Survival of medically treated thalassemia patients in Cyprus. Trends and risk factors over the period 1980-2004

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Background and Objectives. A large number of patients with thalassemia major have been born and treated exclusively in Cyprus. They have been managed according to standard international practice, but few have been transplanted. In 1999, a combination chelation regime with desferrioxamine and deferiprone was introduced. We analyzed survival trends in Cypriots and tried to identify factors associated with prolonged survival.

Design and Methods. We had incomplete information on births pre-1974 and complete information from 1974 onwards. Clinical data were incomplete pre-1980 and complete thereafter. We analyzed data on 539 patients born after 1960 and followed over the period 1980 to the end of 2004.

Results. There were 58 deaths, 31 (53.4%) of which where due to cardiac causes. In the complete birth cohort of 284 patients born after 1974, survival (95% CI) at 10, 20 and 30 years was 100% (0); 98.5% (96.1-99.4) and 92.7% (86.7-96.1) respectively. There was a significant trend of increasing cardiac deaths between 1980 and 2000 (p<0.001) and a decline after 2000 (p=0.06). In multivariate survival analysis, protective effects were found for female sex (hazard ratio, 0.37, 95% CI 0.21-0.66; p<0.001), and post-2000 follow-up (hazard ratio, 0.44, 95% CI 0.20-0.99; p<0.05), but not for genotype, treatment center or birth cohort.

Interpretation and Conclusions. Most patients born after 1974 survive to at least the age of 30. There has been a marked improvement in survival for patients of all ages since 2000, which may be due to the introduction of combination chelation therapy.

Key words: thalassemia, Cyprus, survival, deferiprone.

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Cyprus is a small eastern Mediterranean island, with a population of approximately 800,000 Greek and Turkish Cypriots. The carrier rate for thalassemia is one of the highest reported, at about 15%.[1] Cyprus was the first country to introduce a successful prevention program, based on pre-marital screening, and the annual birth rate has decreased to less than 5 cases per year from an expected 70-80.[1,2] The Greek Cypriot thalassemic patients comprise a discrete, contained group that has been uniformly treated. The long-term clinical outcome of this group has not previously been reported, but this information would be helpful for describing the evolution of thalassemia during standard medical therapy.

Secular trends in thalassemia survival have traditionally been analyzed by means of Kaplan-Meier plots, usually done by comparing different birth cohorts.[3,4] This approach is limited when (i) accurate clinical records are not kept from birth, (ii) there are changes in treatment over time resulting in patients being exposed to different risk/protective factors at different times in their lives, and (iii) more than one factor may affect survival. Multi-variate Cox proportional hazards regression, with right- and left- censoring of data is an appropriate method in this context, since it allows the same patient to contribute to different kinds of exposure over different periods of follow up, and for entry at different times after birth.[5,6]

Iron-induced cardiomyopathy, as a result of inadequate iron chelation therapy, remains the commonest case of death, and this is seen in patients who are unable to adhere to the demanding regime of regular sub-cutaneous desferrioxamine infusions at least 5 nights per week.[3,4] The oral iron chelator deferiprone received a license for second-line chelation therapy from the European Medicines Control Agency in 1999. There is evidence that deferiprone is an effective chelator of cardiac iron and may improve cardiac outcome.[7-10] Furthermore, combining both desferrioxamine and deferiprone is emerging as a means of optimizing chelation.[11-13] In 1999, due to an increasing number of cardiac deaths, the Greek Cypriot clinics developed and implemented a combination chelation therapy protocol for patients at risk of cardiac complications, which was essentially the same as that recently reported from Sardinia.[13] In this study we applied multi-

variate survival analysis in the Cypriot thalassemia cohort to test the hypothesis that survival post-2000 improved relative to 1980-1999.

Design and Methods

Definition of study group and period of follow-up

Patients with β thalassemia major (regular transfusions started before the age of 5) who had been born and treated exclusively in Cyprus (not more than three years in another country) were eligible for inclusion in the study group. Table 1 illustrates the evolution of treatment and the quality of thalassemia records in Cyprus. For the purposes of this study we limited the start of follow-up to 1980, since data on deaths and causes of death pre-1980 were of inadequate quality.

Table 1. Evolution of thalassemia clinics in Cyprus.

