Long term effects of birth order and age at diagnosis in cystic fibrosis; a sibling cohort study.

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Abbreviated title: Effects of age at diagnosis in siblings with CF

Key words: cystic fibrosis, siblings, prognosis, lung function, Pseudomonas aeruginosa

ABSTRACT

Background: Siblings with cystic fibrosis (CF) share many genetic and environmental factors, but may present different phenotypes. Younger sibs are mostly earlier diagnosed with CF than their older sibs, but ~~are often infected~~ might be at risk for an earlier colonization with *P. aeruginosa* (PA) ~~at an earlier age~~ than their older counterparts due to cross-infection within families.

Aims: To analyze the effects of birth order and age at diagnosis on lung function, PA colonization, nutritional status, and survival during the first two decades of life in siblings with CF.

Methods: A retrospective cohort study of 52 sibling pairs was performed in two Dutch CF centers. Data were analyzed both cross-sectionally and longitudinally using Kaplan-Meier curves and modified log-rank tests.

Results: Median age at diagnosis was significantly higher in the older sib compared with the younger sib (3.0 and 0.2 years, respectively, P<0.0001). At the age of 5, 10 and 15 years no difference in lung function was found. However, at the age of 20 years, FEV_1 in older sibs was 19.4% (95% CI: 5.9-32.9%, P=0.007) lower than in younger sibs. In the younger sibs group, FEV_1 at age 20 years was significantly better in those who had a diagnosis before the age of 6 months (difference 22.9%, 95% CI: 0.1-45.8%, P<0.05). In the first 10 years of life the younger sibs tended to be earlier colonized with PA than their older counterparts. No differences in nutritional status and survival were observed.

Conclusion: In this sibling cohort study, an early diagnosis of CF was associated with better lung function ~~during the first~~ after two decades of life. Although younger siblings tended to be colonized with PA at an earlier age, they showed better lung function outcomes. This underscores the importance of early diagnosis with newborn screening and early referral to a specialized center in the prevention of long-term deleterious effects on lung function.

INTRODUCTION

There is considerable variation in the clinical course of disease in cystic fibrosis (CF), even in patients with the same CF genotype,[1] suggesting the influence of other factors such as environment, quality of care and modifier genes.[2-4]

Siblings with CF share the same CFTR mutations, are generally exposed to the same environment, and have similar quality of care. These factors contribute to a high concordance in CF phenotype between CF siblings.[5] However, in countries where newborn screening (NBS) is not available (as in the Netherlands), younger sibs are generally earlier diagnosed with CF than their older sibs.[5,6] Early asymptomatic diagnosis is associated with better lung function,[7-10] nutritional status,[11-14] and survival.[7,9,14-16] This would suggest an advantage for the younger sib.

On the other hand, since cross-infection is known to occur between patients with CF,[17,18] it can be expected that younger siblings may acquire human adapted respiratory pathogens at an earlier age. *P. aeruginosa* (PA) infection is associated with deteriorating lung function and increased morbidity,[19,20] which may result in a more severe CF phenotype in the younger sib.

The aim of this study was to investigate the effects of birth order and age at diagnosis on lung function, PA colonization, nutritional status, and survival in non-twin sibs with CF, aged up to 20 years.

METHODS

The study population consisted of all sibling pairs treated at the CF Centers of the University Medical Center Utrecht (Utrecht, the Netherlands) and the Haga Teaching Hospital (The Hague, the Netherlands). These centers provide care for 580 out of 1250 CF patients who are recorded in the Dutch Cystic Fibrosis Registry. This registry covers 93% of all Dutch CF-patients (www.ncfs.nl). Treatment strategies are comparable in both centers and are based on national guidelines.[21] All patients gave informed consent for the recording of their clinical data in our database and for the use of these data for scientific purposes, including descriptive studies.

In families with more than two affected siblings, only the first two siblings were included in the study. Twins were excluded. Patients born before 1970 or without clinical data before the age of 20 years available were also excluded.

All data were retrospectively collected from patient's medical charts at each center. The diagnosis of CF was confirmed in all patients with sweat testing and/or DNA analysis for CF mutations.

The primary outcome measure was lung function (forced expiratory volume in one second (FEV_1) and forced vital capacity (FVC)). Secondary outcome measures included age at diagnosis, nutritional status (height and weight), PA colonization and survival.

FEV_1 and FVC were measured by a pneumotachograph and converted to percentage of predicted values.[22,23] For cross-sectional analysis, the highest FEV_1 measure within the last year of available data for the younger sib was used. This value was matched with the highest FEV_1 of the older sibling during the year in which he/she reached the same age as the younger sib. Longitudinal measures were derived from all years of available lung function data for each study subject, using the best FEV_1 measurement per year.

Z-scores for height and weight were calculated using standard growth diagrams for the Dutch population.[24] Matching of height and BMI measures between older and younger sibs was similar to those performed for lung function. Data on lung function and nutritional status were available from 1991 onwards.

