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CLINICAL STUDY

Quantitative evaluation of the retinal venous tortuosity in chronic anaemic patients affected by β-thalassaemia major

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Abstract

Aims **Retinal venous tortuosity (RVT) is a common finding in patients affected by different forms of chronic anaemia. The aims of this study were to quantify RVT in anaemic patients with ß-thalassaemia major and to verify whether it is related to some of the following parameters: patient's age, ferritin plasma level, and Desferrioxamine (DFX) daily dosage.**

Methods **A retrospective study was carried out. In total, 36 consecutive thalassaemic patients, treated with polytransfusion regimen and DFX, were age- and sex-matched with a control group of 36 normal subjects. All subjects bilaterally underwent red-free fundus photography, centred on the optic disc. The four main retinal veins were measured with a computer-assisted method.**

Results **Mean venous length in the thalassaemic group was significantly greater than that observed in the control group ($P<0.001$). In thalassaemic patients, no significant correlations between retinal venous length and, respectively, plasma ferritin level and DFX daily dosage were documented. Statistical analysis demonstrated a very significant association between patient's age and increased RVT only in thalassaemic patients ($P<0.001$).**

Conclusions **Our findings demonstrate that patients with β-thalassaemia major have increased RVT, as compared to normal subjects. In this selected anaemic population, patient's age, closely related to anaemia duration, is the only variable responsible for the RVT increment. This clinical sign indicates a long-standing duration of anaemia.**

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Introduction

Patients affected by β-thalassaemia major may develop a variety of ocular abnormalities.[1–3] Some of these alterations, such as cataracts,[4] optic neuropathy,[5–7] visual field defects,[5,8–10] visual electrophysiological changes,[7–11] colour vision abnormalities[6–8,11,12] and decreased visual acuity,[5–8] have been described in the course of iron chelation therapy with Desferrioxamine (DFX). Other more common ophthalmoscopic changes like degeneration of the retinal pigment epithelium,[2,6–10,12] angioid streaks,[2,13] retinal microvascular abnormalities,[1] and venous tortuosity[2,10] seem to be independent from DFX treatment.

The venous tortuosity and engorgement, observed in patients affected by β-thalassaemia major, have been documented throughout both subjective clinical observations[2,14,15] and objective method, which measured the length of the four main venous retinal branches.[16] However, in anaemic patients RVT has also been reported in the absence of other retinal changes.[14,15]

The aims of the present study were to measure the retinal vein length in patients affected by β-thalassaemia major, and to verify whether it is related to some of the following parameters: patient's age, ferritin plasma level, and DFX daily dosage.

Patients and methods

Study population

The study was carried out in patients with β-thalassaemia major, regularly followed at the

Paediatric Department of the S Anna Hospital, Ferrara, and examined at the University Eye Clinic of Ferrara between 1999 and 2000. A total of 36 consecutive patients (22 males, 14 females), aged 17.86 ± 6.63 years (range 7–33 years), suffering from chronic microcystic hypochromic anaemia and regularly transfusing to maintain the mean haemoglobin level at 11.0 g/dl according to national protocols,[17] represented the study group. The mean haematocrit was 29.27 ± 6.54% (range 14–43.9%). The mean serum ferritin level, determined by radioimmunoassay, was 2131.30 ± 1750.87 μg/l (range 450–8750 μg/l). All patients were treated with DFX by subcutaneous infusion at a mean dosage of 59.56 ± 49.89 mg/kg/day (range 27–300 mg/kg/day). Thalassaemic patients were matched with a control group of 36 healthy subjects (22 males, 14 females), mean age 17.88 ± 6.26 years (range 8–36 years), selected from a volunteer population. The data of the two groups were controlled as to distribution normality and variance homogeneity.

In both groups, subjects with glaucoma and hyperopia or myopia greater than 1 diopter (spheric equivalent) were excluded. None of the patients had evidence of systemic disorders other than β-thalassaemia major, to avoid the bias related to the inclusion of diseases known to be associated with retinal vascular abnormalities, such as hypertension, leukaemia, lymphoma, systemic lupus erythematosus, vasculitis, and diabetes mellitus. Each patient underwent a complete ophthalmologic examination, including keratometry, best-corrected visual acuity, tonometry, and biomicroscopy of the anterior segment and of the fundus. Informed consent to participate in this clinical trial was given by each patient after they had received a detailed description of the procedures to be used and the aims of the study.

