PAPERS AND SHORT REPORTS

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A highly cost effective method of mass screening for thalassaemia

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Abstract

A simple, fast, and reliable two step procedure for the detection of non-α-thalassaemias in mass screening programmes is presented. Step 1 consists of a study of red cell morphology and a one tube red cell osmotic fragility test. This step eliminates the non-thalassaemic samples; the rest are processed through step 2, consisting of determination of red cell indices and haemoglobin studies.

Over the past seven years this procedure has been used at this centre in mass screening secondary school students in Latium. Blood samples from 289 763 students were examined, and 6838 cases of thalassaemia detected. It is estimated that 0·35 ± 0·25% of subjects with thalassaemia escaped detection by this procedure.

Introduction

The treatment and retrospective prevention of Cooley's anaemia have severe limitations. Prospective prevention is, at least in principle, the only totally effective way to cope successfully with this disease. Any global programme for prospective prevention requires, as a preliminary step, the reliable identification of young people with thalassaemia (which must then be followed by genetic counselling and antenatal diagnosis). Unfortunately, in most affected areas no mass screenings have been performed despite the fact that several theoretically effective strategies are available.

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We describe a simple, fast, reliable, and low cost mass screening procedure that we have developed over the past 40 years.[1]

Methods

Over seven years' screening for thalassaemias in secondary schools in Latium we took a total of 289 763 blood samples. All school students were systematically examined in their last year of compulsory education (age group 13-14).

STEP 1

Every blood specimen underwent (*a*) a study of red cell morphology, and (*b*) a test of osmotic fragility.

Red cell morphology was examined on a freshly made, unstained blood film, smeared in such a way that it included a monolayer of well spread and distinct erythrocytes; this made it possible to detect anisocytosis, poikilocytosis, red cell fragments, and target cells, as well as hypochromia and an opacity that contrasts with the refractile appearance of normal erythrocytes.

The red cell osmotic fragility test was performed with a single hypotonic saline solution obtained by diluting Tyrode's solution to 40% with water.[2] For each test 5 μl of whole blood was added to 1 ml of this solution and thoroughly mixed. If the erythrocytes are normally resistant the mixture became clear and reddish within 1-2 minutes; if osmotic resistance is increased the suspension remained turbid. A reading was made after an interval of 1-2 hours.

STEP 2

All blood specimens in which abnormalities were found according to the above criteria (in our region about 15%) were processed through a further series of studies. These include determination of *red cell indices* with an electronic red cell counter and *electrophoretic assay for haemoglobin* A_2. The experimental conditions for electrophoretic assay were: cellulose acetate Titan III-H plates; Zip zone chamber of the Helena laboratory; haemoglobin concentration of 5 d/dl in the fresh, stroma free haemolysate; application by a super Z applicator of the Helena laboratory; glycine-NaOH 0·02 buffer at pH 8·7, voltage 280 V; time 15 minutes. A Beckman CDS 200

densitometer with manual scanning was used to measure the density of haemoglobin A_2. When electrophoresis showed the presence of haemoglobin F, this was quantitated by the Betke method.[3]

Results and comment

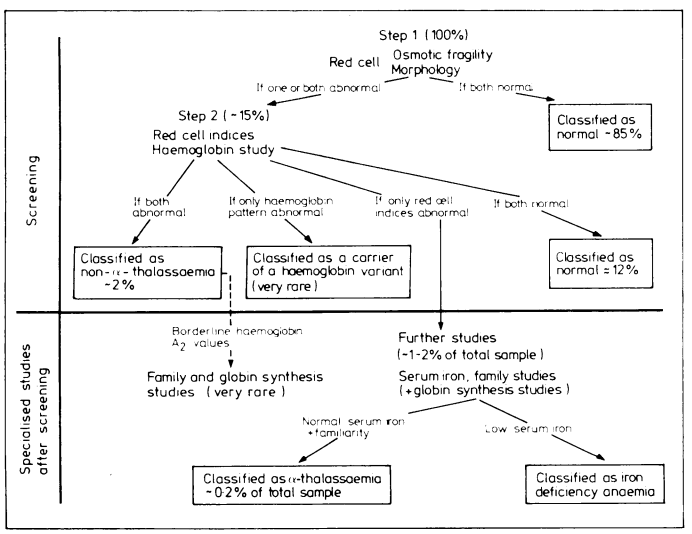
Table I shows that the two tests of step 1 detected virtually all subjects with thalassaemia. There must be liberal admission to step 2, with even a weakly positive result of the red cell fragility test or any very slight alteration of red cell morphology qualifying the sample for step 2. Thus in our study some 15% of samples went on to step 2, even though only one in six of these were eventually identified as having thalassaemias (see figure). Of the two tests of step 1, red cell morphology is more sensitive than osmotic fragility. Of the 575 subjects with β-thalassaemia, 46 had normal osmotic fragility by our one-tube method, but only two had normal morphology. Thus we must regard the test of osmotic fragility essentially as an ancillary cross check for morphology, providing protection from clerical mistakes.

