Journal of Cystic Fibrosis 21 (2022) 111–114

Contents lists available at ScienceDirect

Journal of Cystic Fibrosis

journal homepage: www.elsevier.com/locate/jcf

Overweight and obesity in adults with cystic fibrosis: An Italian multicenter cohort study

Andrea Gramegna[a,b,*], Stefano Aliberti[a,b], Martina Contarini[b], Daniela Savi[c], Giovanni Sotgiu[d], Fabio Majo[e], Laura Saderi[d], Vincenzina Lucidi[e], Francesco Amati[b], Maria Pappalettera[b], Paolo Palange[c], Francesco Blasi[a,b]

[a] Department of Pathophysiology and Transplantation, University of Milan, Milan, Italy
[b] Internal Medicine Department, Respiratory Unit and Cystic Fibrosis Adult Center. Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy
[c] Department of Public Health and Infectious Diseases, "Sapienza" University of Rome, 00185 Rome, Italy
[d] Clinical Epidemiology and Medical Statistics Unit, Department of Biomedical Sciences, University of Sassari, Sassari, Italy
[e] Cystic Fibrosis Unit, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy

ARTICLE INFO

Article history:
Received 31 December 2020
Revised 30 April 2021
Accepted 3 May 2021
Available online 27 May 2021

Keywords:
Overweight
Adults
Cystic fibrosis

ABSTRACT

Background: Over the last decades aggressive interventions have been successful to improve nutritional outcomes in people with cystic fibrosis (CF). As a result, with improvement of life expectancy and new CFTR modulators, overweight and obesity are progressively becoming a source of concern for adult population and in developed countries.

Methods: This was a multicenter, observational, cross-sectional study of 321 adults with CF at three large CF centers in Italy. Patients were divided into three groups according to BMI classes, overweight and obesity (OW) group including patients with BMI ≥25 kg/m^2, normal weight (NW) group with BMI 18.6–24.9 kg/m^2 and underweight (UW) group with BMI ≤18.5 kg/m^2.

Results: We demonstrated that prevalence of OW in adults with CF in Italy is 22%. OW status is independently associated with male sex (OR 3.520, $P = 0.001$), pancreatic sufficiency (OR 2.873, $P = 0.014$) and older age at diagnosis (1.015, $P = 0.042$). BMI correlated with ppFEV1 ($r = 0.337$; $P<0.0001$) with median ppFEV1 significantly higher in patients with OW than comparisons. We also reported preliminary data on unfavorable cardiovascular risk factors in a subgroup of patients, where median blood levels [IQR] of cholesterol and systemic hypertension [%] were significantly higher in the OW group than in the NW and UW.

Conclusions: People with CF and OW is a relevant patient group that might deserve better definition and proper clinical management.

1. Background

Cystic fibrosis (CF) is a life-limiting genetic disease caused by the dysfunction of the CFTR protein, a chloride channel involved in fluid transportation across epithelia [1]. While CF is commonly associated with malnutrition due to both intestinal malabsorption and increased energy expenditure, over the last two decades the prevalence of CF-related malnutrition has significantly decreased as a result of targeted interventions [2]. On the other hand, in consideration of the increase in life expectancy, overweight and obesity are progressively becoming a source of concern for people with CF, especially in adults and high-income countries [3,4]. Furthermore, dietary interventions in CF are based on high-calorie supplementation with no restriction on fat intake in the view that poor nutritional status is associated with worse respiratory outcomes [5,6].

In the general population overweight and obesity are considered relevant risk factors for cardiovascular complications including systemic hypertension and ischemic heart disease. Over the last years overweight and obesity have been recognized as a potential clinical issue for patients with CF [7]. However, the prevalence of overweight and obesity is heterogeneous: several studies have been carried out in North America and United Kingdom, but no data are currently available in Southern Europe. To address this

* Corresponding author.
E-mail address: andrea.gramegna@unimi.it (A. Gramegna).

https://doi.org/10.1016/j.jcf.2021.05.002
1569-1993/

epidemiological gap, a multi-center, observational study was performed in three reference adult CF centers in Italy.

2. Methods

Study design and data collection. This was an observational, cross-sectional study enrolling consecutive adults (≥18 years) with CF at three reference CF centers in Italy (IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan; Ospedale Pediatrico Bambin Gesù, Rome; Policlinico Umberto I, Rome). All clinical data including CF genotype, lung function, respiratory microbiology, comorbidities and CF medications refer to year 2017 and were retrospectively collected by three trained investigators by chart or medical record review between September 2018 and March 2019. All site investigators were provided with a protocol including the following clinical definitions.

