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Original Article

The natural history of cystic fibrosis liver disease a prospective cohort study

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ABSTRACT

Background: Our understanding of the natural history of cystic fibrosis liver disease (CFLD) is limited, leading to uncertainty for patients their families and clinicians when liver abnormalities are identified.
Aim: to determine the incidence of CFLD, identify risk factors and document the natural history of liver abnormalities in cystic fibrosis (CF).
Methods: The Irish longitudinal study of CFLD (ILSCFLD) prospectively enrolled 95% of children with CF in 2007. Their liver disease status was classified as (i) advanced liver disease with portal hypertension (CFLD). (ii) nonspecific cystic fibrosis liver disease (NSCFLD) (iii) no liver disease (NoLD)
Results: 480/522 (91.9%) children were followed for a median 8.53 years IQR 1.28, of whom 35 (7.29%) had CFLD, 110 (22.9%) NSCFLD and 335 (69.79%) had NoLD. At follow-up 28/445 (6.29%) participants without CFLD at baseline, progressed to CFLD (Incidence 7.51/1000 person years (Pyrs) (95%CI 4.99–10.86). Of these 25/28(89.28%) were <10 years. No participant >10 years of age without clinical or radiological evidence of liver disease at baseline progressed to CFLD.
During follow-up 18/35(51.43%) participants with CFLD died or received a transplant, MTx rate 7.75/100 Pyrs (95%CI 4.59–12.25) compared to NSCFLD 2.33/100 Pyrs (95%CI 1.44–3.56) and NoLD 1.13/100 Pyrs (95%CI 0.77–1.59). CFLD was an independent risk factor for mortality in CF. Children with CFLD also had a shorter life expectancy.
Conclusion: The incidence of CFLD was highest in children under10 years. Children over10 years, with normal hepatic function did not develop CFLD. Research to identify the cause and improve outcome should focus on young children.

1. Background

Recent advances in clinical care and new modulator therapies have dramatically improved both quality of life and survival for persons with cystic fibrosis (CF) [1,2],

While liver disease is identified as a significant and sometimes lethal complication of CF, its aetiology is unknown and natural history poorly defined [1,3,4]. Many patients with CF who are found to have

Abbreviation: ALT, Alanine aminotransferase; ALk Phos, Alkaline phosphatase; APRI, ALT to Platelet ratio; AST, Aspartate aminotransferase; BMI, Body mass index; CI, Confidence interval; CF, Cystic fibrosis; CFLD, Cystic fibrosis liver disease; FEV_1, Forced expiratory volume in 1 s; GGT, Gamma glutamyltranspepidase; GPR, GGT to Platelet ratio; ILSCFLD, Irish Longitudinal Study on Cystic Fibrosis Liver Disease; MI, Meconium ileus; NACFF, North American Cystic Fibrosis Foundation; NoLD, No liver disease; NSCFLD, Nonspecific cystic fibrosis liver disease; Pyrs, Person years; SD, Standard deviation; SDS, Standard deviation score.

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biochemical, or radiological abnormalities will not progress to clinically significant liver disease [1,3]. However, a small percentage will develop severe liver disease with portal hypertension. This marked difference in outcome presents a fundamental challenge for clinicians attempting to interpret for patients with CF the clinical impact of radiological or biochemical abnormalities in liver function when they first occur. Determining the age of onset and the incidence of CFLD has always been problematic because of its insidious onset, and the high prevalence of transient liver test abnormalities in children [5] which are thought to reflect intercurrent infection rather than overt liver disease. While the development of the optimal non-invasive test for the diagnosis and monitoring progressive liver disease remains a research priority, significant advances have been made in developing biomarkers of CFLD [6,7] and radiological predictors of advanced liver disease [8]

Building from our previous work [9,10] we established a new national cohort study in 2007 called the Irish Longitudinal Study on Cystic Fibrosis Liver Disease (ILSCFLD). This is a national longitudinal study of persons with CF in which 95% of eligible participants attending paediatric CF centres in the Republic of Ireland in 2007 were enrolled [11]. The aims of this cohort study were threefold: to prospectively determine the incidence of CFLD, identify risk factors for the development liver disease in CF and document the natural history of this condition.

2. Methods

2.1. Study design setting and oversight

This study was approved by the research ethics committee of institutions providing care for persons with CF. (Supplementary Table 1). Parents consented to their child's participation while children under 18 years of age were invited to assent to the study. When participants transitioned to adult care they provided written consent to their ongoing participation in the study.

2.2. Study population

Children with CF under 18 years of age attending any paediatric CF centre (Supplementary Table 1) in 2007 were prospectively enrolled in this study and had a comprehensive assessment of their liver disease status once every five years. To date three cycles of data collection have been completed centred around the years 2007, 2012 and 2017, each taking place over an 18 month period with additional time required for classification of participants liver disease status. The Covid-19 pandemic delayed finalisation of the data and manuscript preparation.

Children with a sweat chloride $>$ 60 mmol/L or in the absence of a sweat test result two disease causing CFTR genetic mutations were enrolled. Participants with other liver diseases, complex genetic or metabolic disorders in association with CF were excluded from the analysis of the outcome of CFLD.

