Title

Chronic infection and inflammation affect exercise capacity in cystic fibrosis

Running head

Infection, inflammation and exercise capacity

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Author contributions

The guarantor of this study is PW who is responsible for the integrity of the work as a whole, from conception and design to conduct of the study and acquisition of data, analysis and interpretation of data and writing of the manuscript. PW, MS, HH, HA, CE designed the study. PW and MS were responsible for data acquisition. PW and CK performed the analysis and PW wrote the manuscript. All authors reviewed and approved the final version.

Conflict of interest declaration

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ABSTRACT

Pulmonary function and nutritional status are important determinants of exercise capacity in patients with CF Studies investigating effects of determinants, such as genotype or infection and inflammation are scarce and have never been analyzed in a multivariate longitudinal model.

A prospective longitudinal cohort study was performed to evaluate whether genotype, chronic inflammation and infection were associated with changes in exercise capacity. Furthermore, we investigated whether exercise capacity can predict clinical outcome. 504 exercise tests of 149 adolescents with CF were evaluated. $VO_{2max/kg}$%pred declined 20% during adolescence and was associated with IgG levels and chronic P. *aeruginosa* infection A lower exercise capacity was associated with a higher mortality, steeper decline in pulmonary function, and greater increase in IgG levels.

Since a decline in exercise capacity during adolescence was negatively associated with IgG levels and chronic P. *aeruginosa* infection, these data emphasize the importance of prevention and treatment of chronic inflammation and infections in patients with CF. Furthermore, a lower exercise capacity was associated with a higher mortality rate, steeper decline in pulmonary function and higher increase in IgG levels with increasing age in adolescents with CF. This stresses the value of regular exercise testing for assessing prognosis in adolescents with CF.

INTRODUCTION

Exercise capacity has been identified as an independent predictor of mortality in patients with cystic fibrosis (CF) (1) (2;3). Regular measurement of maximal oxygen consumption (VO_{2peak}) by a maximal cardiopulmonary exercise test (CPET) has therefore been emphasized as important in evaluating and assessing prognosis and disease management (4). Whether exercise capacity can be used as a prognostic marker for clinical outcomes other than mortality rate, such as pulmonary function, chronic inflammation and infection, is unknown. Previous studies showed that pulmonary function (5-10) and nutritional status (5-8) are important determinants of exercise capacity in patients with CF. Studies investigating effects of other potential determinants, such as the cystic fibrosis transmembrane regulator (CFTR) genotype or markers of infection and inflammation, are scarce and have never been analyzed in a multivariate longitudinal model.

While the severity of the CFTR mutation (mild versus severe) is associated with pancreatic insufficiency, pulmonary function and survival (11;12), data on the effect of CFTR genotype on exercise capacity are scarce (13;14). It has been shown that CFTR is expressed at the sarcoplasmic reticulum of skeletal muscle (15;16). Deficiency of CFTR protein led to elevated intracellular calcium levels and enhanced expression of inflammatory genes, which predisposed to muscle wasting in mice (15). CFTR genotype might therefore be a potential determinant of changes in exercise capacity in patients with CF.

CF is a chronic inflammatory disease characterized by the recruitment of high numbers of neutrophils into the infected lungs and the excessive production of immune modulatory polypeptides. Increased levels of the pro-inflammatory interleukins (IL) IL1-β, IL-6, tumour necrosis factor alpha (TNF-α) and IL-8 are found , whereas levels of the anti-inflammatory cytokine IL-10 are decreased (17). A study in adult patients with CF showed that higher C-reactive protein (CRP), total and specific immunoglobulin G (IgG) and total leukocyte levels were significantly associated with a decreased pulmonary function (18;19). Another study showed that a change in CRP level was negatively associated with exercise capacity, but the effect of CRP level on exercise capacity was never investigated in a multivariate model (21).

Chronic infections with P. *aeruginosa* are associated with a decline in pulmonary function, increased morbidity and mortality (22;23) and are known to be an important inducer of hyper-inflammation in patients with CF (17). However, the independent effects of inflammation and chronic P. *aeruginosa* infection on exercise capacity in patients with CF have never been studied.