PA colonization was considered to be present when >50% of the PA cultures in the preceding year were positive.[25] In the older siblings less than 4 sputum samples per year were available. In these patients the old European consensus definition for chronic PA colonization was used, i.e. at least three positive cultures over ≥6 months with a ≥1 month interval.[26] In patients who were colonized according to this definition, we defined date of PA colonization as date of first positive PA culture plus one year (to make this date similar to the Leeds criteria). In all patients we attempted to perform a sputum culture. When a patient was not able to produce sputum, an oropharyngeal swab was taken.

Based on their functional effects, CFTR mutations were divided in 5 classes.[1] Class I, II and III mutations result in absence of or non-functional CFTR, whereas classes IV and V mutations, allowing some function of CFTR, usually result in less severe disease manifestations.[27] Patients with two class I, II or III mutations were considered to have a severe CFTR genotype, whereas patients with one or two class IV or V mutations were considered to have a mild genotype.[1]

Statistical analysis

Differences between siblings were calculated using paired Student's t-test for normally distributed continuous variables, Wilcoxon signed-rank test for not-normally distributed continuous variables, and McNemar test for discrete variables. In order to test if continuous variables were normally distributed, a Kolmogorov-Smirnov test was performed. The level of significance was 0.05.

Power and sample size were calculated using PASS 2008 (NCSS, Kaysville, UT). Sample size calculation showed that twenty sibling pairs were needed to detect a 20% difference in lung function with an estimated SD of the difference in lung function of 30%, an alpha of 0.05 and a power of 0.8.

Kaplan-Meier curves and modified log rank tests were used to assess whether there were differences in survival and PA colonization by birth order. Specifically, a Cox proportional hazards model (the probability model implicit in the log rank test) was used, with a term denoting sibling pairs, to test for the significance of birth order in the model. This model takes into account the paired nature of the siblings when calculating the standard error for the birth order coefficient.

RESULTS

In total, 52 families with at least one sibling pair with CF met the inclusion criteria. The mean age difference between the two siblings was 3.7 ± 2.2 years.

Patient characteristics are shown in Table 1. Median age at diagnosis was significantly higher in the older sib compared with the younger sib (3.0 and 0.2 years, respectively, P<0.0001).

In 37 pairs (71.2%) the older sib was diagnosed first, in 11 pairs (21.2%) the younger sib was diagnosed first, and in 4 pairs (7.7%) sibs were diagnosed at the same time. Eight of the younger sibs (15.4%) were diagnosed antenatally or shortly after birth before the development of symptoms because of CF in the older sib. Both the first and second diagnosed sibs were immediately followed in a CF center from the diagnosis. ~~PA colonization in first and second diagnosed sibs was 12.5% and 8.3%, respectively.~~ Sputum cultures at diagnosis in first and second diagnosed sibs were positive for PA in 12.5% and 8.3% of the patients, respectively (McNemar test P=0.63).

Older sibs tended to have respiratory symptoms at diagnosis more often (55.8% and 38.5%, respectively, P=0.12) compared with younger sibs (Table 1).

In four sibling pairs no CFTR genotype was determined. Of the remaining 48 patient pairs, 39 (81.3%) had a severe CFTR genotype class.

Lung function testing was performed in 47 sibling pairs. Lung function at last clinical visit for the younger sib tended to be higher than the matched lung function for the older sib (Table 2). FEV_1 was analyzed per 5-year intervals. Unfortunately, for some sibling pairs lung function data were not available at all investigated ages because they had not reached that age or because they were first treated at another center. At the age of 5, 10, 15 and 20 years, for 15, 21, 22 and 22 patients pairs lung function data were available, respectively. FEV_1 at the age of 5, 10 and 15 years was not significantly different between the sibs. However, at the age of 20 years, FEV_1 in older sibs was 19.4% (95% CI: 5.9-32.9%, P=0.007) lower than in younger sibs (Figure 1). At this age, 4 of the older sibs had a better and 18 had a poorer lung function compared to their younger sibs (Wilcoxon signed rank test P=0.007).

In order to test the hypothesis that the better clinical outcome in the younger sibs is caused by earlier diagnosis of CF, we analyzed the effect of age at diagnosis on lung function in the younger sibs using an unpaired Student's t-test. Since the association between age at diagnosis and lung function could be confounded by severity of CFTR genotype,[28] this analysis was only performed in patients with a severe CFTR genotype. FEV_1 analyzed per 5-year intervals demonstrated a significantly higher FEV_1 at the age of 20 years in sibs diagnosed

before 6 months of age compared with those diagnosed after 6 months of age (difference 22.9%, 95% CI: 0.1-45.8%, P<0.05). However, no significant differences were observed at the age of 5, 10 and 15 years.

PA colonization at last visit was not different between the older and younger sibs (Table 2). Also, when differences in age at PA colonization between younger and older sibs were analyzed longitudinally, younger sibs were overall not earlier colonized with PA (Cox proportional hazard 1.60, 95% CI: -0.73-3.52). However, the younger sibs tended to be earlier colonized in the first 10 years of life than their older counterparts (P=0.08), but this difference disappeared in the second decade (Figure 2). Height, weight and BMI did not differ between the older and the younger sibs (Table 2).