2.3. Data collection

As this was a national prospective study CF specialist centres were not required to standardise diagnostic investigations or patient care pathways. All serum derived parameters were determined by standard tests in the laboratory used by each hospital. While there was some variation between CF centres in the range of laboratory and radiological tests performed, or the age investigations were introduced all centres provided care in accordance with best practice guidelines for persons with CF [12]. If laboratory or radiological data for a cycle year were not available, the nearest available year +/- 1was collected.

A data collection pro forma was used to collect details on regularly used medication, days missed from school/work due to CF, together with hospital admissions for CF and the number of courses of IV antibiotics in the previous 12 months. Laboratory data included haemoglobin, white cell count, platelet count, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyltranspepidase (GGT), bilirubin, albumin and fat soluble vitamins. Abnormal laboratory results were recorded only when at least two such abnormalities were observed in the absence of intercurrent illness.

We used 1.5 times the ULN of liver tests to classify liver disease status [13,14]. In addition we calculated GGT to platelet ratio (GPR) and AST to platelet ratio APRI based on upper limits of normal in healthy children [6,7,15]

Forced expiratory volume in 1 second (FEV_1) in children $>$7 who were clinically stable was converted to standard deviation scores (SDS) using the Global Lung Initiative calculator [16] (http://gli-calculator.ersnet.org/index.html)

Ultrasound data were abstracted from radiological reports as follows: liver consistency was classified as homogenous, or heterogeneous with normal or increased echogenicity [17]. Liver contour was characterised as normal or nodular [17]. Where available, liver size was recorded as normal or increased, and spleen length in centimetres was converted to SDS score for children up to 17 years of age using https://radiology-universe.org/calculator/paediatric-spleen-sizes/calculator.php [18].

Where there was uncertainty as to the correct phenotypic classification participants' liver disease statuses they were invited to have a clinical examination for research purposes by experienced paediatric (BB) or adult hepatologist (PAMcC). This determined the most appropriate classification for participants based on the case definition and the hepatologist expert opinion. Where there was uncertainty about the evidence for PH, a nonspecific classification was assigned.

2.4. Phenotypic classification of cystic fibrosis liver disease

The classification of CFLD used in the ILSCFLD is consistent with the North American Cystic Fibrosis Foundation Classification [13], and the Eurocare CF guidelines [14] with some minor modifications in terminology. We used the terms (i) cystic fibrosis liver disease (CFLD) which included those with portal hypertension (PH) or advanced liver disease (ii) nonspecific cystic fibrosis liver disease (NSCFLD) to define those with indeterminate findings that did not meet the criteria for PH. We used the term NSCLFD rather than mild or moderate disease because the long term implications of any liver test abnormality are unknown. Those with normal liver function we classified as having no liver disease (NoLD)

2.5. Criteria for phenotypic classification of liver disease in CF

2.5.1. Cystic fibrosis liver disease (CFLD)

Participants with CFLD had either clinical, radiological or histological evidence of liver disease. Clinical evidence of CFLD was defined as (a) palpable firm liver on clinical examination, (b) in the absence of a firm liver, a clinically enlarged spleen, with or without hypersplenism, having ruled out of other causes of splenomegaly or portal hypertension.

Radiological evidence of CFLD was defined as evidence of portal hypertension with a spleen size $\geq$ 2 standard deviations (SD) above the mean for age, or a spleen size greater than 13 cm in those over 17 years of age [18]. Ultrasonographic liver parenchymal abnormalities alone, while suggestive of CFLD were not included in the definition of CFLD.

Histological evidence of CFLD was confirmed if participants had characteristic evidence of CF-associated hepatobiliary fibrosis on biopsy.

2.5.2. Non specific cystic fibrosis liver disease (NSCFLD)

Participants were classified as NSCFLD if they had clinical, radiological or biochemical liver abnormalities but which did not meet the diagnostic criteria for PH. Clinical characteristics included a palpable soft liver, which was clinically different in nature from the characteristic firm liver of CFLD. Radiological parameters included changes in the appearance of the liver on ultrasound that did not meet the criteria for portal hypertension. Biochemical evidence of liver disease included

persistent abnormalities of liver biochemistry ≥ 1.5 times the upper limit of normal for age and gender [13].

2.5.3. No liver disease (NoLD)

Participants with NoLD had no clinical, radiological or biochemical abnormalities consistent with NSCFLD or CFLD.

Where there was any uncertainty as to the correct phenotype of participants, 2 experienced hepatologist were the final arbiters of the participants liver disease classification.

2.6. Statistical analysis

Continuous variables were reported as median with interquartile range (IQR) and compared using one-way analysis of variance (ANOVA). Categorical variables were presented as numbers and percentages and compared using chi-squared tests, and presented as risk difference.

2.7. Incidence rates

The incidence of CFLD was calculated as the number of new cases of CFLD, with the number of person time years contributed by all children at risk of CFLD as the denominator. Children included in estimation of incidence rates had no evidence of liver disease, or had nonspecific changes which did not meet the criteria for CFLD in 2007. Incident cases were defined by the presence of portal hypertension. Person years (Pyrs) was defined as the contribution by each child in person years from date of enrolment in 2007 until date of follow-up, death or transplantation

2.8. Rates

We combined deaths and transplants (liver or lung) and used the term mortality/transplant (MTx) [19]. MTx rates were calculated using person years (Pyrs) of follow-up from the date of enrolment to date of follow-up, date of death or date of transplantation. The risk factors for MTx were assessed using logistic regression and Cox's proportional hazards models and adjusted for predictive factors including liver disease status (CFLD and NSCFLD) age at baseline, sex, height and pulmonary function SD scores. Survival (dead or alive) was calculated from date of birth to dates of follow-up, death or transplant.

