ORIGINAL CONTRIBUTIONS

Epidemiology and Survival Analysis of Cystic Fibrosis in an Area of Intense Neonatal Screening Over 30 Years

Baroukh M. Assael1,2, Carlo Castellani1, Marisol Barao Ocampo1, Patrizia Iansa1, Andrea Callegaro3, and Maria Grazia Valsecchi3

1 Cystic Fibrosis Center, Verona, Italy.
2 Department of Pediatrics, University of Milano Medical School, Milano, Italy.
3 Department of Clinical Medicine, Prevention and Biotechnologies, Section of Medical Statistics, University Milano-Bicocca, Milano, Italy.

Received for publication October 25, 2001; accepted for publication April 24, 2002.

This population-based study was conducted in Veneto and Trentino (northwestern Italy, population 5 million). In this area, neonatal screening for cystic fibrosis started in 1973 and has been virtually universal since the early 1980s. During this study, the estimated incidence of cystic fibrosis in this region was 1/2,650 livebirths per year. The authors analyzed data on 593 patients born in 1938–2000 and living in the region who were followed by a single referral center. Median time from birth to confirmation of diagnosis after screening was 32 days (range, 0–1,531). For patients whose disease was recognized after symptoms occurred, median age at diagnosis was always less than 1 year. Median survival age was 37.7 years. Long-term survival (to age 20–30 years) was not significantly influenced by mode of diagnosis (screened or unscreened), sex, or age at diagnosis for unscreened patients (<1, 1–5, >5 years). Current survival analysis of three consecutive decades showed that improving survival tended to vanish in the last years of the study. The authors concluded that a regional neonatal screening program allows very early recognition of cystic fibrosis. They could not conclude that neonatal screening improves long-term survival if compared with diagnosis by symptoms in early infancy. Am J Epidemiol 2002;156:397–401.

cystic fibrosis; neonatal screening; survival analysis

Abbreviations: IRT, immunoreactive trypsinogen; SE, standard error.

Cystic fibrosis is one of the many genetically transmitted diseases that are prevalent worldwide. Some four decades ago, the disease was considered almost inevitably lethal, and death was expected in the first decade of life. In the late 1950s, Shwachman and Kulczycki (1), describing their experience with 105 patients, recognized that improved diagnostic methods and early recognition of mild cases had ameliorated the survival of cystic fibrosis patients beyond childhood.

In the subsequent 40 years, survival of cystic fibrosis patients increased progressively, and the proportion of adult subjects is currently high. Geographic differences exist and may be accounted for by genetic background, precocity in diagnosis, diagnostic and selection criteria, and therapeutic approaches.

Despite the availability of cystic fibrosis survival studies (2–4), only a few population-based data have considered progression of the disease into adulthood. Of these, we know of none that has considered cohorts of cystic fibrosis patients diagnosed after neonatal screening programs have been implemented.

Italian legislation has established regional cystic fibrosis referral centers, which are specifically funded to implement screening programs and to promote early recognition of the disease. In Veneto and Trentino, a northeastern region of Italy with approximately 5 million people and an average annual birth cohort ranging from approximately 80,000 newborns in the 1970s to 50,000 in the 1990s, neonatal screening programs have been implemented since 1973 and...
have progressively been improved since then, in the last 10 years reaching virtually the whole cohort of newborns (5). This paper examines the changing epidemiology of the disease in this region over the last 30 years and analyzes the survival of a consecutive series of patients diagnosed and followed in the referral Cystic Fibrosis Center of Verona.

**MATERIALS AND METHODS**

Since it was instituted in the early 1960s, the Center of Verona has followed 1,609 cystic fibrosis patients, 593 of whom were born between July 1938 and April 2000 and resided in the Veneto and Trentino region. The first cystic fibrosis neonatal screening program in this area began in 1973. Until 1981, screening was performed in a cystic fibrosis center to determine albumin levels in dried meconium by using semiquantitative radial immunodiffusion assay. In 1981, immunoreactive trypsinogen (IRT) measurement was adopted; the protocol involved measuring IRT levels at birth and then retesting hypertrypsinemic newborns at 1 month of age. Since the early 1990s, the screening strategy has involved a three-tier system, whose progressive steps are IRT, mutation analysis with complementary meconium lactase determination, and sweat test. If the IRT measurement is greater than the 99.5th percentile, lactase determination and mutation analysis (DeltaF508, DeltaS507, R1162X, 2183AA>G, N1303K, 3849+10KbC>T, G542X, 17171-1G>A, R553X, Q552X, G825E, 711+5G>A, 3132delTG, 2789+5G>A, and W1282X, with an 85 percent detection rate in our area) are performed; if lactase exceeds its threshold level and/or the mutation analysis is positive, the subject undergoes a sweat test.

A computerized database was implemented in 1989 to prospectively include demographic, vital, and several types of clinical data for all patients seen in the center; it was also retrospectively supplied with data on patients followed by the cystic fibrosis center since 1958. Clinical data are recorded at each follow-up visit, and the records are updated continuously under the supervision of a database administrator. We used information extracted from this database for statistical analysis. Except for 4 percent of the patients in the present study who were lost to follow-up, the status of each patient was updated until December 2000.