Two of the older sibs and none of the younger sibs received a lung transplant before the age of 25 years. Three of the older sibs and one of the younger sibs died before the age of 25 years. ~~Survival curves for older and younger sibs are displayed in Figure 3.~~ Survival (no death and no lung transplant) was not significantly worse in the older sibs compared with the younger sibs (Cox proportional hazard: P=0.21).

DISCUSSION

This study on long-term differences in lung function, PA colonization, nutritional status, and survival between older and younger sibs with CF demonstrated that older siblings had a poorer lung function in the second decade of life compared to their younger siblings with CF. Early diagnosis was associated with better lung function outcome. PA colonization was not significantly different between younger and older sibs, but younger sibs tended to be earlier colonized in the first 10 years of life. Older and younger siblings did not appear to have a different height, weight and survival.

In this study, we hypothesized that younger siblings might be at risk of more accelerated lung disease because of exposure to cross-infection from older siblings. On the other hand, we postulated that younger sibs would be earlier diagnosed and would therefore benefit from therapy earlier in life. It was shown that younger sibs tended to be earlier colonized with PA in the first 10 years of life, but that this difference disappeared in the second decade. Despite the higher PA colonization in the first decade, this did not result in a significantly worse lung function in the first decade, probably due to an earlier diagnosis. In the second decade no difference between PA colonization was found between the sibs, and younger sibs had better lung function. This suggests that the net effect of earlier diagnosis in younger CF siblings outweighs the negative effects, including the exposure to CF pathogens from older siblings.

The beneficial effect of younger age at diagnosis on lung function was confirmed by our subgroup analysis in the younger sibs: patients diagnosed before 6 months of age had a significantly better lung function in the second decade of life. This is in accordance with the better prognosis associated with early asymptomatic diagnosis of CF through NBS.[7-10,29] In these early diagnosed asymptomatic patients, early antibiotic therapy, nutritional supplementation, and intensive physiotherapy can delay the progression of lung disease in CF.[29]

A better lung function in younger siblings with CF was also found in a relatively old and small study by Orenstein *et al.*[30] On the other hand, two recent studies could not find significant differences in FEV_1 and FVC between older and younger sibs with CF.[5,6] However, lung function was only measured at the age of 7 and 10 years,[5] and at the age of 8 years,[6] respectively. The age of 10 years may be too young to identify significant differences between the two siblings, which was demonstrated by our longitudinal analysis (difference only found in second decade of life).

Similar to other studies,[5,6] we did find a strong familial tendency for PA colonization. This was probably caused by cross-infections between the siblings. The importance of cross-infections among CF patients within families has already been confirmed by DNA fingerprinting and serology.[18,31] In accordance with other studies,[5,6] we

demonstrated an earlier age at PA colonization in the younger siblings than in the older siblings. However, in all studies the interpretation is limited by the age-of-diagnosis bias. Patients are only tested for typical CF pathogens if they already have a CF diagnosis. Therefore, a positive culture at time of diagnosis prevents accurate determination of true age of infection. Since the older child in a family is always diagnosed at an older age than the younger sibling, regardless of who is the index case, older siblings are more likely to have positive cultures at time of diagnosis, simply due to more time to acquire the pathogens. The age-of-diagnosis bias could only be overcome by a study of CF siblings diagnosed through NBS, but NBS is not yet available in the Netherlands.

The proportion of patients chronically colonized by PA according was rather high for the age of the patients. The high proportion of chronic PA colonization was probably due to the fact that early eradication protocols for PA were introduced only some years ago. Until 4 years ago, there was no segregation policy in The Netherlands and many children visited CF summer camps, which may have contributed to the relatively high PA rates in our cohort.

Since the proportion of patients chronically colonized by PA in this study was high for age, one could speculate that patients who are early diagnosed and consequently early followed in CF centers are at risk of early acquisition of bacterial pathogens, such as PA. However, different recent studies could not demonstrate an association between early diagnosis (and early follow-up in a CF center) and early infection with PA when good infection control practices are followed.[32,33]

In the younger children the definition of chronic colonization by PA were based on Leeds criteria.[25] The validity of this definition has recently been confirmed.[34] The minimum sample number needed to use the Leeds criteria for a given year should be four. Unfortunately, in the past less than 4 sputum cultures per year were performed in our patients and, consequently, the Leeds criteria could not be used in the older siblings. This could have led to under-detection of the PA colonization. The effect on the outcome of our study is probably limited, since the same definition was used within siblings pairs.