The aim of this study was to evaluate whether CFTR genotype, chronic P. *aeruginosa* infection and inflammatory markers , such as total IgG, leukocyte and neutrophil levels are independently associated with longitudinal changes in exercise capacity in adolescents with CF. Furthermore, we investigated whether exercise capacity can be used as a prognostic marker to predict mortality and chronic P. *aeruginosa* infection rate, changes in inflammatory markers, pulmonary function and body mass index (BMI), in adolescents with CF.

MATERIAL AND METHODS

Study design and subjects

A prospective longitudinal cohort study involving adolescent patients with CF was performed. Patients of our CF centre in the Netherlands attend a multidisciplinary examination annually. Pulmonary function tests, a CPET and measurements of inflammation and anthropometrics are routinely performed. Results were prospectively recorded in a database between 1998 and 2008. Sputum cultures taken during the entire year preceding the annual examination and were retrospectively evaluated and also recorded in the database. Additionally, the database contained information about demographics, decease dates and CFTR genotype.

Patients were included when CPET data of at least two multidisciplinary examinations were available. The database contained longitudinal data of 171 adolescents with CF, who performed 567 CPETs. Patients were 12 – 18 years old (96 boys, 75 girls) and were free of pulmonary exacerbations at the time of testing. All patients gave written informed consent for storage and use of their data for scientific purposes and use of the database was permitted by the ethical board of the University Medical Centre Utrecht. All researchers had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

The CPET was performed on an electronically braked cycle ergometer (Ergoline, Cardinal Health, Houten, The Netherlands) according to the Godfrey protocol (24). Patients breathed through a face mask (Hans Rudolph Inc, USA)

which was connected to a calibrated metabolic cart (Oxycon pro, Cardinal Health, Houten, The Netherlands). Volume measurements and breath-by-breath respiratory gas analyses were performed with a flow meter (Triple V volume transducer) and gas analyzer for oxygen and carbon dioxide (Oxycon Pro, Cardinal Health, Houten, the Netherlands). Oxygen consumption, carbon dioxide output and the respiratory exchange ratio (RER) were calculated by a computer from conventional equations. Heart rate (HR) and oxygen saturation were measured continuously during the CPET with a 3 lead electrocardiogram and a pulse oxymeter respectively. CPET results were only included for analysis when the test was performed until maximal exhaustion. Effort was considered to be maximal when one of the following criteria was met: 1.heart rate (HR_{peak}) > 180 beats per minute , 2.respiratory exchange ratio (RER_{peak}) > 1.00=(26). Maximal exercise capacity was calculated as the average value over the last 30 seconds of the test and was expressed as VO_{2peak} ($l.min^{-1}$) and VO_{2peak} corrected for body weight ($VO_{2peak/kg}$; $l.kg^{-1}.min^{-1}$). For the analysis maximal exercise values were expressed as a percentage of predicted (VO_{2peak}%pred and $VO_{2peak/kg}$%pred) by using reference values of Dutch healthy adolescents, which allowed to adjust for age, gender and weight. Except for pre-exercise pulmonary function testing, which was not performed in the healthy Dutch adolescents, exercise test criteria were comparable to the protocol used in the patients with CF to estimate maximal exercise capacity and achieve maximal effort (27).

CFTR mutations were divided into five classes, based on the functional effect of the mildest of the two mutations. Class I, II and III mutations were

categorized as severe and class IV and V as mild. If one or both of the mutations were unknown, the patient was classified as unknown (12).

Nutritional status was expressed as the standard deviation score for body weight (SDS weight), height (SDS height) and body mass index (SDS BMI) based on reference values for healthy Dutch adolescents (28).

Pulmonary function tests were performed prior to the exercise test after inhalation of 800 µg of salbutamol. The forced expiratory volume in 1 second ($FEV_{1;}$ l) was obtained from the best of three maximal expiratory flow-volume curves (Masterscreen; Cardinal Health, Houten, The Netherlands) and expressed as FEV_1%pred (29). All curves were checked for accuracy and repeatability (30).