Time period	*Organized voluntary blood donation for transfusion*	*Desf.*	*Def.*	*New diagnosis register*	*Clinical records*	*Genotyping*
Pre-1974	No	No	No	No	Inadequate	No
1974-1980	Yes	Clinical trial	No	Yes	Inadequate	No
1980-1995	Yes	Standard protocol	No	Yes	Comprehensive	No
1995-2000	Yes	Standard protocol	Clinical trial	Yes	Comprehensive	Yes
Post-2000	Yes	Standard protocol	Combination protocol	Yes	Comprehensive	Yes

Desf.: desferrioxamine; Def.: deferiprone.

Thalassemia treatment

Transfusions were administered infrequently and selectively up to the early 1970s. Thereafter, voluntary blood donation was encouraged and blood was increasingly available for thalassemic children. By the mid 1970, sufficient blood was available to transfuse thalassemic patients to the same level of intensity as in the UK, Italy and North America. From 1980 treatment was administered in four dedicated thalassemia clinics (Nicosia, Limassol, Larnaca and Paphos). Iron chelation with desferrioxamine followed best contemporary practice, and was mostly administered at a dose of 20-50 mg/kg 5-6 times per week as a 10-hour infusion. From 1994, patients with heart failure were treated with intra-venous infusions of desferrioxamine through an in-dwelling line (port-a-cath).[14] A small number of patients were issued with disposable desferrioxamine infusers.[15] In 1999, combination chelation therapy was introduced for patients at high risk of heart failure; the protocol of this combination therapy was essentially the same as that described by Origa *et al.*[13]

DNA analysis

β-thalassemia mutations were detected using a standard methodology at the Cyprus Institute of Neurology and Genetics. The mutations were classified as (i) severe: IVS 1-110, IVS 1-1, IVS 2-745, codon 39; (ii) mild: IVS 1-6,-87, Hb Knossos, Hb Lepore or (iii) not identified.[16]

Statistical analysis

All statistical analyses were carried out in STATA 9.0. Survival analysis was carried out via Cox proportional hazards on left- and right-censored data (multiple-record and multiple-event data). The advantage of this method is two-pronged. The *left-censoring* attribute of the method allows patients to *enter* the database at an age x where x may be considerably greater than 0 (this is the case for individuals who were born before 1980 but who contribute to the analysis only post-1980). The method also allows for the possibility that patients change *exposure* during their lifetimes (this is the case for patients who contribute y years of follow-up data in 1980-1999 and z years post-1999).[5,6] Data were right-censored at the date of bone marrow transplant or on 31st December, 2004. Univariate comparisons of mortality were carried out by means of Poisson regression (cardiac and non-cardiac deaths).

We used multivariate Cox proportional hazards analysis to establish risk/protective factors against mortality in the study cohort.[5,6] The main exposure of interest was follow-up post-2000 relative to 1980-1999. Based on results from previous studies, and known parameters which affect the severity of thalassemia, we controlled for the following potential confounders: age, sex, thalassemia genotype, and the clinic where treatment was delivered. Risk factors for death were assessed via the *hazard ratio* (HR) statistic, as a surrogate of relative risk, where a HR>1 indicates risk and a ratio <1 indicates protection. Multivariate analyses were carried out using forward stepwise methods, retaining variables with p values <0.20.[6]

Ethical considerations

The National Medical Ethics Committee of Cyprus approved the study.

Results

Patients

We identified records for 771 patients. Of these, 46 died prior to 1980, 17 were lost to follow-up before 1980, 50 were treated predominantly in another center or moved to Cyprus during the follow-up, and 45 were not transfused regularly. These patients were excluded from the survival analysis. A further 72 patients were born prior to 1960. There was a higher proportion of

deaths and of milder genotypes in this group compared to those born after 1960 (Table 2). We suspect that some of these patients survived because they did not require regular transfusions in early childhood (i.e. had thalassemia intermedia). Inclusion of these pre-1960 births would have confounded the analysis of survival of well-treated patients, and for these reasons we chose to exclude them from the main analysis. The remaining 539 patients constitute the study group (Table 2). Two hundred and eighty-four patients were registered at birth or at diagnosis after 1st January 1974 and represent a birth cohort. Six children have undergone bone marrow transplantation. The study group is characterized by a relatively narrow age distribution. This is illustrated in Figure 1, which shows that the majority were under 10 years old in 1980, and over 20 years old by 2005.