Similar to other sibling studies,[5,6] we did not demonstrate any significant differences in height or BMI between older and younger sibs, although one of the earlier sibling studies demonstrated a marginally significantly higher height z-score in younger siblings than in older siblings.[6] Other studies[13,29,35] have shown dramatic differences in nutritional status at diagnosis between those diagnosed through screening and those diagnosed as a result of clinical signs and symptoms. However, these differences can be reduced once appropriate dietary intervention, including the provision of pancreatic enzyme replacement therapy, is commenced.[13] That we could not detect any advantage of early diagnosis on nutritional status in younger sibs might be because of a significant catch-up

growth achieved in the early years of life. Finally, in accordance with our study but in contrast with NBS studies, a recent Belgian study could not find significant differences in anthropometric parameters between children who were early and late (<2 and ≥2 years after diagnosis) referred to a CF center. [36]

We found no significant differences in survival between older and younger sibs, but the number of patients investigated may have been too small to detect these differences. Other sibling studies did not investigate differences in survival between older and younger sibs.[5,6,30] However, most of the studies on the effect of NBS found that early diagnosis through NBS is associated with improved child survival.[7,9,14-16]

Although this is the largest sibling study so far on the effect of birth order on lung function in CF patients, our study was limited by the population size. However, power analysis revealed that the number of sibling pairs included was sufficient to detect a 20% difference in lung function between the siblings.

Another limitation may have been the influence of time. Treatment regimes for sibling pairs born in the early seventies were different than the ones for sibling pairs born in the nineties. These differences include improved nutritional management and dietary recommendations, new airway clearance techniques and new antipseudomonal antibiotics.[37] One could hypothesize that having a older sib could have resulted in poorer lung function in younger sibs born in the seventies compared to those born in the nineties because of new antipseudomonal drugs. Unfortunately, the size of our population did not allow us to analyze the influence of date of birth on outcome.

In conclusion, in this sibling cohort study, an early diagnosis of CF was associated with better lung function ~~during the first~~ after two decades of life. Although younger siblings tended to be colonized with PA at an earlier age, they showed better lung function outcomes. This underscores the importance of early diagnosis with newborn screening in the prevention of long-term deleterious effects on lung function.

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ABSTRACT

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Conclusion: In this sibling cohort study, an early diagnosis of CF was associated with better lung function after two decades of life. Although younger siblings tended to be colonized with PA at an earlier age, they showed better lung function outcomes. This underscores the importance of early diagnosis with newborn screening and early referral to a specialized center in the prevention of long-term deleterious effects on lung function.

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There is considerable variation in the clinical course of disease in cystic fibrosis (CF), even in patients with the same CF genotype,[1] suggesting the influence of other factors such as environment, quality of care and modifier genes.[2-4]

Siblings with CF share the same CFTR mutations, are generally exposed to the same environment, and have similar quality of care. These factors contribute to a high concordance in CF phenotype between CF siblings.[5] However, in countries where newborn screening (NBS) is not available (as in the Netherlands), younger sibs are generally earlier diagnosed with CF than their older sibs.[5,6] Early asymptomatic diagnosis is associated with better lung function,[7-10] nutritional status,[11-14] and survival.[7,9,14-16] This would suggest an advantage for the younger sib.

On the other hand, since cross-infection is known to occur between patients with CF,[17,18] it can be expected that younger siblings may acquire human adapted respiratory pathogens at an earlier age. *P. aeruginosa* (PA) infection is associated with deteriorating lung function and increased morbidity,[19,20] which may result in a more severe CF phenotype in the younger sib.

The aim of this study was to investigate the effects of birth order and age at diagnosis on lung function, PA colonization, nutritional status, and survival in non-twin sibs with CF, aged up to 20 years.

METHODS

The study population consisted of all sibling pairs treated at the CF Centers of the University Medical Center Utrecht (Utrecht, the Netherlands) and the Haga Teaching Hospital (The Hague, the Netherlands). These centers provide care for 580 out of 1250 CF patients who are recorded in the Dutch Cystic Fibrosis Registry. This registry covers 93% of all Dutch CF-patients (www.ncfs.nl). Treatment strategies are comparable in both centers and are based on national guidelines.[21] All patients gave informed consent for the recording of their clinical data in our database and for the use of these data for scientific purposes, including descriptive studies.

In families with more than two affected siblings, only the first two siblings were included in the study. Twins were excluded. Patients born before 1970 or without clinical data before the age of 20 years available were also excluded.

All data were retrospectively collected from patient's medical charts at each center. The diagnosis of CF was confirmed in all patients with sweat testing and/or DNA analysis for CF mutations.

The primary outcome measure was lung function (forced expiratory volume in one second (FEV_1) and forced vital capacity (FVC)). Secondary outcome measures included age at diagnosis, nutritional status (height and weight), PA colonization and survival.

FEV_1 and FVC were measured by a pneumotachograph and converted to percentage of predicted values.[22,23] For cross-sectional analysis, the highest FEV_1 measure within the last year of available data for the younger sib was used. This value was matched with the highest FEV_1 of the older sibling during the year in which he/she reached the same age as the younger sib. Longitudinal measures were derived from all years of available lung function data for each study subject, using the best FEV_1 measurement per year.

Z-scores for height and weight were calculated using standard growth diagrams for the Dutch population.[24] Matching of height and BMI measures between older and younger sibs was similar to those performed for lung function. Data on lung function and nutritional status were available from 1991 onwards.

