First premarital screening of thalassaemia carriers in intermediate schools in Latium

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SUMMARY In the 1975 to 1976 school year, under the auspices of the Health Authorities of the Latium Region, the Rome Microcythaemia Centre carried out for the first time a partial screening survey of thalassaemia carriers among the students of the compulsory intermediate school in Latium. This work was the beginning of a new preventive school health service aimed at the prophylaxis of Cooley's disease.

In 23 places investigated in Latium, 17 724 students were examined, 13 354 of whom were in Rome and 4370 elsewhere. The mean percentage of co-operation was 70% and the mean percentage of thalassaemia 2.42%.

Thalassaemic students were invited to attend the centre for a check-up along with their families; about half had already come in by the end of June 1976. All students examined, whether normal or thalassaemic, have received written results of the tests.

The screening survey aroused notable interest and obtained wide approval both at school and at home. The news of being thalassaemia carriers, even if not welcome, was never the cause of family tragedy.

Under the auspices of and with financial support from the Health Authorities of the Latium Region, the Rome Microcythaemia Centre initiated and carried out partial screening for thalassaemia carriers in third-year intermediate students in the school year 1975 to 1976. The centre intends this first screening to be the start of a new preventive school health service to be carried out every year in Latium on all third-year students of the compulsory school. The ultimate aim of this project is the prophylaxis of Cooley's anaemia. The detection of thalassaemia is in fact the indispensable premise for any form of pre- or postmarital prophylaxis of Cooley's anaemia.

Screening stages

First of all, students and teachers of the intermediate schools were informed, class by class, with a brief and simple lecture, of the existence and the dangers of thalassaemia. At the end of the explanatory talk the students received an informative letter for their parents, requesting their permission to carry out the tests, to be brought back to school as soon as possible, duly signed by father or mother.

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Informed consent having been obtained, a team of doctors took 1 ml venous blood from each student in the school dispensary. The samples were transferred that same morning to the centre's laboratory.

All tests were done in each school within about 10 days, and the results were delivered by the centre to the school doctors. A card bearing the result was sent to each non-thalassaemic student, while the thalassaemic or suspected thalassaemic students were sent a letter which, apart from communicating the result as 'suspected thalassaemia', invited the student to come to the centre with the rest of his family for another examination.

The main aim of this second call-up was that of confirming, before definite communication of the results, the effective presence of thalassaemia in the subjects selected by the first screening. This was necessary both to exclude the possibility of mistaken labelling of samples and to identify the cases of thalassaemia which, not being characterised by specific haemoglobin findings, could be confirmed only by the demonstration of the familiarity of the haematological picture seen in the student and by exclusion through serum iron measurement of sideropenic anaemia—a disease which, as is known,

resembles the entire haematological picture of thalassaemia.

Next the idea was to inform the families of their condition and of the need to have information about the marital choices of the thalassaemic offspring; at the same time it was hoped to hear their opinion of the undertaking carried out in the school and their reaction on learning the results. Finally, it was considered advisable to examine immediately the other offspring of these families.

The letters for the positives were delivered elsewhere by the school doctor to those concerned or, better, to their parents, while the negative results were handed out, a few at a time, in class. Thus, recognition of the positives by their classmates was avoided and at the same time the presence of a doctor when the results of the tests were communicated to avoid unnecessary alarm in students and parents.

At the end of the tests the families coming to the check-up received the results from the doctors at the centre. They were first of all informed of the type of thalassaemia they were carrying and, for α -thalassaemia, it was explained that this would not damage offspring in the case of marriage to a β -thalassaemia carrier, but only in the case of marriage to another α -thalassaemia carrier. The results were then issued to all subjects examined, the normals receiving the usual card; the thalassaemics received another type of card which illustrates the danger of mating between two β -thalassaemics and the necessity that thalassaemia carriers should have their future partners examined as soon as possible.

Subjects and methods

Work was begun in Rome and carried out on a vast group of intermediate schools in November 1975. Subsequently another 6 towns in the province of Rome (Fig. and Table 1) were investigated. In the province of Viterbo, thanks to the active collaboration of the local authorities, it was possible to investigate 9 towns; in the two provinces south of Rome (Frosinone and Latina) 5 of the main towns were examined (Fig. and Table 1).

