general severity of sickle cell disease,³ may be due to the relatively small sample size.

Microvascular flow in patients with sickle cell disease is constantly jeopardised by virtue of intracellular polymerisation of deoxyhaemoglobin S,¹⁴ mostly by the cells containing the highest intracellular concentration of haemoglobin S (exclusive of red cell sickling).¹⁵ However, studies of the relationship between the mean corpuscular haemoglobin concentration (MCHC) and indices of disease severity ignore the fact that red cells in patients with sickle cell disease are not homogeneous, but rather comprise several subpopulations with respect to intracellular haemoglobin content.¹⁶ Thus it is not surprising that, although the MCHC was generally higher in severely affected patients, this association was not statistically significant ($p=0.08$).

In this study the patients with severe conjunctival signs has a much broader range of intracellular haemoglobin concentrations (R60 values) and showed a suggestion of a greater degree of haemolysis as reflected by lower haemoglobin values and higher reticulocytes counts. Both the conjunctival sign and conjunctival blood flow in sickle cell patients are affected by local heat, vasoactive substances, sickle cell crisis, and blood transfusions.^{11,12} We speculate that these conjunctival microvascular abnormalities may arise from inappropriate or excessive vasoconstriction owing to the local noxious effects of transient ischaemia mediated by rheologically compromised erythrocytes. The proportional decline in the severity of the conjunctival sign in patients with genetic variants of SS (i.e., SC, S- β^+ -thal, S-HPFH, etc.),^{2,12} in which the intracellular polymerisation of haemoglobin S is inhibited,¹⁷ with the production of a more homogeneous cell population¹⁸ would also support this hypothesis.

Obviously the factors governing red cell heterogeneity in sickle cell disease and their relative impact on the microvasculature will require further exploration. However, this study does show that the severity of the conjunctival sign is strongly correlated with the heterogeneous distribution of intraerythrocytic haemoglobin concentrations. Further studies are in progress to determine whether this sign can be used to monitor therapy targeted at favourably modifying intracellular haemoglobin S levels.

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