
Haemophilia in Greece. A Continuous Struggle for Survival and Quality of Life

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Haemophilia is a very old disease, first described in the Talmud in the 5th century B.C. Haemophilia is a rare disease when compared to the numerous other diseases, which harass humanity and threaten the life of human beings. However, haemophilia is also one of the best examples showing that man can gain a better quality of life in his struggle against the disease.

Haemophilia is a unique example where this struggle can be won by means of^[1]:

- Close cooperation of medical doctors and patient,
- Close cooperation of the various areas of medicine and science in general, and the direct and proper utilization of every scientific and technological progress in the area,
- Mutual trust and affection among the people with haemophilia and all health care workers dedicated to their treatment,
- Proper organization of hemophilia care in Hemophilia Comprehensive Care Centers^[2].

During the 1950's and 1960's there was a tremendous change in the prognosis of the disease thanks to 3 events:

1. Progress in the knowledge of the pathogenetic

mechanisms causing in haemostasis disorders.

2. Progress in the field of Transfusion Medicine and plasma fractionation.

3. Care of the haemophiliacs by specialized and well-organized centers^[1].

Towards the end of the 1970's the application of new technology further improved the quality of life of people with haemophilia.

The 1980's were the decade when high quality of life was achieved by proper and adequate substitution therapy. However, the tragedy of global spread of HIV infection in people treated with plasma derived products shadowed this progress. At the same time, industrial production of safer products was not only triggered but also successfully accomplished. The identification and cloning of the genes coding for factors VIII and IX allowed scientists to perform safe, accurate and early carrier detection, prenatal diagnosis and appropriate genetic counseling.

The 1990's offered the possibility to treat patients with safe products of very high purity either plasma derived or with recombinant technology. Moreover, the progress in antiretroviral treatment retarded the progression of disease (AIDS) in already infected haemop-

hiliacs.

In Greece, care of people with haemophilia started in the 50's and has ever since been continuously offered by dedicated and qualified health care workers following step by step the developments of the international scientific community^[3,4].

According to the registry of the Hemophilia Center of Laikon Hospital Athens Greece, where 80% of Greek hemophiliacs are systematically followed up, the distribution of patients regarding type and severity of disease, date of birth and frequency of inhibitors are presented in Figure 1, 2, 3 and Table 1^[5].

In Greece the frequency of mild haemophilia is rather high. This is related to the presence of large pedigrees of mild Haemophilia A in isolated parts of the country (i.a. Aeani Kozani, Chalkidiki, Island of Cephalonia).

Concerning mortality, in 531 individuals for a total of 8641 person-years, 78 deaths were observed while 30 were expected based on age and sex adjusted population rate. Mortality was almost 8 times higher in patients with severe haemophilia than in the general population and the majority of deaths -54- occurred among patients of this group. On the contrary, patients