Chelation therapy

Desferrioxamine was given as standard chelation therapy between 1980 and 1999. Intensification of desferrioxamine using intravenous infusion through a port-a-cath was required for ten patients between 1990 and 1994, 42 patients between 1995 and 1999, and nine patients between 2000 and 2004. Combination therapy was initiated between 1995 and 1999 in 18 patients, and between 2000 and 2004 in 108 patients. By the end of 2004, 125 (23.2%) patients had been switched to combination chelation therapy. There were 390 patient-years of follow-up on combination therapy, compared with 11,933 on desferrioxamine monotherapy.

Deaths

There have been 59 deaths since 1980. Causes of death in order of frequency were: cardiac 31 (53.4%), infection 7 (12.1%), accident 5 (8.6.%), not known 5 (8.6%), liver disease 3 (5.2%), malignancy 2 (3.5%), other: 5 (8.6%): stroke, hypoglycemia, pulmonary hypertension (congenital heart disease), ruptured appendix, anemia and complications of bone marrow transplantation. The death after bone marrow transplantation is not included in the survival analysis, because this patient's data were censored on the date of transplantation. Death rates (stratified by cardiac and non-cardiac causes) in 5-year time periods are shown in Figure 2. Univariate Poisson regression identified no overall trend in non-cardiac deaths over the entire period (p=0.57), an increasing trend in cardiac deaths between 1980 and 2000 (p<0.001), and a decrease of cardiac deaths of borderline significance after 2000 (p=0.06). For the birth cohort of 284 patients born after 1st January 1974, estimated survival at 10, 20 and 30 years (95% CI) is 100 (100), 98.5% (96.1-99.4) and 92.7% (86.7-96.1). Due to a lack of comprehensive information on births prior to 1974, it was not possible to estimate survival by standard Kaplan Meier analysis for patients born before this date. There were no cardiac deaths in the patients who switched to combination chelation therapy (0 deaths per 1000 person-years follow-up, 95% CI 0-8.2) This was also the case for patients switched to combination thera-

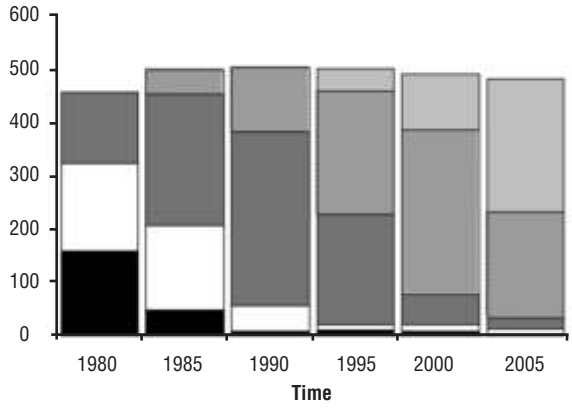

Figure 1. Population size stratified by age group in the study cohort as a function of time: <4 (black), 5 to 9 (white), 10 to 19 (dark gray), 20 to 29 (oblique lines) and 30+ years (light gray).

Table 2. Study sample sizes. Percentages in brackets.

Classification	*Post-1960 birth*	*Pre-1960 birth*	*p values*
Died (cardiac etiology)	31 (6)	7 (10)	0.18[1]
Died (other cause)	27 (5)[2]	13 (18)	< 0.05[1]
Censored at bone marrow transplant	6 (1)	0 (0)	–
Censored at 1/1/2005	475 (88)	52 (72)	–
Total	539 (100)	72 (100)	–
Gender			
Male	281 (52)	45 (62)	0.10
Female	258 (48)	27 (38)	–
Clinic			
Nicosia	230 (43)	33 (46)	0.10
Larnaca	119 (22)	19 (26)	–
Limassol	134 (25)	17 (24)	–
Paphos	49 (9)	1 (1)	–
Other or none given	7 (1)	2 (3)	–
Genotype			
Severe/severe	389 (82)	19 (38)	< 0.001
Severe/mild	85 (18)	24 (48)	–
Mild/mild	0 (0)	7 (14)	–
Unknown	65 (-)	22 (-)	–
Decade of birth			
Pre-1960	–	72 (100)	–
1960 to 1969	132 (24)	–	–
1970 to 1979	324 (60)	–	–
1980 to 1989	58 (11)	–	–
1990 to 1999	21 (4)	–	–
Post 2000	4 (1)	–	–
Patient years of follow-up	12,323	1601	–

[1]Two-sided Fisher's exact p value for comparisons of incidence; otherwise p-values are two-sided Fisher's exact p-values for contingency tables (i.e. for proportions). [2]One additional death from complications of bone marrow transplantation is not included, as the patient was censored at the date of transplant.

py who were excluded from the survival analysis because they were born before 1960, treated previously outside of Cyprus, or were not regularly transfused from childhood. Due to the small number of patient-years of follow-up on combination therapy compared to follow-up on desferrioxamine, there is currently insufficient statistical power for a direct assessment of the independent protective effect of this treatment compared to desferrioxamine.