CRP, total IgG and leukocyte levels were measured in peripheral blood as potential inflammatory determinants of longitudinal changes in exercise capacity. These inflammatory markers were chosen based on literature concerning chronic inflammation in patients with CF (17) and previous studies showing significant correlations between these specific inflammatory markers, and ~~lung~~ pulmonary function or exercise capacity (18-20).

P. *aeruginosa* infection status and decease dates were retrospectively evaluated and recorded in the database. Chronic P. *aeruginosa* infection was considered to be present when >50% of the sputa or cough swab cultures were positive in the preceding year (31). In the older children less than four sputum samples per year were available. In these patients the old European consensus definition for chronic P. *aeruginosa* infection was used, that is, at least three

positive cultures over ≥6 months with a ≥1 month interval (32). Mortality was defined as death or date of lung transplantation, since these patients are expected to die without transplantation.

Statistical analysis

A Kolmogorov-Smirnov test was used to test whether the variables were normally distributed. Patient characteristics were expressed as means and standard deviations (SD) when variables were normally distributed, otherwise as median and interquartile range. Categorical data were expressed as percentage frequency. To evaluate whether CFTR genotype, chronic P. *aeruginosa* infection and inflammation were associated with longitudinal changes in exercise capacity in adolescents with CF, a multivariate linear mixed model (LMM) analysis for repeated measurements was used.[35] This statistical technique has several advantages. (1)Subjects with missing data are not dropped from the analysis. (2)Within-subject changes and standard errors over time are calculated. Large variations at population level, between individuals, therefore do not influence the analysis. This allows us to estimate longitudinal changes over time more accurately compared to a cross-sectional analysis of the data.

Age, FEV_1%pred, CFTR genotype, chronic P. *aeruginosa* infection, CRP, total IgG, leukocyte levels and the interaction terms "IgG levels x chronic P. *aeruginosa* infection", "FEV_1%pred x chronic P. *aeruginosa* infection", "CFTR genotype x P. *aeruginosa* infection" and "Age x P. *aeruginosa* infection" were included as potential independent determinants. The outcome variable was

expressed as $VO_{2max/kg}$%pred(27) to adapt for gender, growth and maturation related differences, which exist between adolescents of different ages.

In order to investigate whether exercise capacity can predict clinical outcome exercise capacity, measured at the first visit, was categorized as low ($VO_{2max/kg}$%pred ≤ 80%) or high ($VO_{2max/kg}$%pred > 80%) and named "exercise capacity group". The LMM analysis for repeated measurements was used to assess whether this variable associated with longitudinal changes in pulmonary function, IgG levels or SDS BMI. This was tested by examining the improvement in model fit after addition of an interaction term to the model (age x exercise capacity group). The interaction term would allow for different slopes over time for both exercise capacity groups. A log rank test was used to examine if there was a difference in chronic P. *aeruginosa* infection or mortality rate between both exercise capacity groups.

All data were analyzed in SPSS 18.0 for Windows (SPSS Inc, Chicago, Ill, USA). A p-value of < 0.05 was considered to be statistically significant. The LMM model was fitted using the Akaike Information Criterion.

RESULTS

Data of 504 CPETs of 149 patients (85 boys, 64 girls) were eligible for inclusion. Mean (SD) duration of follow-up was 2.8 (1.7) years. Table 1 summarizes the baseline characteristics of the patients at the first visit.

Longitudinal changes in $VO_{2peak/kg}$%pred were negatively associated with chronic P. *aeruginosa* infection and total IgG levels, but not with CFTR

genotype, CRP, total leukocyte levels and/ or the interaction terms "chronic P. *aeruginosa* infection x IgG levels", "chronic P. *aeruginosa* infection x FEV$_1$%pred", "CFTR genotype x P. *aeruginosa* infection" and "Age x P. *aeruginosa* infection" (Table 2). A longitudinal annual decline of 3.23% was seen in VO$_{2peak/kg}$%pred, independent of chronic P. aeruginosa infection and total IgG levels. An additional decline of 4.60% in VO$_{2max/kg}$%pred was seen when patients became colonized with P. *aeruginosa* (p=0.007). An increase of 1 g.l^{-1} in total IgG levels was associated with a decline in VO$_{2peak/kg}$%pred of 0.54% (p=0.020). According to the final multivariate mixed model analysis, VO$_{2peak}$%pred of patients with CF at the age of 12 years old is comparable to that of age-matched healthy controls (100%), however it declines by 20% during adolescence (Figure 1).