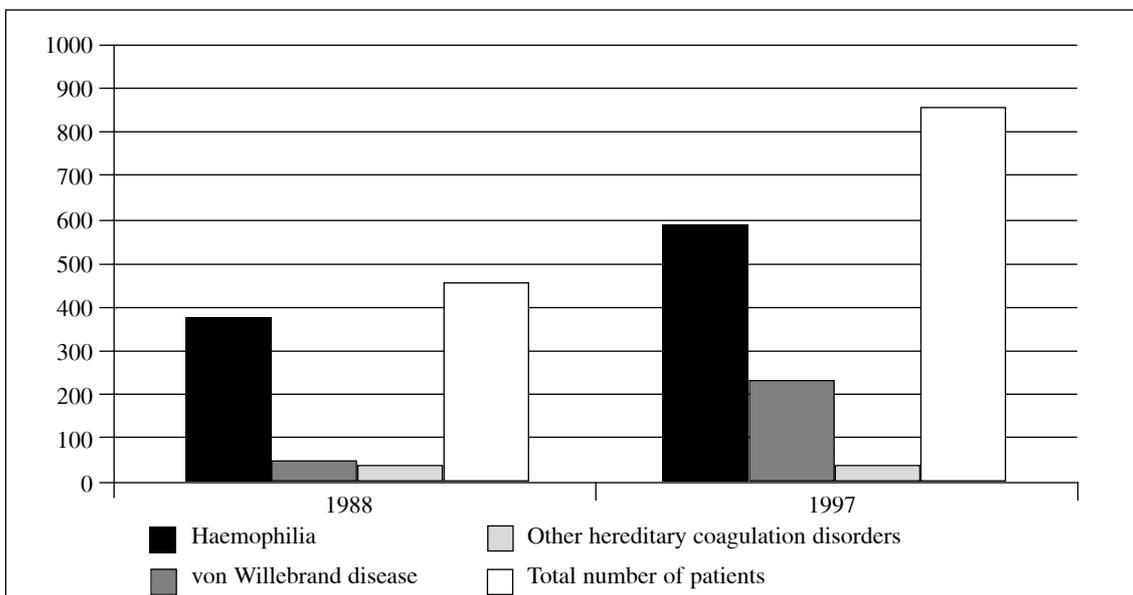


Figure 1. Patients with congenital haemorrhagic disorders in Greece.

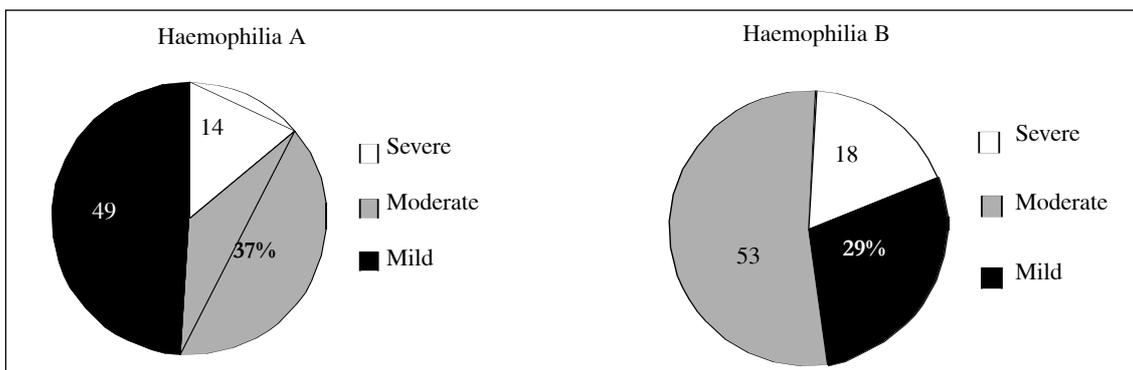


Figure 2. Distribution of patients regarding the severity of the disease according to the recent proposal of SSC of ISTH 2001 (severe: <math>< 0.01\text{ IU/mL}</math> (<math>< 1\%</math>), moderate: $0.01\text{--}0.05\text{ IU/mL}$ (1-5%), mild $0.05\text{--}0.4\text{ IU/mL}$ (> 5% and up to 40%).