3. Results

Five hundred and twenty two participants were enrolled in the first cycle of data collection for ILSCFLD in 2007 (95% target population) [11]. After 10 years (2017) 480/522 (91.95%) participants were included in the analysis to examine the incidence, risk factors and natural history for liver disease in CF. Participants were not included if they (i) had insufficient data to confirm liver disease status in 2017 ($n = 9$) (ii) had evidence of other conditions or medication use which led to uncertainty about the cause of liver abnormalities ($n = 10$), (iii) did not meet criteria for a diagnosis CF ($n = 2$) withdrew ($n = 7$) were lost to follow-up/emigrated ($n = 12$) or not interviewed in 2017 ($n = 2$)

3.1. Baseline characteristics

Baseline characteristics of 480 participants are outlined in Table 1, with 35 (7.29%) participants classified as CFLD, 110 (22.91%) NSCFLD, while 69.79% (335) participants had no evidence of liver disease. The median age in 2007 was 8.75 years (IQR 8.12), median duration of follow-up 8.53 years (IQR 1.28). Participants with CFLD were older ($p < 0.001$), and had a shorter duration of follow-up compared to participants with NSCFLD or NoLD (Table 1). Participants with CFLD were lighter than participants with NSCFLD or NoLD ($p < 0.03$). While the difference in height between the groups did not reach statistical significance ($p < 0.054$), when stratified by age, those over 15 years of age with CFLD were shorter ($p < 0.02$) than participants with NSCFLD or NoLD (data not shown). Participants with CFLD also had worse pulmonary function at baseline compared to those with NSCFLD or NoLD ($p < 0.002$). There was no difference in genotype between the groups.

Table 1
Characteristics of cohort in 2007 based on liver disease classification.

Variables	CFLD	NSCFLD	NoLD	p
Participants (n%)	35 (7.29)	110 (22.91)	335 (69.79)	
Gender Male (n%)	19 (54.29)	64 (58.18)	185 (55.22)	0.84
Age in 2007 (yrs)	13.83 (4.84)	11.37 (6.66)	7.5 (7.92)	0.00
Duration Follow-up (yrs)	7.95 (4.94)	8.51 (1.68)	8.59 (1.14)	0.00
Weight SDS	−1.07(1.47)	−0.53 (1.56)	−0.36 (1.33)	0.03
Height SDS	−0.95(1.54)	−0.68 (−1.62)	−0.53 (1.34)	0.054
BMI SDS	−0.26(1.36)	−0.10 (1.49)	−0.05 (1.29)	0.42
Genotype (Class 1–3)	34 (97.14)	102 (95.33)	304 (69.09)	0.42
FEV_1 SDS	−2.84(3.52)	−1.42 (2.83)	−1.29 (2.75)	0.02
AST	60 (41)	33 (15)	31 (11)	0.00
ALT	51 (42)	26 (14)	22 (13)	0.00
GGT	88.5 (119)	16 (13)	13 (6)	0.00
Alk Phos	353 (200)	256 (132)	236 (95)	0.00
GPR	4.01 (6.64)	0.28 (0.28)	0.23 (0.13)	0.00
APRI	1.45 (1.77)	0.28 (0.15)	0.24 (0.11)	0.00
Albumin	39 (6)	40 (4)	41 (5)	0.00
Platelets	141 (97–236)	324 (279 – 387)	345 (291-409)	0.00
Hospital Days	3.5 (16)	0.0 (10.5)	0.0 (7.0)	0.03
School Days Missed***	10.0 (24.5)	10.0 (25)	8.0 (20)	0.45

Data is presented as Medians and IQR for continuous data.
Abbreviations: ALT (Alanine aminotransferase), ALk Phos (Alkaline phosphatase) APRI (ALT to Platelet ratio) AST (Aspartate aminotransferase), BMI (Body mass index) CFLD (Cystic fibrosis liver disease) FEV_1 (Forced expiratory volume in 1 second) GGT (Gamma glutamyltranspepidase, GPR (GGT to Platelet ratio) NSCFLD (Non specific cystic fibrosis liver disease) NoLD (No Liver Disease) SDS (Standard deviation score).
The number of participants for whom clinical or biochemical data was unavailable at baseline was as follows (n%).
FEV_1 CFLD 2 (5.7), NSCFLD 16 (14.54), NoLD 123 (36.71); AST CFLD 4 (11.42), NSCFLD 32 (29.1), NoLD 100 (29.85); ALT CFLD 6 (17.14), NSCFLD 11(10), NoLD 67 (20%); GGT CFLD 5 (14.28), NSCFLD 25 (22.7), NoLD 77 (22.98), Alk Phos CFLD 0, NSCFLD 8 (7.27), NoLD 35(10.45); GPR CFLD 5 (14.28), NSCFLD 27 (24.5), NoLD 10,429.3); APRI CFLD 4 (11.43), NSCFLD 33 (30), NoLD 112 (33.43); Albumin CFLD 0, NSCFLD 8 (7.2%), NoLD 35 (10.45); Platelets CFLD 1 (2.85) NSCFLD 10 (9.1) NoLD 45(13.43); Hospital days CFLD 5 (14.28), NSCFLD 6 (5.45), NoLD 22 (6.57), School days missed in those over 5 years of age CFLD 6 (17.64), NSCFLD 14.43, NoLD (8.3%).
*** in those over 5 years of age.