Patients with a low VO$_{2max/kg}$%pred at their first visit had a significantly steeper decline in FEV$_1$%pred and a higher increase in total IgG levels during follow-up, compared to patients with a high VO$_{2max/kg}$%pred, which was independent of age and body weight. SDS BMI was not associated (Table 3). Furthermore, a lower VO$_{2max/kg}$%pred at the first visit was associated with a higher mortality rate during follow-up of 80.0% versus 96.3% (Log-rank test, p = 0.018), but not with chronic P. *aeruginosa* infection rate (Log-rank test, p = 0.903).

DISCUSSION

This study showed that exercise capacity of adolescents with CF at the age of 12 years old is comparable to that of age-matched healthy controls, however it declines by 20% during adolescence. This longitudinal decline in exercise capacity was negatively associated with chronic P. *aeruginosa* infection and IgG levels, independent of age, pulmonary function and body weight. CFTR genotype, CRP, leukocyte and neutrophil levels were not associated. Furthermore, this study showed that clinical outcome can be predicted by exercise capacity. A lower exercise capacity was associated with a higher mortality rate, a steeper decline in pulmonary function and a higher increase in IgG levels during follow-up, but not with SDS BMI and P. *aeruginosa* colonization rate.

Inverse correlations between elevated IgG levels and pulmonary function have been reported previously in patients with CF (18;19). IgGs serve to neutralize pathogens such as P. *aeruginosa* and promote its elimination by phagocytes. Elevated P. *aeruginosa* specific IgG levels can be measured before the organism can be isolated from sputum in patients with CF (35). Elevated IgG levels might therefore be a good representative of inflammation and probably also of P. *aeruginosa* infection status. However, in our model chronic P. *aeruginosa* infection and IgG levels did not interact and were independently associated with longitudinal changes in exercise capacity. A possible explanation could be that total IgG levels are rather non-specific for an infection with P. *aeruginosa* as it serves as an antibody for infections in general. P. *aeruginosa* specific IgG levels might interact with P. *aeruginosa* infection

status. Unfortunately, we did not measure   specific P. *aeruginosa* IgG antibodies.

The negative association between inflammation and exercise capacity is compatible with the concept that chronic systemic inflammation results in a reduced exercise capacity due to devastating effects on skeletal muscle (33;34), which is not limited to CF disease (21), but is also present in other chronic inflammatory diseases such as COPD (20). Skeletal muscle mass and function have been shown to be related to exercise capacity in CF (5;36), but were unfortunately not measured in our study. Furthermore, chronic infection and inflammation may lead to increased rates of intravenous treatment and hospitalization, which invalidate patients and lead to a reduction in habitual physical activity levels. Although the effect is relatively weak, habitual physical activity levels have been shown to be a significant predictor of VO_{2peak} in patients with CF (37;38). Recent literature suggests that increasing habitual physical activity levels may have the potential to diminish low-grade inflammation (39). Whether regular exercise can also diminish inflammation in patients suffering from severe infections and inflammation, such as patients with CF, is unknown. Unfortunately, habitual physical activity levels, rate of intravenous treatment and hospitalization were not recorded in the database. Moreover, chronic infection and inflammation can lead to a reduced appetite (40) and therefore to a reduction in body weight, which may lead to a reduction in exercise capacity. However, this is not the most likely explanation as exercise capacity was adjusted for differences in body weight in our model.

Although   CRP levels were found to be negatively correlated   with exercise capacity in patients with CF in an univariate model (21) we did not find an association between CRP levels and longitudinal changes in exercise capacity. CRP is an acute phase reactant, which is predominantly elevated during *acute* infectious diseases and only slightly in patients suffering from *chronic* infectious diseases. IgG levels might be a better representative of chronic inflammation and therefore a better predictor of longitudinal changes in exercise capacity in patients with CF, compared to CRP.