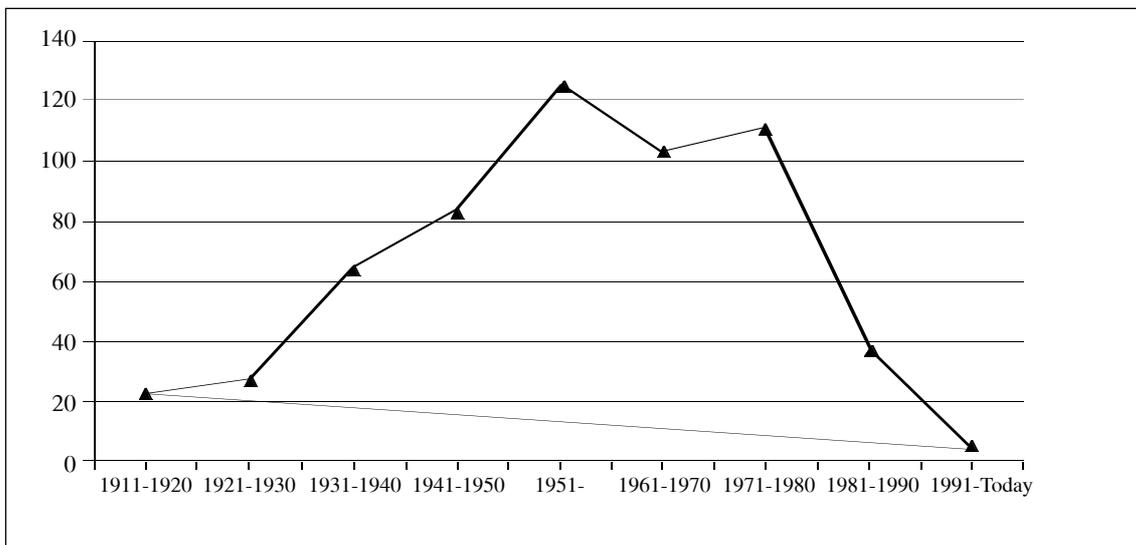


Figure 3. Distribution of haemophiliacs according to date of birth.

Table 1. Inhibitor incidence in 460 cases of haemophilia A*

F VIII inhibitor (+) 38 cases** 7%

F VIII inhibitor (-) 422 cases 93%

* No haemophilia B patient developed inhibitor.

** Only 14 pts out of 38 were high responders.

with mild and moderate haemophilia did not experience excess mortality. Mortality was by far the highest in HIV (+) haemophiliacs after the mean date of infection (Table 2)^[6].

The most common cause of death among our patients was AIDS (Table 3). Cerebral haemorrhage followed in frequency and was the commonest cause among HIV (-) patients^[5].

As pointed out in Figure 4, the distribution of haemophiliacs according to their educational status shows a significant increase of the number of patients with higher education (High School, Technological Institute or University graduates) while a decrease in the number of Elementary School graduates is also noted. This is due to several factors such as better substitution therapy, early and systematic prophylactic treatment of haemarthroses and to psychological support provided by the Haemophilia Center. These results are

indicative of the high significance of offering motivation to young people. Moreover, it should be noted that the number of unemployed haemophiliacs has almost disappeared.

The majority of death occurred between the ages of 30 and 49. Main causes of death in this age are the transfusion transmitted diseases (hepatitis B and C, HIV infection) (Table 3).

The follow-up of the defective gene in order to establish or exclude carrier status and to perform prenatal diagnosis was systematically applied since 1986^[7,8]. Two hundred fourteen unrelated pedigrees of Greek and Cypriot origin and 1058 DNA samples isolated from the peripheral blood of 338 patients, 570 relatives and 81 reference subjects and from 69 chorionic villus samples were studied^[9].

Five polymorphisms were used to perform linkage analysis in Haemophilia A: F VIII/BcII, F VIII/CA13, F VIII/XbaI, F VIII/BglII and F VIII/TaqI. Their heterozygosity in the Greek population is 49%, 60%, 47%, 50% and 78% respectively. In Hemophilia B five markers were used, F IX/dup, F IX/HhaI, F IX/TaqI, F IX/MnlI and F IX/XmnI, presenting in the Greek population a heterozygosity rate of 36%, 48%, 45%, 45% and 41% respectively. In von Willebrand disease the two multiallelic VNTRs of exon 40 of the vWF gene were used in the 143 individuals studied. A real cumu-

Table 2. Mortality by severity and HIV status^[6]

Group	Patient-years (yrs)	Standardized mortality ratio*	95% confidence intervals	Number of deaths
ALL				
Severe	4072	7.9	6.0-10.4	54
Moderate	1565	1.2	0.6-2.2	11
Mild	3003	0.9	0.5-1.6	13
Total	8641	2.6	2.1-3.3	78
HIV (+)				
Severe	1010	17.9	12.7-24.5	39
Moderate	256	14.7	4.8-34.3	5
Mild	175	11.4	4.2-24.6	6
Total	1443	16.4	12.2-21.6	50

* Expected mortality based on national Greek mortality rates of 1970, 1980 and 1985 in 5-years age classes. Therefore, rates are adjusted for age, sex and calendar period.