The screening was carried out in two stages. In the first stage, for all the samples taken daily (from 200 to 400), osmotic fragility was studied with the technique standardised by Silvestroni and Bianco (1945), and red cell morphology was examined on an unstained slide.

A first discrimination was made with these two blood tests, that is the samples which showed an increased cell fragility and altered morphology of red cells, or either of these two characteristics, and which, therefore, had the greatest likelihood of being thalassaemic, were selected. The experience of Silvestroni and Bianco (1973), in 30 years' work on thalassaemias in Italy, demonstrates this (Table 2): in fact, 88% of carriers of the various different varieties of thalassaemia have both the above characteristics, 10% only one, and 2% neither one nor the other.

With the simultaneous use of the two above tests 98% of thalassaemia carriers were, therefore, already selected in the first stage of screening: this is the *first group* of subjects sent to the second stage of screening.

Fig. Cities and towns investigated in the provinces of Latium. Percentage frequencies of thalassaemia in third-year intermediate students.

Table 1 Results of screening for thalassaemia among 3rd-year intermediate students in provinces of Rome, Viterbo, Latina, Frosinone

Place	No enrolled	No. tested	% tested	Thalassaemic carriers	Percentage thalassaemia
<i>Province of Rome</i>					
Rome	18 551	13 354	72	312	2.33
Velletri	525	340	64	8	2.35
Colleferro	324	185	57	2	1.08
Civitavecchia	515	292	56	6	2.05
Artena	160	159	99	2	1.25
Valmontone	167	113	67	2	1.76
Segni	118	93	78	3	3.22
	20 360	14 536	71	335	2.30
<i>Province of Viterbo</i>					
Viterbo	782	735	93	18	2.44
Bagnai and Vitorchiano	62	56	90	2	3.57
Celleno, Graffignano, Grotte S. Stef.	87	74	85	2	2.70
Canepina	50	44	88	3	6.81
Canino and Cellere	80	67	83	4	5.97
Tarquini	144	83	57	1	1.20
	1 205	1 059	87	30	2.83
<i>Province of Latina</i>					
Fondi	424	290	68	13	4.48
Formia	470	332	70	10	3.01
Gaeta	388	245	63	6	2.44
	1 282	867	67	29	3.44
<i>Province of Frosinone</i>					
Frosinone	624	451	72	13	2.88
Pontecorvo	404	226	56	9	3.98
Cassino	1 133	585	52	13	2.22
	2 161	1 262	58	35	2.77
General total =	25 009	17 724	70	429	2.42

With the 2% residue containing α -thalassaemia carriers (which cannot be detected by any haematological or electrophoretic investigation when we are dealing with latent α -thalassaemia) and carriers of isolated increase of HbA₂ (which can be identified only by way of the electrophoretic examination of Hb), we attempted to select at least some of these latter subjects by sending to the second stage of screening all those cases with even minimum alterations of the red cell morphology. On the average, 5 to 6% of the daily samples were taken and on the whole about 900 subjects (*second group*). Finally, with the aim of further checking the screening method adopted, over the course of the whole survey 1000 subjects who had turned out to be completely normal

in the two preliminary blood tests were chosen at random and subjected to the second stage of screening (*third group*).

In the second stage of screening all the samples from the first and second group underwent, on the same day as sampling, more complete blood tests (Hb measurement, red cell count in 0.9 and 0.36% saline, red cell morphology in counting chamber, haematocrit). The next day the same samples were submitted to electrophoretic examination of haemoglobin which, besides giving diagnostic confirmation, also typed the thalassaemia.