PA colonization was considered to be present when >50% of the PA cultures in the preceding year were positive.[25] In the older siblings less than 4 sputum samples per year were available. In these patients the old European consensus definition for chronic PA colonization was used, i.e. at least three positive cultures over ≥6 months with a ≥1 month interval.[26] In patients who were colonized according to this definition, we defined date of PA colonization as date of first positive PA culture plus one year (to make this date similar to the Leeds criteria). In all patients we attempted to perform a sputum culture. When a patient was not able to produce sputum, an oropharyngeal swab was taken.

Based on their functional effects, CFTR mutations were divided in 5 classes.[1] Class I, II and III mutations result in absence of or non-functional CFTR, whereas classes IV and V mutations, allowing some function of CFTR, usually result in less severe disease manifestations.[27] Patients with two class I, II or III mutations were considered to have a severe CFTR genotype, whereas patients with one or two class IV or V mutations were considered to have a mild genotype.[1]

Statistical analysis

Differences between siblings were calculated using paired Student's t-test for normally distributed continuous variables, Wilcoxon signed-rank test for not-normally distributed continuous variables, and McNemar test for discrete variables. In order to test if continuous variables were normally distributed, a Kolmogorov-Smirnov test was performed. The level of significance was 0.05.

Power and sample size were calculated using PASS 2008 (NCSS, Kaysville, UT). Sample size calculation showed that twenty sibling pairs were needed to detect a 20% difference in lung function with an estimated SD of the difference in lung function of 30%, an alpha of 0.05 and a power of 0.8.

Kaplan-Meier curves and modified log rank tests were used to assess whether there were differences in survival and PA colonization by birth order. Specifically, a Cox proportional hazards model (the probability model implicit in the log rank test) was used, with a term denoting sibling pairs, to test for the significance of birth order in the model. This model takes into account the paired nature of the siblings when calculating the standard error for the birth order coefficient.

RESULTS

In total, 52 families with at least one sibling pair with CF met the inclusion criteria. The mean age difference between the two siblings was 3.7 ± 2.2 years.

Patient characteristics are shown in Table 1. Median age at diagnosis was significantly higher in the older sib compared with the younger sib (3.0 and 0.2 years, respectively, P<0.0001).

In 37 pairs (71.2%) the older sib was diagnosed first, in 11 pairs (21.2%) the younger sib was diagnosed first, and in 4 pairs (7.7%) sibs were diagnosed at the same time. Eight of the younger sibs (15.4%) were diagnosed antenatally or shortly after birth before the development of symptoms because of CF in the older sib. Both the first and second diagnosed sibs were immediately followed in a CF center from the diagnosis. Sputum cultures at diagnosis in first and second diagnosed sibs were positive for PA in 12.5% and 8.3% of the patients, respectively (McNemar test P=0.63).

Older sibs tended to have respiratory symptoms at diagnosis more often (55.8% and 38.5%, respectively, P=0.12) compared with younger sibs (Table 1).

In four sibling pairs no CFTR genotype was determined. Of the remaining 48 patient pairs, 39 (81.3%) had a severe CFTR genotype class.

Lung function testing was performed in 47 sibling pairs. Lung function at last clinical visit for the younger sib tended to be higher than the matched lung function for the older sib (Table 2). FEV_1 was analyzed per 5-year intervals. Unfortunately, for some sibling pairs lung function data were not available at all investigated ages because they had not reached that age or because they were first treated at another center. At the age of 5, 10, 15 and 20 years, for 15, 21, 22 and 22 patients pairs lung function data were available, respectively. FEV_1 at the age of 5, 10 and 15 years was not significantly different between the sibs. However, at the age of 20 years, FEV_1 in older sibs was 19.4% (95% CI: 5.9-32.9%, P=0.007) lower than in younger sibs (Figure 1). At this age, 4 of the older sibs had a better and 18 had a poorer lung function compared to their younger sibs (Wilcoxon signed rank test P=0.007). In order to test the hypothesis that the better clinical outcome in the younger sibs is caused by earlier diagnosis of CF, we analyzed the effect of age at diagnosis on lung function in the younger sibs using an unpaired Student's t-test. Since the association between age at diagnosis and lung function could be confounded by severity of CFTR genotype,[28] this analysis was only performed in patients with a severe CFTR genotype. FEV_1 analyzed per 5-year intervals demonstrated a significantly higher FEV_1 at the age of 20 years in sibs diagnosed before 6 months of age compared with those diagnosed after 6 months of age (difference 22.9%, 95% CI: 0.1-45.8%, P<0.05). However, no significant differences were observed at the age of 5, 10 and 15 years.

PA colonization at last visit was not different between the older and younger sibs (Table 2). Also, when differences in age at PA colonization between younger and older sibs were analyzed longitudinally, younger sibs were overall not earlier colonized with PA (Cox proportional hazard 1.60, 95% CI: -0.73-3.52). However, the younger sibs tended to be earlier colonized in the first 10 years of life than their older counterparts (P=0.08), but this difference disappeared in the second decade (Figure 2). Height, weight and BMI did not differ between the older and the younger sibs (Table 2).