Table 3. Causes of death in patients with congenital haemorrhage diathesis up to 1997^[5]

Causes of death	No of deaths	%
Cerebral haemorrhage	22	14.8
Bleeding	6	4.0
HIV infection	58	39.0
Liver disease	9	6.0
Cancer-lymphomas	11	7.4
Other (renal failure, diabetes, pneumonia)	12	7.4
Unknown	23	15.5

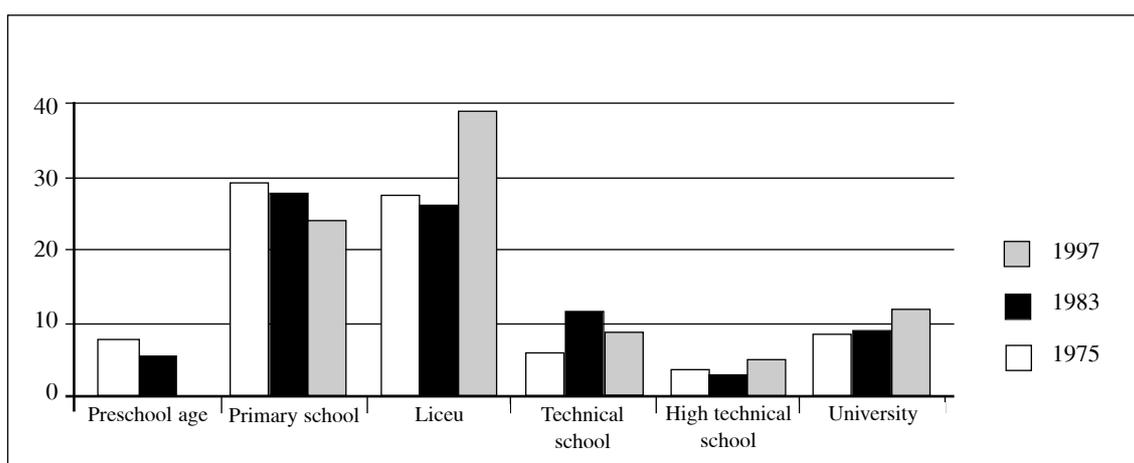


Figure 4. Comparative distribution of people with haemophilia according to educational status according to the 1975, 1983 and 1997 registries^[5].

lative polymorphism information content of 94% was observed when both polymorphisms were studied in each pedigree including at least two affected individuals^[10,11].

The causative mutation was identified in 40% of the patients with Haemophilia A. Among patients with severe type of the disease 35% show an inversion of a major part of F VIII gene, detected by southern blotting, radiolabelling and autoradiography^[12]. All 71 patients with mild Haemophilia A and a common origin from the village Aiani in Northern Greece, share an A > T transversion at codon 280 resulting in the replacement of Asn 280 by Ile, that can be detected by PCR and digestion with BamHI. The 8 generations and more than 600 individuals pedigree of Aiani is the largest regarding the number of patients ever reported in the literature (Figure 5)^[13]. Additionally, we identified:

a. A deletion longer than 27 kb in a family including three patients all having developed inhibitors against F VIII, and

b. The substitution of C 6977 by T that causes premature termination of transcription and was detected after screening by PCR and digestion with TaqI of the CpG hot spots in exons 18.24 and 26 of F VIII gene in 95 families. Moreover a single point mutation was identified in the promoter region of a haemophilia B family characterised by progressive increase of F IX levels by age (F IX Leyden)^[14-16].

According to the above-mentioned information we decided on the strategy of Carrier Detection of Haemophilia A and B in Greece. In families with known causative mutations direct testing is performed. In the rest of the families, linkage analysis is used. In Haemophilia A the following polymorphisms are studied sequentially. F VIII/BcII, F VIII/CA 13 and F VI-II/TaqI by PCR, F VIII/BgIII, F VIII/TaqI and F VI-II/XbaI by southern blotting. In cases that extragenic markers are used, a probability of carriership is calculated taking under consideration the phenotypic data. In cases that linkage analysis is meaningless or noninformative, the answer is based exclusively on phenotypic data given that the reliability of the answer is > 95%. By the use of this strategy 94% of the 146 potential carriers tested received an answer and in 82% the reliability levels were > 99%.

In linkage analysis of Haemophilia B all five polymorphisms are studied simultaneously and in noninformative cases, either, SSCP analysis is performed or the phenotypic data are evaluated. By the use of this strategy 93% of the 42 potential carriers tested received an answer and in 76% the reliability levels were > 99%.