The blood tests were done using conventional methods: the Hb was given in g/dl by the Drabkin method, the red cell count was done with the optical

Table 2 Detection of thalassaemia through testing of red cell fragility and morphology

Osmotic fragility	Alterations of red cell morphology	Type of thalassaemia	Percentage of all thalassaemias
Decreased	Present	β -thalassaemia α -thalassaemia $\delta\beta$ -thalassaemia with high Hb F $\delta\beta$ -thalassaemia with Hb Lepore Thalassaemia with Hb H	88
Decreased	Absent	β -thalassaemia α -thalassaemia	5
Normal	Present	β -thalassaemia α -thalassaemia	5
Normal	Absent	β -thalassaemia α -thalassaemia	2

microscope, and the haematocrit value was determined by the capillary method. For the osmotic fragility test the method of Silvestroni and Bianco (1945) was used; this is done in 0.36% hypotonic solution and allows the results to be obtained in only a few minutes. In fact, the blood is diluted in a red cell count pipette with a solution made up of 4 parts of Tyrode saline and 6 parts of distilled H₂O; within a very short time if the red cells have a normal osmotic fragility, the contents of the pipette become clear; if the opposite is obtained, the red cells have an increased osmotic fragility and the liquid remains opalescent.

The red cell morphology was examined, in the first stage of screening, on the unstained blood slide. For this examination it is necessary for the slide to be very thin and for the cells to be visible in a single layer and well separated from each other. In the second stage, that is in subjects fully examined from a haematological point of view, the red cell alterations were checked again in the counting chamber.

The electrophoretic study of Hb was carried out with the technique of microzone on cellogel at alkaline pH, standardised in the Rome Centre by Bianco *et al.*, (1972) and already widely tested. The percentage of haemoglobin fractions was obtained from the stained and diaphanised strip of cellogel on a Beckman R-110 densitometer. The amount of Hb F was determined in the cases of evident increase by the 1-minute alkaline denaturation technique of Singer *et al.* (1951), and, in the cases of more modest increase, the HbF was assayed on a slide by the acid elution technique of Kleihauer *et al.* (1957).

Results

The techniques and procedures used in screening made it possible to identify among the students examined at least 98% of thalassaemia carriers and to type each case of thalassaemia. The subjects selected in the first stage on the basis of the osmotic fragility tests and red cell morphology were, with rare exceptions, all thalassaemia carriers. From the subjects in the second group (who were carriers of only very slight red cell alterations and of no other thalassaemic haematological characteristics) the electrophoretic study of Hb made it possible to identify still more cases of β thalassaemia (3 out of a total of 429 thalassaemics), rather more classifiable among the cases of isolated increase in HbA₂. In the third group (subjects negative for both haematological tests, taken at random in the course of the whole screening) out of a total of 1000 examined no case of thalassaemia was found which could be recognised by electrophoretic examination of Hb.

In the course of the whole screening 17 724 third-year intermediate students were examined in various places (Fig. and Table 1).

In 94 intermediate schools in Rome, 13 354 students were examined out of a possible 18 551 enrolled students, that is 72%. Out of the total 13 354 students examined, 312 thalassaemia carriers were identified, that is 2.33%. There is, however, a distinct difference in the various schools: in fact, frequencies were found of 0.6 to 1% and of 6.5 to 6.8%.

In the province of Rome a total of 1182 students were examined out of 1809 enrolled, that is 65%. The frequency of thalassaemia fluctuated from 1.08% to 3.22%.

In the province of Viterbo the percentage of co-operation was everywhere very high (average 87%) and altogether 1059 students were examined. The frequency was 2.44% in Viterbo, 2.83% of the whole, and in the other towns of the province it fluctuated between 1.2% and 6.8%.

In the province of Latina 3 towns were investigated and 867 students were examined of 1282 who were enrolled (67.6%). The frequency of thalassaemia fluctuated between 2.3% and 5.1%, with an overall mean of 3.34%.

In the province of Frosinone 1262 students were examined out of 2161 enrolled (58%) in the intermediate schools of Frosinone, Pontecorvo, and Casinò. The frequency of thalassaemia fluctuated between 0.5% and 4.8%, with an overall mean of 2.77%.