3.2. Incidence of CFLD

Fig. 1 outlines the change in liver disease status and MTx during follow-up. Of 445 participants who were classified as either having no evidence of liver disease ($n = 335$) or nonspecific changes ($n = 110$) in 2007, 28/445 (6.29%) participants developed CFLD with portal hypertension by 2017. This comprised 15/110 (13.63%) participants who had NSCFLD and 13/335 (3.88%) participants who had NoLD at baseline in 2007. Of the 28 children who progressed to CFLD during follow-up 15/28 (53.6%) were categorised as CFLD in the first 5 years of follow-up, while 13/28 (46.4%) were assigned a CFLD classification in the second 5 years. Those participants with NSCFLD at baseline were more likely to be categorised as CFLD in the first 5 year cycle ($n = 10$; 66.6%)

The incidence of CFLD with portal hypertension was 7.51/1000 Pyrs of follow-up (95%CI 4.99–10.86). The incidence rate for participants <10 years of age in 2007 was 10.77/1000 Pyrs (95%CI 6.97–15.9) compared to 2.13/1000 Pyrs (95%CI 0.42–6.23) in participants >10; incidence rate difference 8.63 (95%CI 3.77–13.55 $p < 0.05$). No participant older than 10 years in 2007 who was classified as NoLD

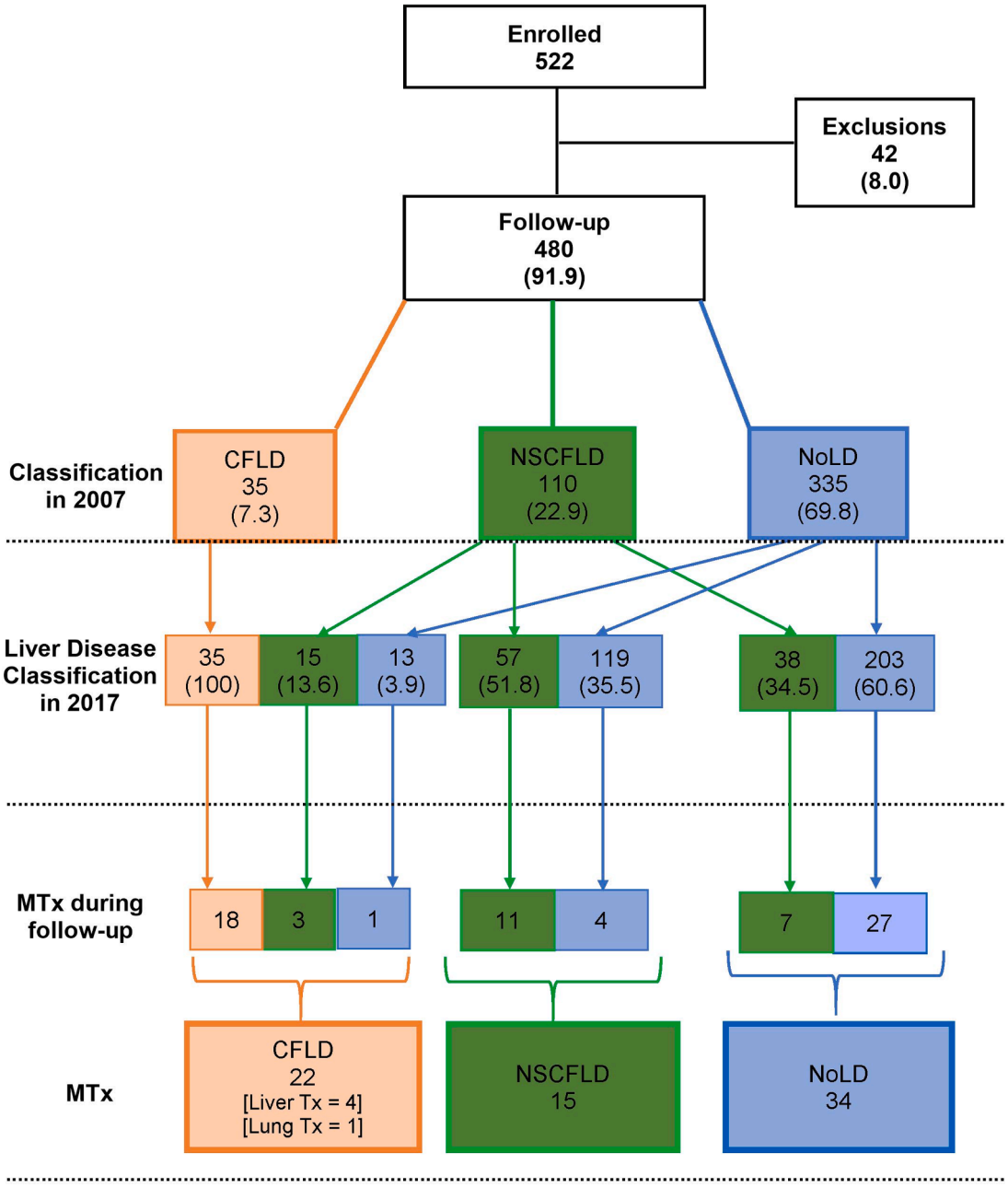

Fig. 1. Schematic representation of change in Liver disease classification (n%) and mortality for each group during follow- up. CFLD = Cystic fibrosis liver disease, NSCFLD = Nonspecific cystic fibrosis liver disease, No LD = No liver disease. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