The entire coding region of F IX gene of 27 unrelated patients with Haemophilia B was screened by SSCP analysis applied by both a radiolabelling and a semi-automatic silver staining method. Direct DNA sequencing was applied in 10 patients and allowed the identification of 7 different mutations. One of them is a novel de novo frameshift thymidine deletion, two are nonsense mutations (codons 44 and 252) and 4 are missense mutations causing the substitution of Arg -4 by Trp or Gln, of his 221 by Arg and of Arg 248 by Gln. The His 221 substitution has not been previously described and is of great importance, since this residue is part of the catalytic triad of F IX^[10].

The first step of a successful prenatal diagnosis is to determine before pregnancy for each carrier which of the above-mentioned methodologies will be justified and applicable when required. The feasibility of prenatal diagnosis was examined in 230 carriers of Haemophilia A or B. Molecular Biology methods are applicable to 96% of the cases after chorionic villus sampling in the 10th week of gestation. In 87% the diagnosis is absolutely reliable. For the rest of the women the only choice is cordocentesis by foetoscopy in the 20th week of gestation and subsequent coagulation measurements in foetal blood samples. In 38 out of the 69 chorionic villus samples (35 female and 34 male foetuses) sex determination was carried out successfully by tracing Y chromosome sequences by PCR or by karyotyping. The application of this method was established in all cases, since it contributes significantly to speeding up the prenatal diagnosis procedure. In 26 of the 34 male foetuses testing was carried out by intragenic markers, in one the inversion of F VIII gene was identified for the first time in the family, in 5 cases extragenic markers were used and in 2 cases inadequate sampling lead to the decision to proceed to diagnosis in the 20th week by foetal blood sampling. In 2 families that asked for it, the result of extragenic markers analysis was verified by coagulation measurements in foetal blood.