Two of the older sibs and none of the younger sibs received a lung transplant before the age of 25 years. Three of the older sibs and one of the younger sibs died before the age of 25 years. Survival (no death and no lung transplant) was not significantly worse in the older sibs compared with the younger sibs (Cox proportional hazard: P=0.21).

DISCUSSION

This study on long-term differences in lung function, PA colonization, nutritional status, and survival between older and younger sibs with CF demonstrated that older siblings had a poorer lung function in the second decade of life compared to their younger siblings with CF. Early diagnosis was associated with better lung function outcome. PA colonization was not significantly different between younger and older sibs, but younger sibs tended to be earlier colonized in the first 10 years of life. Older and younger siblings did not appear to have a different height, weight and survival.

In this study, we hypothesized that younger siblings might be at risk of more accelerated lung disease because of exposure to cross-infection from older siblings. On the other hand, we postulated that younger sibs would be earlier diagnosed and would therefore benefit from therapy earlier in life. It was shown that younger sibs tended to be earlier colonized with PA in the first 10 years of life, but that this difference disappeared in the second decade. Despite the higher PA colonization in the first decade, this did not result in a significantly worse lung function in the first decade, probably due to an earlier diagnosis. In the second decade no difference between PA colonization was found between the sibs, and younger sibs had better lung function. This suggests that the net effect of earlier diagnosis in younger CF siblings outweighs the negative effects, including the exposure to CF pathogens from older siblings.

The beneficial effect of younger age at diagnosis on lung function was confirmed by our subgroup analysis in the younger sibs: patients diagnosed before 6 months of age had a significantly better lung function in the second decade of life. This is in accordance with the better prognosis associated with early asymptomatic diagnosis of CF through NBS.[7-10,29] In these early diagnosed asymptomatic patients, early antibiotic therapy, nutritional supplementation, and intensive physiotherapy can delay the progression of lung disease in CF.[29]

A better lung function in younger siblings with CF was also found in a relatively old and small study by Orenstein *et al.*[30] On the other hand, two recent studies could not find significant differences in FEV_1 and FVC between older and younger sibs with CF.[5,6] However, lung function was only measured at the age of 7 and 10 years,[5] and at the age of 8 years,[6] respectively. The age of 10 years may be too young to identify significant differences between the two siblings, which was demonstrated by our longitudinal analysis (difference only found in second decade of life).

Similar to other studies,[5,6] we did find a strong familial tendency for PA colonization. This was probably caused by cross-infections between the siblings. The importance of cross-infections among CF patients within families has already been confirmed by DNA fingerprinting and serology.[18,31] In accordance with other studies,[5,6] we

demonstrated an earlier age at PA colonization in the younger siblings than in the older siblings. However, in all studies the interpretation is limited by the age-of-diagnosis bias. Patients are only tested for typical CF pathogens if they already have a CF diagnosis. Therefore, a positive culture at time of diagnosis prevents accurate determination of true age of infection. Since the older child in a family is always diagnosed at an older age than the younger sibling, regardless of who is the index case, older siblings are more likely to have positive cultures at time of diagnosis, simply due to more time to acquire the pathogens. The age-of-diagnosis bias could only be overcome by a study of CF siblings diagnosed through NBS, but NBS is not yet available in the Netherlands.

The proportion of patients chronically colonized by PA according was rather high for the age of the patients. The high proportion of chronic PA colonization was probably due to the fact that early eradication protocols for PA were introduced only some years ago. Until 4 years ago, there was no segregation policy in The Netherlands and many children visited CF summer camps, which may have contributed to the relatively high PA rates in our cohort.

Since the proportion of patients chronically colonized by PA in this study was high for age, one could speculate that patients who are early diagnosed and consequently early followed in CF centers are at risk of early acquisition of bacterial pathogens, such as PA. However, different recent studies could not demonstrate an association between early diagnosis (and early follow-up in a CF center) and early infection with PA when good infection control practices are followed.[32,33]

In the younger children the definition of chronic colonization by PA were based on Leeds criteria.[25] The validity of this definition has recently been confirmed.[34] The minimum sample number needed to use the Leeds criteria for a given year should be four. Unfortunately, in the past less than 4 sputum cultures per year were performed in our patients and, consequently, the Leeds criteria could not be used in the older siblings. This could have led to under-detection of the PA colonization. The effect on the outcome of our study is probably limited, since the same definition was used within siblings pairs.

Similar to other sibling studies,[5,6] we did not demonstrate any significant differences in height or BMI between older and younger sibs, although one of the earlier sibling studies demonstrated a marginally significantly higher height z-score in younger siblings than in older siblings.[6] Other studies[13,29,35] have shown dramatic differences in nutritional status at diagnosis between those diagnosed through screening and those diagnosed as a result of clinical signs and symptoms. However, these differences can be reduced once appropriate dietary intervention, including the provision of pancreatic enzyme replacement therapy, is commenced.[13] That we could not detect any advantage of early diagnosis on nutritional status in younger sibs might be because of a significant catch-up

growth achieved in the early years of life. Finally, in accordance with our study but in contrast with NBS studies, a recent Belgian study could not find significant differences in anthropometric parameters between children who were early and late (<2 and ≥2 years after diagnosis) referred to a CF center. [36]

We found no significant differences in survival between older and younger sibs, but the number of patients investigated may have been too small to detect these differences. Other sibling studies did not investigate differences in survival between older and younger sibs.[5,6,30] However, most of the studies on the effect of NBS found that early diagnosis through NBS is associated with improved child survival.[7,9,14-16]

Although this is the largest sibling study so far on the effect of birth order on lung function in CF patients, our study was limited by the population size. However, power analysis revealed that the number of sibling pairs included was sufficient to detect a 20% difference in lung function between the siblings.