For all places investigated the overall mean of co-operation was 70%. In all 429 thalassaemia carriers were identified and the average frequency of thalassaemia was 2.42%. The most frequent variety in all places was β -thalassaemia, followed by α -thalassaemia and thalassaemia with Hb Lepore (Table 3). In this survey no cases were found of $\delta\beta$ -thalassaemia with normal levels of HbA₂, and raised Hb F and no cases of $\delta\beta$ -thalassaemia with normal levels of HbA₂ and Hb F.

Up to 30 June 1976 (that is the end of the school year) 50% of the families of thalassaemic students had already come into the centre for tests. An investigation carried out during the check-up revealed that all these families had greatly appreciated the survey carried out

Table 3 Varieties of thalassaemia found among 3rd year intermediate students in provinces of Rome, Viterbo, Latina, and Frosinone

Place	β -thalassaemia	α -thalassaemia	Hb Lepore
Rome	287	19	6
Province of Rome	22	1	
Province of Viterbo	30		
Province of Latina	28	1	
Province of Frosinone	35		
Total	402	21	6

in the schools, and all declared that they preferred to know the condition of their children exactly than to remain in a dangerous ignorance. In no case did the news of being thalassaemic, even though not welcome, disturb the serenity of the family. A telephone inquiry to families still absent from the check-up revealed that except for some cases of real difficulty the most frequent cause of absence from the check-up was negligence or ignorance of the problem and not a refusal to participate in the project. After the telephone calls many of these families came to the centre for tests.

Conclusions

The results of this first experiment of screening for thalassaemia in the intermediate school can be considered satisfactory on the whole. That does not mean that it was an easy task. In the beginning, especially, the difficulties and obstacles were considerable and sometimes insurmountable, and the work throughout the whole project was difficult.

The average percentage of co-operation of 70% shows the degree of interest that the new undertaking (though aimed at populations to a large extent still ignorant of the existence of the thalassaemias) aroused in students and their families. An analysis of the single results shows that where the job of information was exhaustive and well done (or the population was already conscious of the problem) consent always reached a high level of up to 95 to 100%. To this fact is added the high percentage of thalassaemic families who quickly responded to the invitation to come to the centre for a check-up of all the members, and the observation that among the families still absent after the first request the most frequent causes of absence were ignorance or negligence and never refusal to take part in the scheme; this indicates the lack, in many families today, of a eugenic awareness and therefore the need for a campaign for more complete information; it does not reflect the failure or uselessness of school screening.

On the whole these results suggest that the survey should be repeated every year and that in a short time it will cover the whole region of Latium and a very high percentage of students.

It is not possible to foresee at the moment what will be the future behaviour of today's adolescents when approaching marriage, faced with the choice of a criteria of prophylaxis of Cooley's disease. In those cases in which the parents asked whether there were alternative solutions to the prophylaxis of Cooley's disease, the possibility of prenatal diagnosis was discussed. Parents were generally not interested in this method, however, perhaps because the problem was not a real one for the family. But in those cases with

thalassaemic children the parents were prepared to propose a test for thalassaemia to the prospective partner of their thalassaemic child. The centre's future programme plans to have a yearly check-up of thalassaemic families in Latium and to keep permanently in touch with thalassaemic families, with the aim of continuing to help the adolescents of today over the years up to the age of marriage.

If this programme is carried out regularly, in a few years' time it will be possible to know whether the population has accepted premarriage counselling for the prevention of Cooley's disease or has preferred other means of defence.

However, independently of the choices of the young thalassaemics on the subject of prophylaxis of Cooley's disease, it is evident that the global screening plan suggested and carried out by the Rome Microcythaemia Centre does a useful task in the field of social and preventive medicine in that it identifies and informs thalassaemics before marriage; and it gives them freedom and responsibility for making a choice of a partner without thalassaemia, or, after marriage, the freedom and responsibility for controlling procreation.

The present model of collaboration between a private organisation and Government Health Authorities can in future be modified and improved, but has already, in its present form, given proof of working efficiently. For this reason it has seemed worth while to bring it to the attention of the Italian regions and of foreign countries which are equally interested in the problem of thalassaemia.

As far as the region of Latium is concerned, the local authorities have decided to go ahead with annual screening for thalassaemics in schools as a regular school health service.

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