TABLE I—*Sensitivity of step 1 in screening for β-thalassaemia**

Survey	No of subjects	No of β-thalassaemic heterozygotes	
		Finally identified	Missed on step 1
Sardinia (first study[4])	1003	158	2
Latium (first study[5])	4095	132	0
Sardinia (second study†)	917	221	0
Latium (second study‡)	3895	62	0
Total	9912	575	2 (0·35%)§

*All subjects were investigated by step 1 alone and by a complete analysis (step 2). Results were compared only after all tests had been carried out.
†In preparation.
‡Unpublished data collected during the seventh year of screening.
§Upper percentage confidence limit of this estimate is 0·84%—namely, $0{\cdot}35 + 1{\cdot}96 \times 0{\cdot}25$.
The only two cases missed on step 1 were part of the first (oldest) screening.

Our electrophoretic procedure, apart from detecting haemoglobin variants, easily discriminated between cases of β-thalassaemia and normals, since the distribution of densitometric values of haemoglobin A_2 in the two groups are clearly separated.[6] The rare borderline cases are diagnosed unambiguously by a study of family history and globin synthesis in each case. The cases of δβ-thalassaemia, Lepore thalassaemia, and haemoglobin H disease are easily identified. Thus electrophoresis identifies all carriers of any form of non-α thalassaemia, which is the goal of the screening. Most of the remaining samples turned out to have perfectly normal patterns of haemoglobin and haematological indices, and were thus conclusively classified as normals. About 1-2% of the total sample required further investigations (serum iron assay, familial and, in some cases, globin synthesis studies) to distinguish between α-thalassaemia and iron deficiency anaemia (figure).



Flow chart showing results of mass screening for thalassaemia.

Table II summarises the results of this method over seven years in all schools of our region.

TABLE II—*Results of seven years' screening for thalassaemias in secondary schools in Latium*

Provinces	No tested	No of thalassaemias					Percentage with thalassaemia (± SE)
		β	δβ	Lepore	α	Total	
Rome	219272	4479	34	56	587	5156	2·3 (± 0·03)
Latina	32194	763	3	13	123	902	2·8 (± 0·09)
Viterbo	15354	291	1	2	40	334	2·2 (± 0·12)
Frosinone	20222	373	1	4	34	412	2·0 (± 0·10)
Rieti	2721	25			9	34	1·2 (± 0·21)
Total	289763	5931	39	75	793	6838	2·4 (± 0·03)

Discussion

The procedure described appears to fulfil all criteria required for mass screening for thalassaemia, and it may be applied even with few laboratory facilities and trained personnel. The two tests of step 1 are so simple and rapid that a team of three operators can process 400-500 samples a day under field conditions. A one month course is sufficient to learn how to carry out these two tests and to interpret results.

The only cases that remain undetected by this method are those with an isolated high haemoglobin A_2[7] and the so called "silent" thalassaemias, which can be identified only through biosynthetic studies. In Latium about 0·3% of the non-α thalassaemias (13 out of the 3000 cases identified among about 60 000 subjects) had an isolated high haemoglobin A_2. The "silent" thalassaemias are probably even more rare: none of the mothers of about 500 patients with Cooley's anaemia examined by us in the past 30 years had this type of β-thalassaemia.

Apart from the salaries of the necessary personnel, the cost of step 1 is minimal. The unit cost is about £1·10, so the total spent on 42 000 tests was £46 200. The step 2 tests are more expensive (unit cost of about £3·50), but the advantage of our approach is that they were performed on a selected subsample only. Thus in our study 8000 samples were studied for £28 000. The all inclusive cost per year of our screening (about 50 000 students) was about £74 000. If, as a result of our screening, even just one birth of a patient with thalassaemia major was prevented (out of the eight or nine expected to be born in Latium every year), the cost incurred would be fully recovered.