Chronic infection with P. *aeruginosa* was associated with a decline in $VO_{2peak/kg}$%pred of 4.60%, independent of age, IgG levels, pulmonary function and nutritional status. Chronic P. *aeruginosa* infection may have an effect on exercise capacity by weakening of the diaphragm. In a mouse model, it was shown that a pulmonary infection with P. *aeruginosa* preferentially weakened the diaphragm, an effect not directly correlated with the degree of pulmonary inflammation (41). Chronic infections with P. *aeruginosa* were found to be associated with other clinical variables as well, such as a decline in pulmonary function, morbidity and mortality (22;23).

We did not find an association between genotype and $VO_{2peak/kg}$%pred in our study, which is consistent with the results of Kaplan et al. (13), but in contrast with the findings of Selvadurai et al. (14), who showed that patients with a mild mutation (Class IV and V) had a better exercise capacity than patients with a severe mutation (Class I, II or III). Kaplan et al. compared two groups of patients who were either homozygous (n = 10) or heterozygous (n = 20) for the ΔF508 mutation. The study was limited by its small sample size.

Additionally, all heterozygous patients were pooled in one group, independent of the classification of the second mutation. The study of Selvadurai et al. was limited because the effect of CFTR genotype on exercise capacity was only univariately examined. Analysing the effect of CFTR genotype on exercise capacity in a longitudinal multivariate model therefore adds to our current knowledge. Moreover, identification of genetic modifiers might be of importance in elucidating the association between genotype and phenotypical differences among patients.

Furthermore, our longitudinal study showed that exercise capacity can be used as a prognostic marker for clinical outcome. The value of cardiopulmonary exercise testing in predicting survival has been evaluated previously. Consistent with our results, these studies showed that exercise capacity was associated with mortality rate (1-3). Life expectancy has largely improved the last decades in patients with CF, but it is still reduced compared to the healthy population. Additionally, the clinical course differs greatly between patients. Therefore, determining prognosis is an important issue in disease management. A number of variables, such as pulmonary function (1;42), sex (43), nutritional status (42) and chronic infection with P. *aeruginosa* (22;23) have been related to prognosis. Exercise capacity is an important addition to these predictors because it represents a patients functional capacity. We are the first to show that exercise capacity can also predict changes in pulmonary function and inflammation with increasing age in adolescents with CF. Exercise capacity measured at the first visit was not associated with differences in chronic P. *aeruginosa* infection rate.

Many patients were already colonized with P. *aeruginosa* at the start of the study, which reduced the a priori chance for a new colonization event.

The longitudinal cohort study design is one of the key strengths of this study. Longitudinal changes can be estimated more accurately as subject-specific changes over time can be estimated. However, a few limitations should be considered. We did not analyze all potential confounders, such as habitual physical activity levels, muscle mass and/or function and effects of other inflammatory markers like IL-6. IL-6 is known to be involved in exercise metabolism (44), but is unfortunately not routinely measured.

In conclusion, this longitudinal cohort study showed that the exercise capacity of patients with CF declines during adolescence compared to age-matched healthy controls, which is negatively associated with total IgG levels and chronic infection with P. *aeruginosa*. Since a negative association was observed between exercise capacity and markers of chronic inflammation and infection, these findings emphasize the importance of prevention and aggressive treatment of chronic inflammation and infections in adolescents with CF. Furthermore, a lower exercise capacity is associated with a higher mortality rate, a steeper decline in pulmonary function and a greater increase in total IgG levels with increasing age in adolescents with CF. This stresses the significance of regular cardiopulmonary exercise testing for assessing the prognosis of adolescents with CF.

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TABLES

Table 1: Patient characteristics

Variable	
Patients (n)	149
CPETs (n)	504
Sex (% male)	57.1
CFTR genotype classes	
Class I - III (severe) (%)	83.2 [§]
Class IV – V (mild) (%)	10.1 [§]
Unknown (%)	6.7 [§]