Multivariate analysis of survival

Results of univariate and multivariate Cox proportional hazards analysis of risk factors are shown in Table 3. Sex, birth after 1974, and follow-up after the year 2000 were significant predictors of survival at the univariate level. The final multivariate model demonstrated that women had less than half the risk of death compared to men, and the risk was also less than half in all patients followed after 2000 than in those followed-up during the previous decade. Genotype, clinic where treatment was given, and birth cohort pre/post 1974 were not independently related to survival. The analysis was repeated including patients born before 1960, and the results were essentially the same, showing independent protective effects for female sex and follow-up after 2000. We also analyzed the data using the parametric Weibull model (the baseline hazard is parametrized as a Weibull function of age) and found significant independent protective effects for younger age (Weibull parameter 3.84, 95% CI 2.95-5.00), female sex (hazard ratio 0.36, 95% CI 0.21-0.65) and follow-up post-2000 (hazard ratio 0.34, 95% CI 0.17-0.69).

Table 3. Univariate and multivariate Cox proportional hazards survival analysis for deaths due to any cause.

Comparison	*Univariate*		*Multivariate*	
	HR	*95% CI*	*HR*	*95% CI*
Sex				
Females vs males	0.37	0.21-0.66#	0.37	0.21-0.66#
Period of follow-up				
1980-89 vs 1990-99	1.21	0.59-2.49	1.02	0.45-2.31
2000-04 vs 1990-99	0.39	0.19-0.81°	0.44	0.20-0.99*
Clinic				
Larnaca vs Nicosia	1.06	0.53-2.13	–	–
Limassol vs Nicosia	1.35	0.73-2.51	–	–
Paphos vs Nicosia	0.67	0.20-2.22	–	–
Genotype				
S/M vs SS	0.99	0.42-2.30	–	–
Birth cohort				
Post-74 vs Pre-74 birth	0.52	0.28-0.97*	0.74	0.33-1.65

*HR: hazard ratio; * p<0.05, °p<0.01, #p<0.001; CI: confidence interval. S/S: homozygous severe genotype; S/M: double heterozygous severe and mild genotype.*

Discussion

In this study, we report very good survival rates for people with thalassemia born in Cyprus after 1974. This cohort is particularly informative, since it consists of all new births diagnosed amongst Greek Cypriots, and is not subject to selection bias. Very few patients in Cyprus have been treated with bone marrow transplantation, thus we have an estimate of the best outcome with conventional therapy. This outcome compares favorably with that recorded in the UK and Italy,[3,4] and with contemporary transplant outcomes.[17,18]

We have also shown a significant trend of increasing cardiac mortality between 1990 and 1999, despite the use of intensive intravenous desferrioxamine in those individuals most at risk. This observation is consistent with a recent study from London showing that intensive desferrioxamine is not sufficent to prevent cardiac deaths in those who are unable to adhere to the therapy.[19] The trend in cardiac deaths appears to be reversing in the final 5 years of follow-up, between 2000 and 2005 (Figure 2). The multivariate analysis shows a significant independent protective effect on survival for the period 2000-2004.

Survival analysis of groups of thalassemic patients is problematic. Traditional Kaplan-Meier survival analysis requires that all patients be followed from birth, and

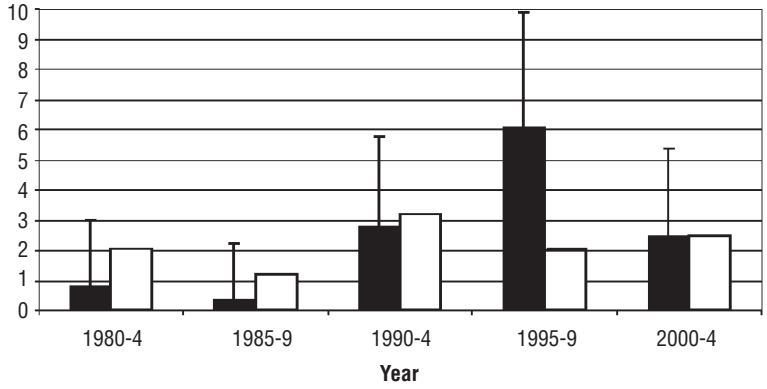

Figure 2. Death rates per 1000 person-years during 5-year periods of follow-up. Cardiac (black), and non-cardiac (white) causes. Error bars represent upper 95% confidence limits. To simplify the illustration, error bars have been omitted from non-cardiac deaths, since there were no significant time-related trends in these data.