Retinal venous tortuosity testing

The RVT was investigated with a quantitative analysis based on the assumption that a longer vein travels a redundant path to reach the same end point. Pupillary dilatation was induced in both groups with a 1.0% tropicamide ophthalmic solution. In all subjects a bilateral 50° red-free fundus photograph was taken, centring the optic disc (Topcon IMAGEnet 1024, TOPCON Corporation, Tokyo, Japan) and printed in its standard one-image format. In thalassaemic patients and control subjects, retinal venous length was evaluated employing a modification of the method suggested by Kagan *et al*[18] and Aisen *et al*.[16] In each photograph, two concentric circumferences of 25 and 180 mm in diameter were marked, employing AutoCAD 2000 software (AUTODESK S.p.A., Milan, Italy); the smaller one was located within the optic disc margin and centred on the papillary vascular stem. The four main retinal veins of both eyes were chosen for the measurement. Venous length was recorded by means of the AutoCAD 2000 system, tracing the course of each of the four vessels between their two points of intersection with the inner and outer circumferences (Figure 1 a,b). The measurements of the four retinal veins were averaged for each patient.

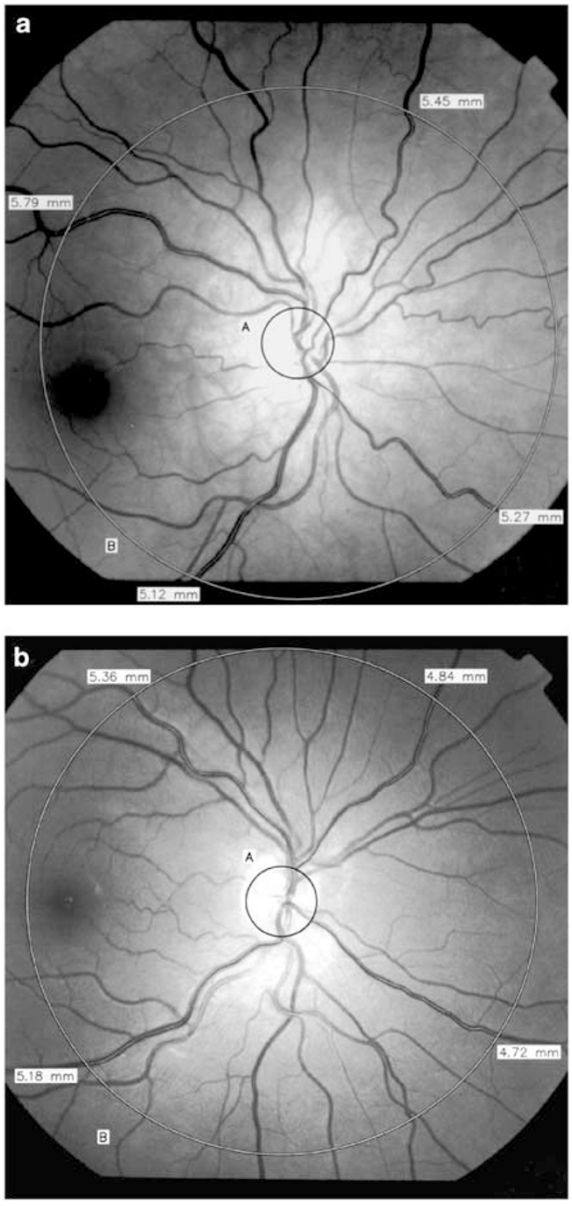

Figure 1 (a) Fundus photograph of an emmetropic patient affected by β-thalassaemia major. (b) Fundus photograph of an emmetropic, healthy age- and sex-matched subject. In both study and control groups, two concentric circumferences of 25 (a) and 180 mm (b) in diameter were marked over each fundus photograph and the four main retinal veins were measured.

All RVT measurements were proportionally corrected for the refractive error of each eye. Photographs of the TOPCON camera model eye were performed simulating emmetropia or ± 1 diopter ametropia. In each photograph, a straight line was traced between the same two points, randomly chosen, and the obtained mean magnification value was then employed to correct the recorded vessel length measurements for each refractive error. In the Topcon IMAGEnet 1024 system the degree of magnification was $\times 1.0285$/diopter. Masked evaluation of venous lengths was performed by the same two investigators (CI and FP), each photograph being assessed by numeric coding. To evaluate the reproducibility of the RVT measurement method, a randomised subset of photographs was analysed more times by the same investigators, and the obtained results were compared to verify inter- and intra-observer variability.