Variable	First visit
Age (years)	13.29 (1.24) [*]
Weight (SDS for age)	-0.83 (0.93) [*]
Height (SDS for age)	-0.75 (1.00) [*]
BMI (SDS for age)	-0.59 (0.97) [*]
FEV_1%pred (% predicted)	83.23 (18.04) [*]
Chronic P. *aeruginosa* infection (%)	43.62 [§]
Total IgG level ($g.l^{-1}$)	12.39 (3.94) [†]
CRP level ($mg.l^{-1}$)	6.77 (7.49) [†]
Total leukocyte level ($x10^9.l^{-1}$)	9.31 (3.58) [†]
VO_{2peak} ($l.min^{-1}$)	1.76 (0.49) [*]
VO_{2peak}%pred (% predicted)	91.66 (28.57) [*]
$VO_{2peak/kg}$ ($ml.kg^{-1}.min^{-1}$)	41.46 (8.89) [*]

VO$_{2peak/kg}$%pred (% predicted)	96.20 (18.29) *
W$_{peak}$ (Watt)	142.41 (38.16) *
W$_{peak}$%pred (%)	74.75 (20.72) *

Data are presented as; * = mean (SD), † = median (IR), § = percentage of total group. Definition of abbreviations: n = number of subjects; FEV$_1$ = forced expiratory volume in 1 second; VO$_{2peak}$ = Peak oxygen consumption.

Table 2: Multivariate linear mixed model (~~LMM~~) analysis evaluating effect of CFTR genotype, chronic P. *aeruginosa* infection and inflammation on longitudinal changes in exercise capacity

Variable	Estimate	95% CI	SE	p-value
Intercept	134.53	121.14 to 147.91	6.81	0.000
Age (years)	-3.23	-3.90 to -2.56	0.34	0.000
FEV$_1$%pred (% predicted)	0.17	0.09 to 0.26	0.04	0.000
Chronic P. *aeruginosa* infection (%)	-4.60	-7.96 to -1.25	1.70	0.007
Total IgG level (g.l^{-1})	-0.54	-0.99 to -0.09	0.23	0.020

Fixed dependent variable: VO$_{2peak/kg}$%pred. Fixed independent variables: age, FEV$_1$%pred, CFTR genotype (mild vs. severe), chronic P. aeruginosa infection, CRP, IgG level, Leukocyte level, "chronic P. aeruginosa infection x IgG", "chronic P. aeruginosa infection x FEV$_1$%pred", "CFTR genotype x P. aeruginosa infection" and "Age x P. aeruginosa infection". Random factor: Intercept. Patient not colonized with P. aeruginosa was coded as 0 and infection

was coded as 1. Definition of abbreviations: SE = standard error, CI = confidence interval.

Table 3: Multivariate linear mixed model analysis evaluating whether exercise capacity can predict longitudinal changes in pulmonary function, total IgG levels or SDS BMI

Dependent variable	Variable	Estimate	95% CI	SE	p-value
FEV$_1$%pred (%)	Intercept	89.83	76.30 to 103.36	6.87	0.000
	Age	-1.35	-2.29 to -0.41	0.48	0.005
	Exercise capacity group x age	1.03	0.53 to 1.54	0.26	0.000
IgG level (g.l^{-1})	Intercept	3.98	4.31 to 9.64	1.35	0.000
	Age	0.52	0.33 to 0.71	0.10	0.000
	Exercise capacity group x age	-0.19	-0.29 to -0.08	0.06	0.001
SDS BMI	Intercept	-1.47	-2.05 to -0.89	0.29	0.000
	Age	0.05	0.00 to 0.09	0.02	0.030
	Exercise capacity group x age	0.02	-0.01 to 0.05	0.01	0.062

Fixed dependent variables: FEV1%pred, IgG level or SDS BMI. Fixed independent variables: Age, "Exercise capacity group (low = 0, high = 1) x age". Random factor: Intercept. Exercise capacity was coded as 0 or low when VO$_{2max/kg}$%pred ≤ 80% or as high when VO$_{2max/kg}$%pred > 80%. Definition of abbreviations: SE = standard error.

FIGURES LEGENDS

Figure 1: Longitudinal decline in VO$_{2max/kg}$%pred during adolescence in patients with CF

Grey dots represent measured VO$_{2peak/kg}$%pred. Lines represent calculated VO$_{2peak/kg}$%pred based on the final multivariate mixed model analysis: mean (solid line), +/- 1 SD (dotted line), +/- 2 SD (dashed line).