progressed to CFLD by 2017 ($n = 77$); median age at follow-up 22.86 years IQR 5.2.

3.3. Risk factors for the development of CFLD

Participants who developed CFLD during follow-up were younger at baseline in 2007 (median age 6.21 (IQR 5.04) compared to those who did not develop CFLD 8.58 (IQR 8.0) years, $p < 0.001$ Table 2). Amongst those who progressed to CFLD during follow-up 25/28 (89.28%) participants were <10 years in 2007.

Those who developed CFLD were more likely to be male (21/28 (75%) $p < 0.047$), had a higher incidence of meconium ileus (35.71% Vs 19.66, $p < 0.053$), and had higher liver enzymes, APRI and GPR (Table 2) compared to those who did not progress to CFLD. There was no difference at baseline in pulmonary function or nutritional parameters between those who progressed to CFLD and those who did not (Table 2)

Using logistic regression analysis age under 10 years and the presence of NSCFLD were independent risk factors for the development of CFLD (Table 3). The adjusted odds ratio for male sex in the model was 2.44 (95%CI 0.97–6.09 $p = 0.056$) which while not reaching statistical significance suggests that male sex could be a risk factor for CFLD. Meconium ileus was not a risk factor for the development of CFLD in the model (Table 3).

We examined two other models of risk factors for CFLD. In supplementary Table 2a we report that GGT above the upper limit of normal [15] was an independent risk factor with age <10 years for the development of CFLD. In a model examining compound biomarkers APRI and GRP we show that GRP > 0.682 was an independent risk factor with age <10 years for the development of CFLD with PH ((Adjusted odds ratio 11.55 (95%CI 2.32–57.57)Supplementary Table 2b)) APRI was not a

Table 2
Characteristics at baseline (2007) of participants who progressed to CFLD with PH compared to those who did not develop advanced liver disease.

Variables	Progressed to CFLD with PH	No change / Improvement LD status	Risk difference	p
N (%)	28 (6.29)	417 (93.7)		
Age 2007 (Years)	6.21 (5.04)	8.58 (8.09)		0.00
Follow-up (Years)	8.38 (1.52)	8.58 (1.21)		0.73
Genotype (Class 1–3)	28 (100)	378 (92.8)		N E
Height	−0.69 (1.79)	−0.59 (1.34)		0.57
Weight	−0.51 (1.29)	−0.40 (−0.38)		0.72
BMI	−0.51(2.17)	−0.40 (1.38)		0.84
FEV1	−1.16 (1.21)	−0.01 (4.7)		0.13
AST	37 (21)	31 (11)		0.02
ALT	28.0 (22)	23.0 (14)		0.13
GGT	19 (20)	14 (6.5)		0.056
Albumin	40 (8)	41 (5)		0.82
Platelets	354 (135)	339.5(118)		0.74
APRI	0.27 (0.13)	0.25 (0.11)		0.32
GPR	0.47 (0.36)	0.24 (0.13)		0.044
Gender Male	21 (75%)	228 (54.68)	4.86 (0.54–9.18)	0.047
M Ileus	10 (35.71)	82 (19.66)	5.77 (−0.99–12.53)	0.053
Baseline LD status	15 (53.7)	95 (22.78)	9.75 (3.0- 6.49)	0.00
NSCFLD	13 (46.43)	332 (77.22)		
NoLD				
Outcome.				
Dead	4 (14.29)	49 (11.75)	1.42 (−6.07-8.9)	0.7
Age at Death (years)	6.83 (6.33)	13.25 (5.0)		0.11

Data for continuous variables is presented as medians and interquartile range (IQR) and analysed with one way analysis of variance Categorical variables are presented as proportions and risk difference between the groups are presented. Mean differences are not presented.
Abbreviations: ALT (Alanine aminotransferase), ALk Phos (Alkaline phosphatase) APRI (ALT to Platelet ratio) AST (Aspartate aminotransferase), BMI (Body mass index) CFLD (Cystic fibrosis liver disease) FEV_1 (Forced expiratory volume in 1 second) GGT (Gamma glutamyltranspepidase, GPR (GGT to Platelet Ratio); IQR Interquartile range, M Ileus (Meconium ileus); NE Not evaluable (100% of those who progressed to CFLD had Class 1–3 genotype) NSCFLD (Nonspecific cystic fibrosis liver disease) NoLD (No liver disease) PH Portal hypertension SDS (Standard deviation score).
The number of participants for whom clinical or biochemical data was unavailable at baseline was as follows (n%).
FEV_1 Advanced liver disease with PH 14 (50.0) NoLD or improvement 135 (29.98).
AST Advanced liver disease with PH 7 (25.0); NoLD or improvement 125 (29.97).
ALT Advanced liver disease with PH 3 (10.71) NoLD or improvement 75 (17.98).
GGT Advanced liver disease with PH 5(17.85) NoLD or improvement 97 (23.26).
Albumin Advanced liver disease with PH 1 (3.57) NoLD or improvement 42 (10.07).
Platelets Advanced liver disease with PH 3 (10.71), NoLD or improvement 43 (10.31) GPR Advanced liver disease with PH 7(25) NoLD or improvement 104 (24.94) APRI Advanced liver disease with PH 7 (25) NoLD or improvement 138 (33.09).

risk factor in this model.