Cumulative survival probability at different ages was evaluated statistically with the Kaplan-Meier estimator. Age at death or age at the last follow-up, for living patients or patients lost to follow-up (censored data), was calculated. For cystic fibrosis patients diagnosed by neonatal screening or by meconium ileus, the entry point was date of birth, while entry was delayed to date of diagnosis for patients not observed from birth (left truncation). These latter subjects entered the risk set at the age at which they were first diagnosed. The log-rank test was used to compare survival across different groups.

The alternative current survival method was also adopted, with the specific aim of describing survival changes by calendar period. For each calendar period, the survival estimates were obtained after left truncation and right censoring of the follow-up observations at the beginning and end of the period, respectively (6). Although this method in principle enables the “current” picture of survival to be given by analyzing mortality in patients at risk during a limited time period, small numbers may limit its application. In the present study, we applied this method by defining 10-year periods and by considering death rates by 5-year age classes.

The incidence of disease was calculated as the ratio between 1) the number of patients detected by neonatal screening plus the false negatives at neonatal screening recognized later in life and 2) the total number of screened newborns in the last 7 years. Sensitivity, specificity, and positive predictive value of the screening strategy in the latest years were 96 percent, 99 percent, and one third, respectively (7).

**RESULTS**

**Incidence and distribution**

The estimated incidence of cystic fibrosis in our region was 1/2,650 livebirths per year during the course of our study. This incidence resulted from 129 diagnoses in 1993–1999 over a total of 341,947 screened neonates; the rate of screening ranged from 98.4 to 99.1 percent in the last 7 years. Median time from birth to confirmation of diagnosis after screening was 32 days (range 0–1,531).

In this study, we considered the series of 593 patients (283 males and 310 females) born and residing in Veneto and Trentino and diagnosed at the Cystic Fibrosis Center of Verona from 1958 to 2000. Overall, 301 patients were diagnosed by screening, 248 because of symptoms, and seven because of familiarity (defined as having a first- or second-degree relative affected by cystic fibrosis or known to be a carrier); 35 were neonates with meconium ileus and two were male adults with congenital bilateral atresia of the vas deferens. Figure 1 shows the distribution of patients diagnosed by screening or because of symptoms, according to year of diagnosis. Neonatal screening programs led to an increasing proportion of disease being diagnosed at birth. Overall, in the Cystic Fibrosis Center of Verona, median age at diagnosis has been low, decreasing from 0.58 years in 1963–1972 to 0.09 years in 1993–2000, while mean age at diagnosis increased during these periods from 2.33 to 4.14 years, respectively. This finding occurred because mean age at diagnosis by symptoms changed from
12.5 years (1963–1972) to 14.8 years (1993–2000) as a result of late recognition of patients exhibiting mild forms of the disease and two cases identified because of male infertility.

Survival analysis

Ninety-nine patients died at a median age of 18.3 years (15 years for males and 19 years for females). The overall cumulative probability of survival at 20 and 30 years of age was 80.4 percent (standard error (SE), 2.3 percent) and 63.0 percent (SE, 3.5 percent), respectively, and the median survival age was 37.7 years. Survival was not significantly different between males and females ($p = 0.42$), with a 20- and 30-year survival probability of 80.7 percent (SE, 3.2 percent) and 69.4 percent (SE, 4.6 percent), respectively, for males and 80.0 percent (SE, 3.3 percent) and 57.9 percent (SE, 4.9 percent), respectively, for females (figure 2). For males and females who were screened, survival at age 20 years was 85.6 percent (SE, 4.9 percent) and 76.9 percent (SE, 8.1 percent), respectively, and the difference was not significant ($p = 0.74$).

Figure 3 shows the survival curves according to cause of diagnosis; we excluded two cases of adult male infertility (diagnosed at 31 and 30 years of age) and seven patients diagnosed because of familiarity (none of the excluded patients had died by the time we completed our study). There was no significant difference across the various groups ($p = 0.21$). The group of patients who visited the referral center because of symptoms or familiarity was divided according to age at diagnosis. For those diagnosed after age 5 years, survival was slightly better (figure 4), but the difference for the other two groups did not reach statistical significance ($p = 0.25$, test for trend).

It seemed interesting to analyze the data from 1973, the year in which screening was first implemented. To allow sufficient follow-up, analyses were limited to the cohort of 309 patients born in 1973–1992. Of these patients, 190 were diagnosed by screening and 113 because of symptoms at a median age of 0.99 years (range, 0.002–2.8) and 1.3 years (range, 0.03–23.53), respectively. Survival did not differ significantly between these two main subgroups ($p = 0.13$) and, at age 20 years, was 80 percent (SE, 4 percent) and 76 percent (SE, 5 percent) for subjects diagnosed by screening and symptoms,
respectively. A clear “period effect” was observed in this cohort (309 patients), since survival was significantly better in patients diagnosed in 1983–1992 versus 1973–1982 ($p = 0.0013$) (figure 5). This finding was also true for the subgroup of 190 patients screened at birth ($p = 0.04$).