Statistical analyses

Analyses were performed using two statistical packages: SYSTAT (SYSTAT Inc., Evanstone, IL) and STATGRAPHICS (STSC Inc., Rockville, MD). The performer of statistical analyses was masked.

The distribution normality and variance omoscedasticity of the populations data were controlled by the Kolmogorov–Smirnov test and Bartlett's test, respectively. One-way analysis of variance (ANOVA) was used to compare study and control groups. The same test was employed to assess the inter- and intra-observer variability in the RVT measurements ($P<0.05$ was considered significant).

In the study population, a multiple linear regression analysis was performed to ascertain the role of ferritin plasma level, DFX daily dosage, and age of patients as RVT risk factors. Simple linear regression analysis was then employed in both groups to disclose a difference, if any, between RVT values, when they were correlated with age, and to display graphically this linkage. Upon completion of the statistics, masking was broken and assay results were matched to clinical diagnosis. A probability of $P<0.05$ was considered statistically significant.

Results

The age, sex, and mean venous length of control and study groups are shown in Table 1, together with the plasma ferritin mean value and daily subcutaneous DFX mean dosage of thalassaemic patients. The statistical comparison between male, female, total patients and control subjects did not show any significant difference of age distributions.

No significant inter- and intra-observer difference between RVT measurements occurred, probably because of the very simple methodological approach employed.

The mean venous length in the study group was greater than that measured in the control group. The

Table 1 Mean age and mean venous length comparisons between male, female, total patients and control subjects. Mean plasma ferritin level and mean DFX daily dosage of thalassaemic patients.

Parameter	*Study group*			*Control group*			*P**
	No.	*Mean*	*SD*	*No.*	*Mean*	*SD*	
Age (years)							
Males (M)	22	17.86	6.62	22	18.09	6.66	NS
Females (F)	14	17.42	5.80	14	17.57	5.81	NS
Entire group	36	17.69	6.23	36	17.88	6.26	NS
Retinal venous length (mm)							
Males (M)	22	5.50	0.49	22	5.06	0.36	<0.01
Females (F)	14	5.29	0.45	14	5.01	0.45	NS
Entire group	36	5.42	0.48	36	5.04	0.39	<0.001
Ferritin plasma level (μg/l)							
Males (M)	22	1887.77	1385.95				
Females (F)	14	2514.00	2211.88				
Entire group	36	2131.30	1750.87				
DFX daily dosage (mg/kg/day)							
Males (M)	22	66.97	61.70				
Females (F)	14	49.91	17.66				
Entire group	36	59.56	49.89				

*One-way analysis of variance between male, female, and total groups; NS, not significant.

Table 2 Multiple linear regression analysis employed to evaluate three clinical variables as possible risk factors for an increased retinal venous tortuosity in patients affected by β-thalassaemia major (multiple correlation coefficient, R^2=0.607584).

Independent variable	*Coefficient*	*SE*	*t-value*	*P*
Age	0.058646	0.008805	6.6606	<0.001
Ferritin plasma level	0.000010	0.000032	0.3081	NS
DFX daily dosage	0.001841	0.000108	1.7044	NS

NS, not significant.

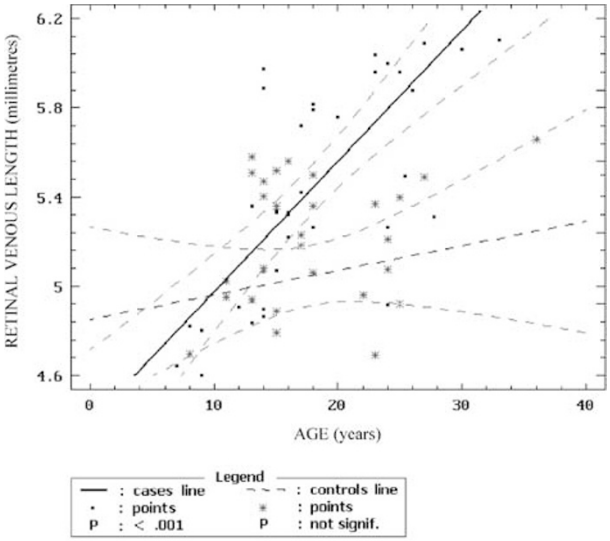

Figure 2 Comparison of the linear regression plots of retinal venous length *vs* age in thalassaemic patients (cases line) and normal subjects (controls line).

difference between the vascular measurements of thalassaemic and control subjects was significant ($P<0.001$). In the study group, the mean venous length was 5.422 ± 0.483 mm, and 5.048 ± 0.396 mm in the control one (Table 1).