3.4. NSCFLD during follow-up

Amongst 355 participants with NoLD in 2007 119 (35.5%) displayed nonspecific evidence of liver disease at follow-up in 2017 (Fig. 1). The median age in 2007 of the 119 participants who progressed to NSCFLD was 5.92 (IQR 6.33) years, of whom 89 (74.79%) were under 10 years.

Table 3
Logistic regression analysis of risk factors for progression to CFLD.

	Progression to CFLD with PH n%	No change / Improvement in LD status n%	Adjusted odds ratio 95%CI	p
Participants	28 (6.29)	417 (93.7)		
Age				
< 10 yrs	25 (9.19)	247 (90.81)	9.77	0.00
≥ 10 yrs	3 (1.73)	170 (98.27)	(2.72–35.09)	
Gender				
Male	21 (8.43)	228 (91.57)	2.44	0.056
Female	7 (3.57)	189 (96.43)	(0.97–6.09)	
M. Ileus				
Yes	10 (10.87)	82 (89.13)	1.43	0.4
No	18 (5.10)	335 (94.90)	(0.59–3.47)	
Baseline Liver				
Status	15 (13.64)	95(86.36)	6.48	0.00
NSCFLD	13 (3.88)	322(96.12)	(2.76–15.20)	
NoLD				

Logistic regression model examining the baseline characteristics which predict the development of CFLD presented as odds ratios and 95% CI using logistic regression. Other factors examined but not included in final model height, weight, BMI, and genotype.
Abbreviations LD Liver disease, MI Meconium ileus, NSCFLD Non specific cystic fibrosis liver disease, NoLD No liverdDisease, PH Portal hypertension.

Of 110 participants classified as NSCFLD in 2007 (Fig. 1) 57 (51.8%) remained stable NSCFLD and 38 (34.5%) had no evidence of liver disease at follow-up. Those who displayed stable evidence of NSCFLD were older in 2007 (median age 12.0 IQR 6.5) compared to those who progressed to CFLD (median age 7.75 years IQR 5.33 $p < 0.00$) with equal proportions of male and female participants.

3.5. Outcome for participants with CFLD

The combined death and transplant (liver or lung) rate (MTx), based on participants' liver disease classification in 2007 were evaluated after 8.53 years (IQR 1.28) of follow-up. By 2017, the MTx in participants with CFLD was 18/35 (51.43%) compared to 21/110 (19.09%) in participants with NSCFLD and 32/335 (9.55%) with NoLD ($P < 0.001$). The MTx rate for participants with CFLD was 7.75/100 Pyrs (95%CI 4.59–12.25) follow-up, for those with NSCFLD 2.33/100 Pyrs (95%CI 1.44–3.56) and 1.13/100 Pyrs (95%CI 0.77–1.59) for those with NoLD. Children under 15 years of age with CFLD had a higher MTx rate than those with NoLD or NSCFLD and MTx amongst girls was higher (Supplementary Table 3).

3.6. Risk factors for MTx and survival in CF

CFLD was a statistically significant risk factor for MTx in CF, and was independent of age, female sex and pulmonary function (Table 4).

Table 4
Risk factors for Mortality in participants with CFLD.

Variable	Odds ratio. 95% CI	p
CFLD vs NoLD	9.19 (2.83–29.86)	0.00
NSCFLD vs NoLD	1.66 (0.69–3.97)	0.19
Sex (Female)	2.46 (1.12–5.42)	0.02
Age	1.00 (0.89–1.13)	0.95
Pulmonary Function	0.35 (0.26–0.46)	0.00
Height SDS	0.80 (0.57–1.14)	0.22

Wald Chi-square probabilities for the significance of the impact of each baseline factor on the mortality status (alive/dead) at follow-up, using a logistic regression model.
Abbreviations: CFLD Cystic fibrosis liver disease, NSCFLD Nonspecific cystic fibrosis liver disease, NoLD No liver disease.

Survival time was censored at time of follow-up assessment for participants who were still alive. Controlling for baseline risk factors of age, sex, height and pulmonary function the presence of CFLD shortened survival time independent of other risk factors. Fig. 2 presents survival estimates for the cohort and in Supplementary Table 4 median survival times with IQR and 95%CI (where estimable) are presented. The median survival time for participants with CFLD in 2007 was 8.66 years (IQR not estimable), compared to 10.86 (IQR 0.23) for those with NSCFLD. Median survival for those with NoLD cannot be estimated as there were too few events for analysis.