Another limitation may have been the influence of time. Treatment regimes for sibling pairs born in the early seventies were different than the ones for sibling pairs born in the nineties. These differences include improved nutritional management and dietary recommendations, new airway clearance techniques and new antipseudomonal antibiotics.[37] One could hypothesize that having a older sib could have resulted in poorer lung function in younger sibs born in the seventies compared to those born in the nineties because of new antipseudomonal drugs. Unfortunately, the size of our population did not allow us to analyze the influence of date of birth on outcome.

In conclusion, in this sibling cohort study, an early diagnosis of CF was associated with better lung function after two decades of life. Although younger siblings tended to be colonized with PA at an earlier age, they showed better lung function outcomes. This underscores the importance of early diagnosis with newborn screening in the prevention of long-term deleterious effects on lung function.

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Table 1. Baseline demographics of the study population.

Variable	Younger sib	Older sib	P-value[a]
N	52	52	
Males (%)	21 (40.4)	20 (38.5)	1.00
Pancreatic insufficiency (%)	48 (92.3)	48 (92.3)	1.00
Age at diagnosis (yrs), median (range)	0.2 (0.0-10.7)	3.0 (0.0-13.7)	<0.0001
Presenting symptoms of CF:			
Meconium ileus (%)	11 (21.2)	8 (15.4)	0.51
Failure to thrive (%)	22 (42.3)	26 (50.0)	0.50
Respiratory symptoms (%)	20 (38.5)	29 (55.8)	0.12
Genotype:			
Homozygous ΔF508 (%)	28 (53.8)		
Heterozygous ΔF508 (%)	15 (28.8)		
Other (%)	5 (9.6)		
Not determined (%)	4 (7.7)		
Genotype classes:			
Class I-III (%)[b]	39 (75.0)		
Class IV-V (%)[c]	9 (17.3)		
Not determined (%)	4 (7.7)		

[a]From Wilcoxon signed rank test for age at diagnosis, McNemar test for all other analyses. [b]Both alleles belonging to class I – III. [c]At least one allele belonging to class IV or V.

Table 2. Disease severity outcome measures at the last visit for the younger sib and at the visit for the older sib at which he/she had the same age as the younger sib. Data are presented as mean (SD), unless otherwise specified.

	Younger sib	Older sib	P-value[a]	Younger sib higher (N)[b]	Older sib higher (N)	P-value[c]
Age, years (SD)	15.2 (6.5)	15.2 (6.5)	1.00			
FEV_1, % predicted (SD)[d]	81.3 (22.9)	74.2 (32.4)	0.11	30	17	0.15
FVC, % predicted (SD)[d]	88.5 (17.6)	82.0 (25.8)	0.11	28	19	0.11
FEV_1/FVC (SD)[d]	77.2 (13.2)	73.7 (13.7)	0.08	28	19	0.10
P. aeruginosa colonization (%)	33 (63.5)	34 (65.4)	1.00			
Height for age, z-score (SD)	-0.76 (0.98)	-0.61 (1.13)	0.31	20	32	0.27
Weight for height, z-score (SD)	-0.23 (0.92)	-0.12 (1.17)	0.55	25	27	0.80
BMI, kg/m^2 (SD)	18.6 (2.8)	18.8 (3.1)	0.66	24	28	0.96

BMI, body mass index; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity.

[a]From McNemar test for PA colonization, paired t-test for all other analyses. [b]Column shows number of patients having a higher value than their older sib. [c]Wilcoxon signed rank test. [d]N=47.

Figure legends

Figure 1. Lung function measured at five-year intervals. At the age of 5, 10, 15 and 20 years, for 15, 21, 22 and 22 patients pairs lung function data were available, respectively. Bold line indicates mean FEV_1. FEV_1 at the age of 20 years was significantly lower in older siblings compared with their younger counterparts (difference 19.4%, 95% confidence interval: 5.9–32.9, P=0.007).

Figure 2. Kaplan-Meier curves of age at PA colonization by birth order, measured in 39 sibling pairs. Cox proportional hazard P=0.24.