The statistical analysis, performed within the thalassaemic series, did not show any significant correlation between retinal venous length and, respectively, plasma ferritin level and daily DFX dosage. The age of patients was the highest risk factor associated with a markedly increased RVT only in thalassaemic subjects ($P<0.001$) (Table 2). In fact, the linear regression analysis, performed on the control group, was not significant; also when dividing into male and female subgroups, the statistical results remained the same. Conversely, within the thalassaemic series the correlation between age and RVT was significant ($P<0.001$), and ageing correlated significantly with the augmented venous length (Figure 2). This direct relationship was observed through the linear regression analyses of the study group, also separating male ($P<0.001$) and female ($P<0.01$) patients (data not presented).

Discussion

Retinal venous changes, such as alterations in calibre and tortuosity, have been observed in a great variety of systemic disorders, i.e., diabetes mellitus, hypertension, leukaemia, Waldenström's macroglobulinaemia, primary antiphospholipid syndrome, myeloma, and sickle cell disease.[18–24] Retinal abnormalities, including venous tortuosity and engorgement, have long been observed to be associated with anaemia.[1,14–16] Although there are previous studies demonstrating the occurrence of increased RVT in patients affected by several forms of chronic anaemia,[2,3,12] in none of these studies had a quantitative evaluation of this tortuosity been performed. Aisen *et al*[16] measured the main retinal venous length in a heterogeneous group of anaemic patients, comparing this datum to that recorded in a normal population. In this study, the authors did not find any significant correlation between patient's age and venous length, probably because they enrolled a small sample (35 subjects) of nonhomogeneous patients (including seven cases of acute anaemia).[16]

Disorders of the cellular and plasmatic composition of the blood may affect its flow characteristics, viscosity, and coagulability. In the course of anaemia, the circulating red blood cell mass decreased, with a consequent reduction in oxygen concentration and a parallel increase in carbon dioxide and other retinal metabolites. Thus, a critical reduction of the haematocrit value may be responsible for alterations in calibre, length, colour, and permeability of the retinal vasculature, as rightly pointed out by Aisen *et al*,[16] who demonstrated a significant inverse correlation between RVT and haematocrit in their series. The same pathogenetic mechanism was observed during an acute carbon monoxide intoxication.[25] The occurrence of dilated and tortuous veins in anaemic patients is well documented, as is the clinical feature of central retinal vein occlusion.[26–28] The anaemic hypoxia itself, followed by a functional endothelial default of the retinal vessels,

seems to be the leading cause of these funduscopic pictures.[26] The presence of retinal haemorrhages is associated with both the primary agent of anaemia and related haematological changes, including leukaemia, thrombocytopenia, and macroglobulinaemia. In 1967, Duke-Elder and Dobree[19] postulated that progressive anaemic condition results in an impaired oxygenation of retinal capillaries, which in turn induces firstly an augment of permeability, and then haemorrhages. The incidence of retinopathy is closely related to the intensification of the anaemic status.[30] Furthermore, neither severe anaemia nor thrombocytopenia alone usually causes retinopathy. Rubenstein *et al*[31] observed that anaemic hypoxia is not able to induce retinal haemorrhage, as long as a sufficient amount of platelets present to ensure the integrity of the capillary endothelium. Other studies, which evaluated the thrombocytopenia and/or thrombocytosis influence on the occurrence of retinal changes, did not provide any definite clinical conclusion.[1,29] On the other hand, the reduction of the haematocrit reading is not always correlated either with an increased haemorrhagic predisposition[1,28–31] or with greater retinal venous tortuosity.[32] The haematocrit reading of our thalassaemic patients was widely unstable, closely depending on the blood abstraction moment in comparison with transfusion timing. For this reason, also in the present study, the haematocrit level cannot be reliably investigated as a possible RVT pathogenetic factor.

At the time of our ophthalmoscopic examination, all the thalassaemic patients of the study group had been regularly transfused to support a minimal, fairly constant, haemoglobin level. In this homogeneous thalassaemic population, polytransfusion regimen was able to keep the haemoglobinaemia at around 11.0 g/dl,[17] and no patient showed any sign of anaemic retinopathy, except for an increased RVT.