The coordinating center (Milan) received the study approval by the local Institutional Ethical Committee, followed by the approval of the other local ethical committees. This project was not funded and relied upon voluntary site and investigator participation.

Study groups. Three groups were selected according to the BMI classes as defined by WHO [8]: overweight and obesity group (OW) including patients with BMI ≥25.0 kg/m^2, normal weight group (NW) including patients with BMI 18.6–24.9 kg/m^2, and underweight group (UW) including patients with BMI ≤18.5 kg/m^2.

Study definitions. CF was defined according to the last diagnostic criteria [9]. Chronic infection was defined by the isolation of *P. aeruginosa* and other common CF bacteria (including *S. maltophilia, A. xylosoxidans, B. cepacia* complex) in sputum culture on two or more occasions at least 3 months apart over a 1-year period [10]. A pulmonary exacerbation was defined according to Fuchs criteria [11]. Frequent exacerbators were defined as patients with ≥3 pulmonary exacerbations per year. CFTR residual function mutations (RFM) were defined according to the mutation list included in clinical trials [12]. Referring to comorbidities, pancreatic insufficiency (PI) was defined in patients treated with pancreatic enzymes, while CF-related diabetes (CFRD) was diagnosed according to standard criteria. The definition of CF related liver disease (CFLD) included both liver steatosis and fibrosis. Total serum cholesterol levels were expressed as mg/dL and systemic hypertension was defined according to the presence of an antihypertensive therapy.

Statistical analysis. Qualitative and non-parametric quantitative variables were summarized with frequencies and medians (interquartile ranges, IQR), respectively. Differences between groups were assessed with chi-squared or Fisher exact test for qualitative variables and with Mann-Whitney for non-parametric quantitative variables, respectively. BMI was correlated with continuous variables with the Spearman correlation. A two-tailed p-value was considered statistically significant when less than 0.05. All statistical computations were performed with IBM SPSS, Statistics for Mac, version 22.0.

3. Results

Patient cohort and prevalence of overweight status. A total of 321 patients (median [IQR] age: 38 [32–45] years; 41% female] were enrolled. Among them, 21% patients had at least one RFM and 59.5% had PI. A total of 146 (45.5%) patients had chronic P. aeruginosa infection and the median (IQR) exacerbation frequency was 1 (0–3) per year. Median (IQR) BMI was 22.39 (20.9–24.4) kg/m^2. OW, NW, and UW groups included 71 (22.1%), 238 (74.1%), and 12 (3.8%) patients, respectively. In the OW group, 8 patients had BMI ≥30 and met the WHO definition for obesity.

Clinical characteristics among groups. When compared with NW and UW groups, patients with OW were more likely to be male (76% VS. 55.8% VS. 16.7%; P <0.0001) and diagnosed at an older age (median [IQR] age: 26 [7–34] VS. 9 [1–28] VS. 9 [0.5–22] years; P: 0.003), as reported in Table 1. The prevalence of patients homozygous for p.Phe508del mutation in the OW group was lower in comparison with NW and UW groups (1.4% VS. 9.7% VS. 8.3%; P: 0.043). BMI correlated with ppFEV1 (r: 0.337; P <0.0001, Fig. 1). Median ppFEV1 was significantly higher in the OW group (median [IQR]: 91 [75–99] VS. 70 [51–91] VS. 49 [44–76]; P <0.001). The OW group showed a lower prevalence of chronic respiratory infection with P. aeruginosa (33.8% VS. 47.9% VS. 66.7%; P: 0.036) and a lower number of pulmonary exacerbations (median [IQR]: 1 [0–2] VS. 1 [0–3] VS. 3 [1.25–5.5]; P: 0.004) and of hospitalizations in the previous 12 months (median [IQR]: 0 [0–0] VS. 0 [0–1] VS. 1 [0.25–2]; P <0.0001). Frequent exacerbators were less prevalent in the OW group (16.9% VS. 27.7% VS. 50.0%; P: 0.010). Patients with CF and OW were less likely to have PI (33.8% VS. 64.2% VS 100%; P: 0.003). CFRD and osteopenia were less prevalent in the OW group (15.5% VS. 28.1% VS. 50%; P: 0.017; and 25.7% VS. 47.2% VS. 70%; P: 0.001, respectively); CFLD had higher prevalence in the OW group (59.1% VS. 45.4% VS. 25%; P: 0.035). A subgroup analysis in Milan investigated total cholesterol levels in blood and systemic hypertension: median (IQR) cholesterolemia was 158 (132–198) mg/dl, with the OW group showing higher levels (median [IQR]: 187 [136–218] VS. 154 [128–188] VS. 157 [143–183] mg/dl; P: 0.007); while systemic hypertension was more prevalent in the OW group than in the other groups (12.7% VS. 4.6% and 8.3%; P: 0.025).