Figure legends

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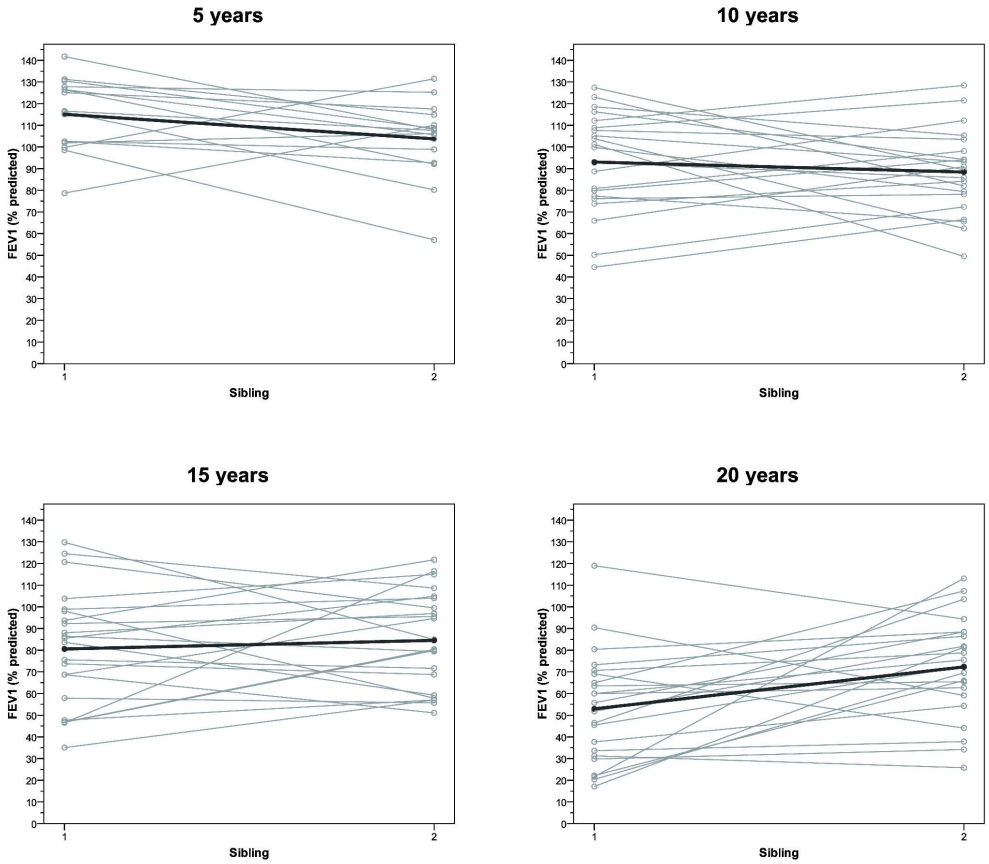

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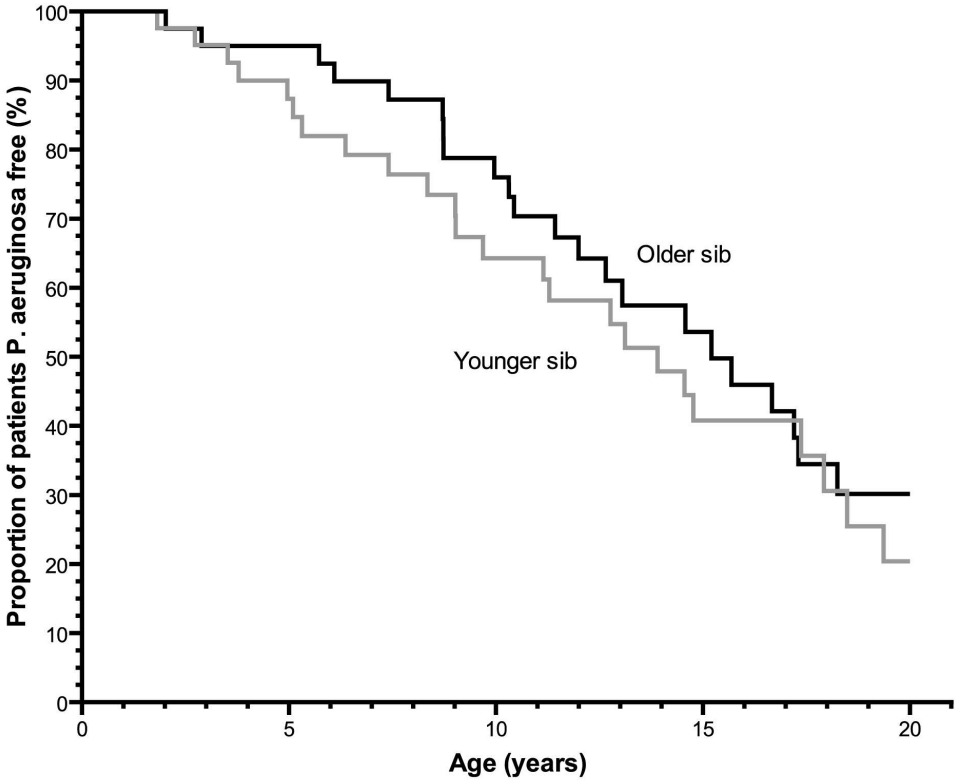

Figure 2. Kaplan-Meier curves of age at PA colonization by birth order, measured in 39 sibling pairs. Cox proportional hazard P=0.24.
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