In the management of β-thalassaemia major, different transfusion schemes are currently employed, with baseline haemoglobin levels ranging from 8 to over 12 g/dl. These polytransfusion regimens, maintaining a relatively high level of haemoglobin, provide an effective erythropoiesis suppression and can allow a significant reduction in blood consumption.[33–37] High-transfusion schemes are the most advantageous, but unavoidably resulting in a mild and chronic anaemic state, in which red cells are unable to adapt to the decline in haemoglobin that occurs during the intertransfusion interval.[38,39] This condition, at the level of retinal vasculature, results in a tissular hypoxia, responsible for increased venous tortuosity, which represents an adaptation to the altered physiologic state.

According to previous observations,[2,3,12,16] our findings also demonstrate the lack of any correlation between increased RVT, serum ferritin level, and DFX dosage (Table 2).[2,10,13,32] Furthermore, we document that the patients with β-thalassaemia major, treated with the same long-standing high-transfusion programme, show a progressive age-related RVT increase, while the advancing age of the normal subjects is not associated with any modification of the retinal veins length. Since none of these selected thalassaemic subjects was affected by other systemic or ocular conditions known to be associated with retinal vascular changes and abnormal haemostasis, it may be concluded that, in this form of chronic anaemia, patient's age, closely related to anaemia duration, is the only variable able to increase the retinal veins length. Thus, RVT being an index of the exposure of retinal tissue to hypoxia, this objective method of quantitative evaluation of the retinal veins length could represent a useful tool for further monitoring all forms of chronic anaemia.

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References

1 Holt JM, Gordon-Smith EC. Retinal abnormalities in diseases of the blood. *Br J Ophthalmol* 1969; **53**: 145–160.
2 Gartaganis S, Konstantinos I, Papageorgiou O *et al*. Ocular abnormalities in patients with beta thalassaemia. *Am J Ophthalmol* 1989; **108**: 699–703.
3 Sorcinelli R, Sitzia A, Figus A *et al*. Ocular findings in beta-thalassaemia. *Metab Pediatr Syst Ophthalmol* 1990; **13**: 23–25.
4 Bloomfield SE, Markenson AL, Miller DR *et al*. Lens opacities in thalassaemia. *J Pediatr Ophthalmol Strabismus* 1978; **15**: 154–156.
5 Borgna-Pignatti C, De Stefano P, Broglia AM. Visual loss in patient on high-dose subcutaneous Desferrioxamine. *Lancet* 1984; **1**: 681.
6 Lakhanpal V, Schocket SS, Jiji R. Desferrioxamine (Desferal)-induced toxic retinal pigmentary degeneration and presumed optic neuropathy. *Ophthalmology* 1984; **91**: 443–453.
7 Olivieri NF, Buncic RB, Chew E *et al*. Visual and auditory neurotoxicity in patients receiving subcutaneous Desferrioxamine infusions. *N Engl J Med* 1986; **314**: 869–873.
8 Davies SC, Hungerford JL, Arden GB *et al*. Ocular toxicity of high-dose intravenous Desferrioxamine. *Lancet* 1983; **2**: 181–184.
9 Rubinstein M, Dupont P, Doppee JP *et al*. Ocular toxicity of Desferrioxamine. *Lancet* 1985; **1**: 817–818.
10 Arden GB, Wonke B, Kennedy C *et al*. Ocular changes in patients undergoing long-term Desferrioxamine treatment. *Br J Ophthalmol* 1984; **68**: 873–877.