Finally, to better evaluate the period effect, we estimated “current” survival based on the entire case series, according to three calendar periods (1973–1982, 1983–1992, and 1993–2000). As shown in figure 6, a marked improvement in survival was present in the first period but seemed to vanish in the latest decades.

DISCUSSION

Our study was conducted under probably unique conditions representing the epidemiology of cystic fibrosis in a geographic area of prolonged and intense neonatal screening and very early postnatal diagnosis. To our knowledge, it is the first survival analysis into adulthood of cystic fibrosis patients screened at birth since the early 1970s.

The estimated incidence of this disease in this region is one of the highest reported so far (8) and is higher than the overall incidence reported for Italy (9), where screening programs still have not been implemented at the national level. Since subjects were diagnosed quite early in life, it is likely that our study limited the risk of “survivor bias.” In 1993–1999, our screening program covered virtually the entire birth cohort (98.8–99.1 percent), but high screening rates were achieved only in the 1980s, approximately 10 years after implementation. This accounts for the fact that we were still diagnosing patients born in previous years. Of the 40 patients diagnosed because of symptoms or familiarity in the last 7 years considered, four had a false-negative screening result at birth.

Historically, the demographics of the Veneto and Trentino region have been stable, and no ethnic mixing has happened until recently. Some immigration is occurring now and might lead to future changes in the genetic determinants of cystic fibrosis survival. As of the end of our study, only six patients had immigrated from other part of the world, and no patient from our center migrated from or to other Italian centers. The number of patients lost to follow-up (4 percent) was also limited and should not have affected our analysis.

The median survival age (37.7 years) of our population is one of the highest reported so far and compares well with data from other industrialized countries. Fitzsimmons (2) found a median survival age of 28 years for US patients; in Canada, Corey and Farewell (3) calculated that the median survival age was 36.7 and 27.8 years in 1985–1989 for male and female patients, respectively, a strong improvement relative to 1970–1974. Data from Dodge et al. (4) showed that, in the United Kingdom, the current median survival age was 30 years in 1994. According to a study published in 1996, the current probability of surviving 40 years was 83.3 percent in Denmark (10); although this study found a dramatic improvement in survival of adults with cystic fibrosis, the findings were based on a small number of patients older than age 40 years and might be too optimistic.

As underlined by other authors (11), we confirm that calendar period is one of the best prognostic factors in cystic fibrosis. That is, patients diagnosed in more recent years live longer than those diagnosed in past decades. In our study, this finding was true even for patients screened at birth, strengthening the view that the overall approach to the disease improved survival in the last years. Various factors definitely changed over the period 1970–1980, including earlier recognition of the disease; aggressive antibiotic treatment in Pseudomonas-colonized, albeit asymptomatic patients; an aggressive nutritional approach; and noninvasive ventilation as a bridge to lung transplantation. No single procedure by itself seems to account for such improvements. However, our data and those from the United Kingdom
registry (11) indicate that the margin of improvement in the 1980s became relatively small. Several cystic-fibrosis-related complications such as diabetes, progressive liver disease and cirrhosis, and multiresistant bacteria appear during the second-to-third decade of life; thus, it is difficult to predict whether survival of adult cystic fibrosis patients will still improve.

To what extent early diagnosis influences the long-term prognosis of cystic fibrosis is still unclear. Early postnatal diagnosis or neonatal screening has been shown to improve nutritional status in infancy and early childhood, lower rates of hospitalization, and lead to better clinical scores in childhood (12–21). Importantly, on the other hand, data from the US Cystic Fibrosis registry (Wang et al. (21)) recently showed that early diagnosis (<6 weeks of age) does not influence the age of colonization by *Pseudomonas aeruginosa*, a major factor in lung disease progression. To our knowledge, data from this registry have not yet been used for long-term survival analysis.

In our population, neonatal screening was not associated with better long-term survival. However, ours was not a prospective comparative study, and our conclusion should be interpreted with caution. There have been major changes with time. The screening test adopted in the 1970s was used to test for albumin in meconium and could find only those patients whose exocrine pancreas was already insufficient at birth, a marker of worse outcome. Thus, the two groups were not strictly comparable, since genotypes with two severe mutations were found to be more frequent in the “screening” group than in the “symptoms” group (data not shown). Therefore, despite the well-known limits of the genotype-phenotype correlation, it could be argued that the “screening” group has more disadvantageous genotypes compared with the other groups. On the other hand, neonatal screening also identifies patients with mild or even asymptomatic forms, who have a better prognosis than “symptomatic” patients do.

An effect of gender on the survival of cystic fibrosis patients was identified in some (3, 4, 21) but not all (22) previous studies. Although we found a slightly higher mortality rate for prepubertal males, we could not confirm any important gender difference regarding long-term survival. We conclude that the period effect is still the strongest determinant in cystic fibrosis survival into adulthood, even in an area in which neonatal screening has been performed over the last 30 years.

REFERENCES