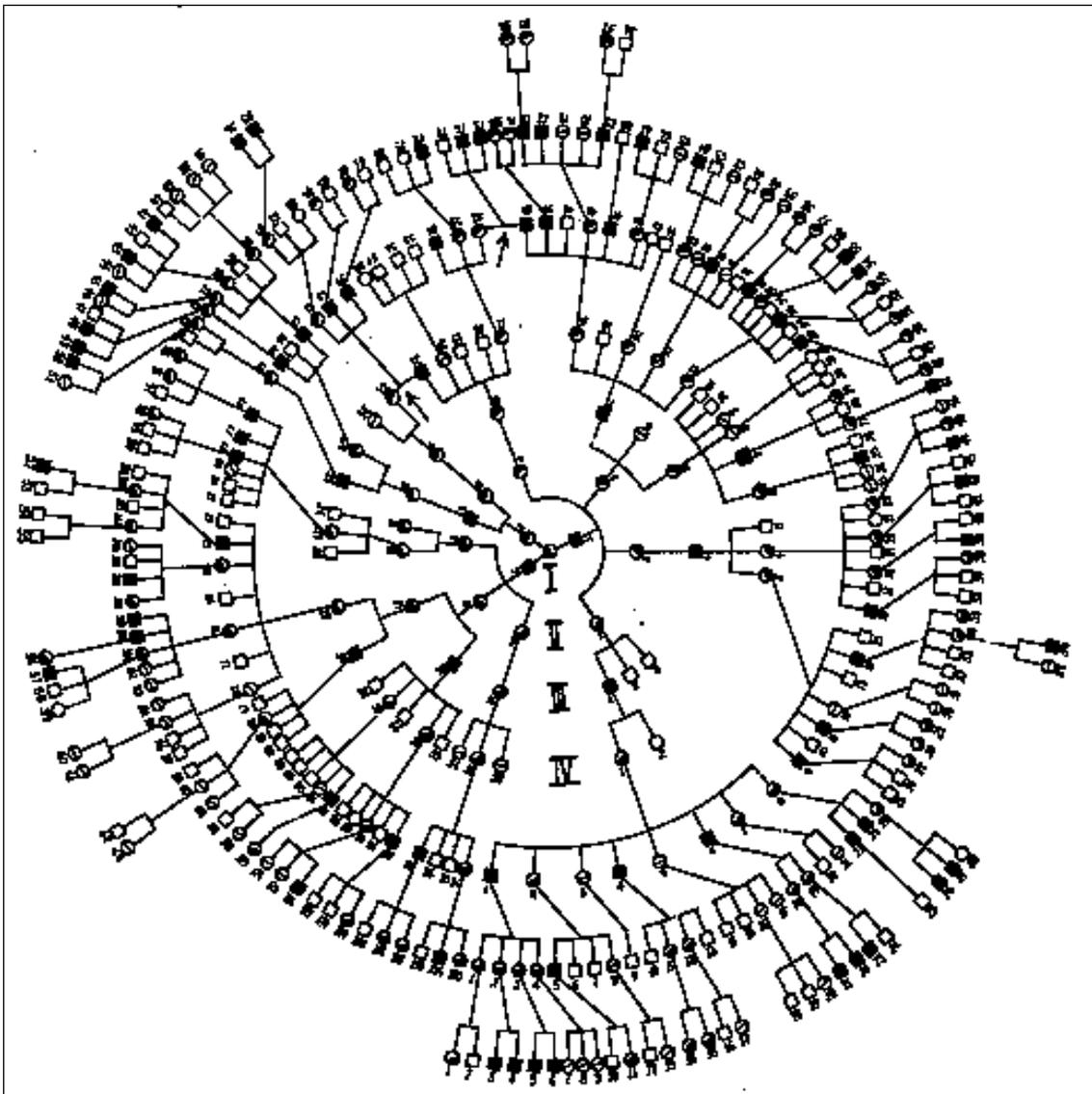


Figure 5. Major Aiani pedigree^[13].

In the autosomal von Willebrand disease the genotype-phenotype relation is not always clear and therefore prenatal diagnosis is usually meaningless since the limits between carrier and patient are often rather vague. However, by linkage analysis it was proved that 14 individuals have not inherited the defective gene and that 15 still asymptomatic cases carry the abnormal genotype.

The use of molecular biology methodologies in family studies in Greece and genetic counseling in the

main hereditary hemorrhagic disorders has been proved highly beneficial, mainly because it allowed carrier detection in almost all cases, an achievement which was not feasible with the conventional methods. Moreover this approach increased reliability in the majority of the cases to levels > 99% and facilitated prenatal diagnosis in the 1st trimester of gestation.

New patients with congenital haemorrhagic disorders will continue to be born, both because it is impossible to foresee new mutations and isolated cases and

because many women either avoid for various reasons prenatal diagnosis or decide not to terminate pregnancies of affected foetuses. However, it is an obligation of every Haemophilia Treatment Center to inform reliably all potential carriers for their carrier status and to guarantee a rapid and reliable prenatal diagnosis to all women who ask for it.

Today haemophiliacs are capable of taking their place as active and competent members of the community and of living virtually normal lives. As children they are able to participate in all but a very few of the activities of their peers. They can take up training for their chosen occupation, and look forward to a working life unmarred by excessive absence from work. Life expectancy in severe hemophilia, which was only 11 years at the turn of the previous century, varies now between 58 and 63 years. Supplies of F VIII/IX have successively increased both in Western Europe and the USA in spite of some temporary shortage for precaution in safety.

Patients with acquired F VIII/IX antibodies, for whom no treatment was previously available, can now be catered for with newly developed methods both for the treatment of their acute bleeding episodes and for the permanent removal of their antibodies (i.e., induction of immune tolerance).

Optimum management of hemophilia is achieved through specialized interdisciplinary comprehensive care centers, such as those that have now been set up in most countries in the USA and Canada, and to a certain extent also in developing countries. In Greece, four centers are now functioning (3 in Athens, 1 in Salonica) appointed by Ministerial Degrees (Ministry of Health).

In the future radical cures for hemophilia may become possible, and both cell transplantation and gene therapy are currently being seriously discussed among researchers in the field. Methods for the propagation, replication and transplantation both of foetal and mature somatic cells and new techniques for gene substitution are under development and application. These developments are fundamental to the planning of various strategies for the primary treatment of such genetic disorders as hemophilia A and B.

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