11 Gelmi C, Borgna-Pignatti C, Franchin S *et al*. Electroretinographic and visual-evoked potential abnormalities in patients with beta-thalassaemia major. *Ophthalmologica* 1988; **196**: 29–34.
12 Dennerlein JA, Lang GE, Stahnke K *et al*. Ocular findings in Desferal therapy. *Ophthalmologe* 1995; **92**: 38–42.
13 Aessopos A, Stamatelos G, Savvudes P *et al*. Angioid streaks in homozygous beta-thalassaemia. *Am J Ophthalmol* 1989; **108**: 356–359.
14 Trevor-Roper PD. Blood dyscrasis and the reticulo-endothelial system. In: Sorsby A (ed). *Modern Ophthalmology*. JB Lippincott: Philadelphia, 1972, pp 509–522.
15 Ballantyne AJ, Michaelson IC. Disorders of the blood and blood-forming organs. In: Ballantyne AJ, Michaelson IC (eds). *Textbook of the Fundus of the Eye*, 2nd ed. Williams & Wilkins: Baltimore, 1970, pp. 287–299.
16 Aisen ML, Bacon BR, Goodman AM *et al*. Retinal abnormalities associated with anaemia. *Arch Ophthalmol* 1983; **101**: 1049–1052.
17 De Sanctis V, Zurlo MG, Senesi E *et al*. Insulin dependent diabetes in thalassaemia. *Arch Dis Child* 1988; **63**: 58–62.
18 Kagan A, Aurell E, Tibblin G. Signs in the fundus oculi and arterial hypertension: unconventional assessment and significance. *Bull WHO* 1967; **36**: 231–241.
19 Duke-Elder S, Dobree JH. The blood diseases. In: Duke-Elder S (ed). *System of Ophthalmology*. CV Mosby: St Louis, 1967, pp 373–407.
20 Duke-Elder S, Dobree JH. Metabolic diseases. In: Duke-Elder S (ed). *System of Ophthalmology*. CV Mosby: St Louis, 1967, pp 408–501.
21 Leishman R. The cardiovascular system. In: Sorsby A (ed). *Modern Ophthalmology*. JB Lippincott: Philadelphia, 1972, pp 447–508.
22 Chester EM. Retinal venous diseases. In: Chester EM (ed). *The Ocular Fundus in Systemic Diseases*. Year Book Medical Publishers: Chicago, 1973, pp 116–126.
23 Margulies LJ. Ocular manifestations of cardiovascular and haematological disorders. *Curr Opin Ophthalmol* 1994; **5**: 99–104.
24 Castanon C, Amigo MC, Banales JL *et al*. Ocular vaso-occlusive disease in primary antiphospholipid syndrome. *Ophthalmology* 1995; **102**: 256–262.
25 Ferguson LS, Burke MJ, Choromokos EA. Carbon monoxide retinopathy. *Arch Ophthalmol* 1985; **103**: 66–67.
26 Kirkham TH, Wrigley PFM, Holt JM. Central vein retinal occlusion complicating iron deficiency anaemia. *Br J Ophthalmol* 1971; **55**: 777–780.
27 Kurzel RB, Angerman NS. Venous stasis retinopathy after long-standing menorrhagia. *J Reprod Med* 1978; **20**: 239–242.
28 Mansour AM. Aplastic anaemia simulating central vein retinal occlusion. *Am J Ophthalmol* 1985; **100**: 478–479.
29 Foster RM. The incidence of retinal haemorrhages in severe anaemia. *Trans R Soc Trop Med Hyg* 1970; **64**: 99–101.
30 Merin S, Freund M. Retinopathy in severe anaemia. *Am J Ophthalmol* 1968; **66**: 1102–1106.
31 Rubenstein RA, Yanoff M, Albert DM. Thrombocytopenia, anaemia, and retinal haemorrhage. *Am J Ophthalmol* 1968; **65**: 435–439.
32 Fletcher ME, Farber MD, Cohen SB *et al*. Retinal abnormalities associated with anaemia. *Arch Ophthalmol* 1984; **102**: 358.
33 Freedman MH. Management of beta-thalassaemia major using transfusions and iron chelation with deferoxamine. *Transfus Med Rev* 1988; **2**: 161–175.
34 Fosburg MT, Nathan DG. Treatment of Cooley's anaemia. *Blood* 1990; **76**: 435–444.
35 Piomelli S. Management of Cooley's anaemia. *Baillieres Clin Haematol* 1993; **6**: 287–298.
36 Cazzola M, De Stefano P, Ponchio L *et al*. Relationship between transfusion regimen and suppression of erythropoiesis in beta-thalassaemia major. *Br J Haematol* 1995; **89**: 473–478.
37 Cazzola M, Borgna-Pignatti C, Locatelli F *et al*. A moderate transfusion regimen may reduce iron loading in beta-thalassaemia major without producing excessive expansion of erythropoiesis. *Transfusion* 1997; **37**: 135–140.
38 Tassipoulos T, Kaltsoya-Tassipoulos A, Alchanati N *et al*. Anaemia 2,3-DPG and tissue oxygenation in beta-thalassaemia heterozygotes. *Nouv Rev Fr Hematol* 1982; **24**: 359–362.
39 Correra A, Graziano JH, Seaman C *et al*. Inappropriately low red cell 2,3-diphosphoglycerate and p50 in transfused beta-thalassaemia. *Blood* 1984; **63**: 803–806.