Four participants (22.2%) who progressed to CFLD during the study died. The median age at death of participants who progressed to CFLD was (6.83 years IQR 6.33) compared to 13.25 years (IQR 5.0) in 49 participants without liver disease (p = NS Table 2).

Of those with CFLD who died during follow-up 9/18(50%) died from hepatic causes of whom 4/9 received a liver transplant while 8/18 (44.4%) died from pulmonary complications of whom 1 received a lung transplant. One participant died from other causes.

4. Discussion

The aetiology of CFLD is unknown and its natural history is poorly defined presenting a significant challenge for CF clinicians when biochemical or radiological abnormalities occur. In this study we report prospectively collected epidemiological data from a national cohort of participants with CF with over 90% follow-up after 10 years. The incidence of CFLD was 7.51/1000 person years of follow-up (95% CI 4.99–10.86), with children <10 years at greatest risk of developing CFLD. Children >10 years at baseline with no evidence of liver abnormalities did not develop liver disease during follow-up. We also confirm that participants with CFLD had a higher MTx rate and a shorter life expectancy than participants who did not develop CFLD.

Previous studies have reported divergent incidence rates for liver disease in CF [19–21]. Differences in study design, diagnostic criteria and referral pathways may explain the differences in incidence rates reported [19–21]. As a prospective national cohort study with defined protocols for the classification of liver disease in CF, supported by specialist hepatology examination, these are the first comprehensive data on the incidence of CFLD.

This study provides reassurance for clinicians and families of children who have no evidence of liver disease (biochemical or radiological) by 10 years of age that they are very unlikely to develop CFLD in the future. The age group at greatest risk of developing CFLD is uncertain [19,20,22–24] with some studies even reporting adult onset liver disease [21,25]. Most recently Siegal et al. report that age is protective against the development of advanced liver disease [26]. In our study we show that children who were <10 years at baseline had the greatest risk of developing CFLD. Only 3 (10.7%) children >10 years in 2007 progressed to CFLD and all had evidence of NSCFLD in 2007. This has implications for CF care, because in adults with no history of liver test abnormalities annual liver ultrasounds may be unnecessary.

The implication of nonspecific radiological or biochemical findings (NSCFLD) for an individual has long challenged clinicians caring for people with cystic fibrosis. Many studies have either excluded this group, and included only those with CFLD and portal hypertension, or fail to explicitly state how those with NSCFLD were categorised in the analysis. We found that the majority of participants who had NSCFLD at enrolment (95/110, 86.4%) did not develop CFLD at follow-up. However, NSCFLD was an independent risk factor for the development of CFLD as 15/110 (13.6%) of those with NSCFLD developed CFLD compared to 13/335 (3.8%) of those with no evidence of liver disease. (Table 2)

Transient elastography has been widely used in the diagnosis of a range of liver diseases in adults [27–29] and children [30,31], and there is good evidence that TE can easily identify those with advanced liver disease [29]. However, evaluating TE in over 200 healthy children we found that TE lacked precision [32], and therefore were unable to include TE in of our protocol for the this long-term follow-up study.

There is now good evidence that CFLD with PH is associated with increased mortality. While the MTx rates vary it must be borne in mind