that all be exposed to the same risk/protective factors throughout the period of follow-up. This is more the rule than the exception, especially when patients come and go from clinics, and may receive different therapies at different times during their follow-up. We analyzed the data using *left-censorship* at 1980, principally because of the lack of good quality clinical data before this date. A considerable number of transfusion-dependent patients born prior to 1974 would have died prior to 1980, and they are not included in our analysis. This precludes the construction of Kaplan Meier curves for birth cohorts prior to 1974, but does not affect our conclusions about factors associated with survival after 1980. The Seven Centre Italian Study and the UK Thalassaemia Register were also left-censored. In the UK, patients were followed from the age of 12 onwards,[4] and in the Italian study, patients born before 1960 were excluded, and follow-up was initiated in 1970.[3] The limits on our study group are not dissimilar to those for these two established cohorts.

The improved survival after 2000 is unexpected, as one might expect patients to become progressively more vulnerable to the toxic effects of iron on the myocardium and conducting system of the heart as they age. One possible explanation is that an age-restricted cohort is being observed going through periods of different levels of risk: low risk, age 5-15, high risk age 15-25, and low risk again after age 25, and that the protective effect observed after 2000 is due to the fact that the majority of the cohort has passed through the adolescent phase, which is acknowledged to be a time of poor adherence to iron chelation therapy.[20] However, our multivariate analysis does not support this hypothesis, since follow-up post-2000 is protective independently of birth cohort (Table 3). It is also interesting that the results of the UK study do not show a plateau in the survival curves of post-1965 birth cohorts, suggesting that in the UK, the risk of mortality for non-adherent adolescents continues through into adulthood.[4]

An alternative hypothesis is that combination chelation therapy has a specific protective effect. This could be because adherence to treatment is improved, and/or because deferiprone has a cardioprotective effect in iron-overloaded patients. After 15 years of continued 5-6 nightly subcutaneous desferrioxamine infusions, it is reasonable to expect *treatment fatigue* leading to increasing non-adherence. This might be corrected by offering an alternative iron chelation regime consisting of fewer infusions together with daily oral therapy. We have not collected data systematically on the adherence to chelation with desferrioxamine compared to deferiprone, however, direct observations from the clinics, and results of a questionnaire survey indicate that oral chelation and combination therapy with reduced frequency of desferrioxamine infusions is much preferred.[21]

Our results are also consistent with recent observations that deferiprone has a specific cardioprotective effect.[7-10] In our study, there were no deaths among patients on combination therapy, and there was a large reduction in overall mortality, despite only a minority of patients being treated. We suggest that this is the case because (i) combination treatment is not randomly allocated, but selected for those considered most at risk, (ii) the clinicians' selection of those at risk has a high degree of sensitivity, and (iii) the treatment is highly effective in preventing cardiac deaths, the commonest cause of mortality in this study.

Female sex was reported to be protective in a large Italian multicenter study,[3] and this has now been confirmed in our multivariate analysis. This is an important observation and requires further investigation. One possible explanation is that females adhere better than males to recommended chelation therapy. Although this seems a reasonable hypothesis, we are not aware of any published data addressing this issue. Indeed, there was no difference in mean serum ferritin levels between males and females during follow-up in the Italian study, suggesting that iron stores did not differ significantly between the sexes.[22] An alternative explanation involves the protective effects of estrogen on the cardiovascular system, which may be mediated through short-term effects on nitric oxide production leading to vasodilatation and a longer-term modulation of the response to vascular injury.[23]

In conclusion, this observational study shows that excellent outcomes can be achieved with medical management in a small country with relatively limited medical resources. It supports previous observations on the beneficial effect of alternative chelation strategies including oral deferiprone. Longer follow-up of the patients in this study is needed to assess whether the protective effect after the year 2000 is specifically due to the introduction of combination chelation therapy.

PT, PGC and MA contributed to the study design, conduction, analysis and writing; PGC contributed to statistical analysis, SC, MH, AK, EP,NP, MP, KS, GS and MS contributed to study planning, case ascertainment, data collection, and interpretation of results. This work was undertaken as part of a contract awarded to PT by The Ministry of Health of The Republic of Cyprus.

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