The potential of this procedure to prevent the birth of patients with Cooley's anaemia is not appreciably lowered by its failure to detect the cases of isolated high haemoglobin A_2 and the silent β-thalassaemias if every carrier of β-thalassaemia allows his or her partner to undergo full investigation.

Other screening strategies have been proposed. Discriminant analyses based on red cell indices[8 9] are quite expensive and fail to detect some 2% of thalassaemias.[5] Kattamis *et al*[10] have carried out a trial of screening in Lesbos using a one tube osmotic fragility test alone at a saline concentration of 3·6 g/l: they identified 71 out of 72 cases of β-thalassaemia. In our population the failure rate of the osmotic fragility test alone is much higher—about 8%. In Sardinia, where the incidence of thalassaemia is extremely high (20-25%), a screening procedure in which red cell indices, electrophoresis of haemoglobin, and microchromatographic assay of haemoglobin A_2 are carried out on every sample has been adopted.[11] Clearly the approach guarantees the identification of all cases of β-thalassaemia but it is necessarily slow and very expensive (about £4·30 per blood specimen).

The best choice among various screening strategies depends on several variables, such as the incidence of thalassaemia and of other haemoglobinopathies, the frequency of iron deficiency anaemia, the ratio between the size of the area and the availability of laboratory facilities, and economic resources. Thus we do

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not suggest that our approach is necessarily always the best. Table III summarises the strategies recommended for different circumstances. The approach taken by Cao *et al*[11] is likely to be more suitable in areas of South East Asia with very high

TABLE III—*Recommended strategies of screening for β-thalassaemias in different areas*

Percentage of thalassaemia in area	Size of population	Availability of resources	Procedure	Percentage undetected thalassaemics	Mean cost per sample (£)
< 10	> 1 M	Poor	Step 1 for whole sample and step 2 on 15% of whole sample	0·34	1·1
10-20	< 1 M	Reasonable	Steps 1 and 2 on whole sample	0	3·5
≥ 10 abnormal haemoglobins	< 1 M	Poor	Electrophoresis on whole sample	0	1·8

incidence of α-thalassaemia and β-thalassaemia, of haemoglobin E, and of iron deficiency anaemias. For areas with intermediate incidence, however, such as most of Italy and probably most of southern Europe, the approach we have described would seem to provide a high level of reliability with a low cost.

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Influence of Doppler ultrasound on fetal activity

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Abstract

A randomised and double blind study of 100 subjects and 50 controls was performed to confirm or refute a report from Cardiff in 1975 that continuous Doppler ultrasound, as used in fetal heart rate monitoring, increases fetal movement by over 90%. The results showed such an effect to be most unlikely (power > 0·99). A total of 150 pregnant mothers recorded fetal movements for 30 minutes while connected to a specially modified cardiotocograph, the ultrasound being switched on at random for either the first or second 15 minutes in 100 of the patients. The mean difference in 15 minute movement count, with and without ultrasound, among the 100 patients was 0·2 of a movement (SD 12·7; p > 0·6 by two tailed Wilcoxon matched pairs signed ranks test). The control group showed a mean difference of 2·6 movements (SD 12·1; p > 0·2).

Results of a pilot study suggested that the observations in the earlier report may have been influenced by mechanisms unrelated to ultrasound.

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Introduction

In 1975 David *et al* reported that exposure of the fetus to Doppler ultrasound caused an increase in fetal activity of over 90%.[1] Four years later, however, two other studies were published,[2 3] and neither of these was confirmatory. Unfortunately none of these studies was performed double blind and each included only a small number of subjects; there were also appreciable differences between them in experimental design.

Because of the important implications of the results of David *et al* we decided that a large, randomised, controlled, and double blind experiment should be carried out, but whose design should deliberately be kept sufficiently similar to that of David *et al* to permit a meaningful comparison of results. In order to optimise the design of the main study and provide preliminary information on fetal movement we also mounted a pilot study.

Pilot study

Fetal movements felt by 50 mothers or seen by an observer watching the abdomen were recorded separately on a two channel chart recorder using hand held push buttons. *No* ultrasound equipment was

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