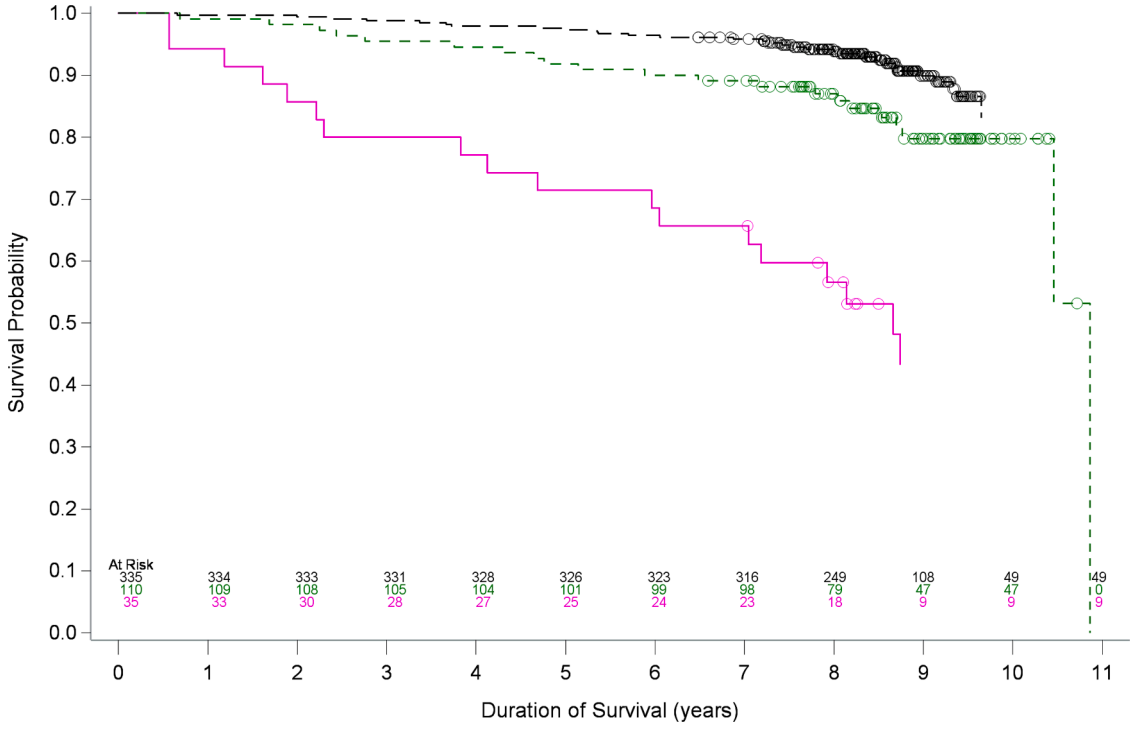

Fig. 2. Cox proportional hazards regression model assessing the impact of CFLD on survival time, while controlling for baseline factors of age, sex, height and pulmonary function. CFLD = Cystic fibrosis liver disease, NSCFLD = Nonspecific cystic fibrosis Llver disease, No LD = No liver disease. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

that the actual numbers of deaths are small and the standard deviation wide. What is compelling is that the difference in MTx rates, and survivorship between those with CFLD and those with CF but no liver disease remains despite advances in CF care [33]. In a retrospective study from the Netherlands Pals et al. reported a MTx rate of 33.6% (22 deaths, 10 transplants).over a 6 year period with the highest mortality rate the under 25 age group [34]. All CFLD patients who had received a transplant prior to the study period were excluded which suggests that the overall MTx rate is much higher. While the MTx rate reported by Cipolli et al. [19] in a 2 centre cohort of children diagnosed by newborn screening was lower than ours at 23.5% (12/51), possibly because of the lack of adult follow-up, it is does compare with the MTx rate we report in those who developed CFLD during a 10 year follow-up period (4/28 (14.3%).

While this prospective study confirms the increased mortality it also reports that CFLD shortens life expectancy in CF both for those who had CFLD at enrolment and for those who progressed to CFLD during follow-up. The median age of death of 4 (22.2%) children who developed new onset CFLD during the study was (6.83 IQR 6.33) years compared to 13.25years (IQR 5.0) in 49 participants who did not develop liver disease. (p = NS Table 2).

This study has a number of strengths including that it is a national census of people with CF in Ireland which followed participants through the transition to adult services and achieved over 90% follow-up. It is based on routinely collected annual assessment data supported by a hepatology examination to confirm the phenotypic classification of liver disease. It could be suggested that our findings are confounded by geography and genetics. However, our recent systematic review provides evidence that the prevalence of CFLD is similar across different continents and that the outcome for CFLD is poor compared to those with no evidence of liver disease regardless of location [33].

There are also limitations to this study: firstly liver disease in CF is complex process with a broad spectrum of disease manifestations in which CFLD with PH represents a small proportion of those with clinically significant CFLD. While in this study we show that those with NSCFLD have a higher MTx than those with NoLD a longer follow-up is required to determine the ultimate impact of nonspecific liver disease changes on outcome. Additional follow-up will also help elucidate if early onset of CFLD impacts mortality. It is possible that those with a more severe liver disease phenotype are diagnosed at younger age. Finally, the current gold standard of a clinical algorithm [13,14] which relies on evidence of portal hypertension, does not reflect the uncertainty of the diagnostic process in clinical practice. Reliance on existing imperfect diagnostic laboratory and imaging tools leave open the possibility of categorisation errors. However we are confident that the inclusion of a clinical examination by experienced hepatologists to address this issue greatly strengthens our study.

5. Conclusion

This study provides reassurance for children >10 years and their families with normal liver function, that the risk of CFLD is low. These data also indicate that the majority of those >10years with nonspecific liver abnormalities will remain stable. However, the stability of NSCFLD demonstrated in this study is over a relatively short period of adolescence and young adulthood and further follow-up is required.

In contrast the risk of developing clinically significant liver disease with portal hypertension is highest in children under 10 years of age. Therefore we should focus research on identifying the cause and improving the outcome for CFLD on young children.

Irish cystic fibrosis liver disease research group

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Credit authorship contribution statement

Marion Rowland: Conceptualization, Methodology, Supervision, Validation, Formal Analysis, Data Curation, Visualization, Writing original draft, Funding acquisition. **Jennifer Drummond**: Methodology, Data Curation, Investigation, Validation, Writing-review and editing. **Lucy Connolly**: Investigation, Software, Validation, Data curation Writing-review and editing. **Erika Daly**: Formal Analysis, Validation, Visualization, Writing-review and editing. **P Aiden McCormick**: Methodology, Supervision, Resources, Investigation, Visualization, Writing-review and editing. **Billy Bourke**: Conceptualisation, Supervision, Resources, Investigation, Visualisation, Writing-original draft preparation. **The Cystic Fibrosis Registry of Ireland**: Resources and Writing-review and editing. **Cystic Fibrosis Liver Disease Research Study Group**: Resources and Writing-review and editing.

Data Transparency Statement

Due to the sensitive personal and identifiable nature of participant data in this study it is not possible to share the data at this time.

Publication policy

Journal of Cystic Fibrosis publication policies have been reviewed and complied with.

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Declaration of Competing Interest

None declared by any of the authors.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jcf.2023.07.002.

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