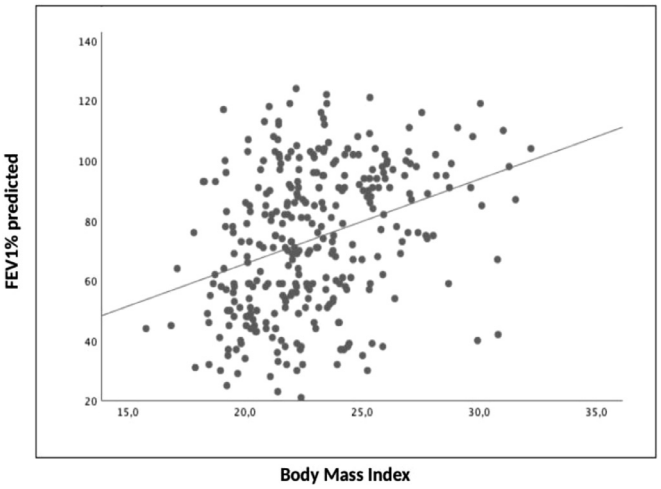

Fig. 1. Linear correlation between BMI and FEV1% predicted in all study cohort.

Factors associated with OW. Multivariate regression analyses showed a set of factors associated with OW in CF adults as independent predictors of overweight status, including male sex (OR 3.52; P: 0.001), pancreatic sufficiency (OR 2.87; P = 0.014), and older age at diagnosis (1.02; P = 0.042) (Table 2).

4. Discussion

This study demonstrated that 1) prevalence of OW in CF adults in Italy is up to 22%; 2) OW status is independently associated with male sex, older age at diagnosis, and pancreatic sufficiency; 3) the OW group showed higher prevalence of cardiovascular and metabolic risk factors.

To our knowledge, this is the first epidemiological study on OW in a cohort of CF adults in Southern Europe. With the present experience, we confirmed the significant prevalence of OW in a large population of adults with CF from Italy.

Table 1
Clinical characteristics of patients with OW and comparison between groups.

Variables	Body Mass Index (BMI)			p-value
Demographics	Underweight (BMI<18.5) (n = 12)	Normal weight (BMI 18.6–24–9) (n = 238)	Overweight (BMI≥25) (n = 71)	
Male, n (%)	2 (16.7)	133 (55.8)	54 (76)	<0.0001
Median (IQR) age, years	40 (33–48)	37 (31–44)	38 (33.5–45)	0.127
Median (IQR) age at CF diagnosis, years	9 (0.5–22)	9 (1–28)	26 (7–34)	0.003
p.Phe508del homozigosity, n (%)	1 (8.3)	23 (9.7)	1 (1.4)	0.043
At least one RFM, n (%)	0 (0)	52 (21.84)	15 (21.1)	0.234
Reason for CF suspicion (only for diagnoses different than NBS)				
Bilateral bronchiectasis, n (%)	8 (66.7)	118 (49.6)	30 (42.2)	0.055
Pancreatitis, n (%)	0 (0)	5 (2.1)	3 (4.2)	0.449
CBAVD, n (%)	0 (0)	24 (10)	18 (25.3)	<0.0001
Respiratory involvement and standard microbiology				
Mean (IQR) FEV1,%	49 (44–76)	70 (51–91)	91 (75–99)	<0.001
FEV1 <80% predicted, n (%)	5 (41.7)	119 (50)	19 (26.7)	0.003(1)
FEV1 <50% predicted, n (%)	3 (25)	45 (18.9)	6 (8.4)	0.057(2)
Chronic infection, n (%)	11 (91.7)	200 (84)	53 (74.6)	0.132
Chronic infection *P. aeruginosa*, n (%)	8 (66.7)	114 (47.9)	24 (33.8)	0.036(3)
Median (IQR) exacerbation previous year	3 (1.25–5.5)	1 (0–3)	1 (0–2)	0.004
Patients with 2+ exacerbations/previous year, n (%)	6 (50)	86 (36.1)	17 (23.9)	0.046(4)
Patients with 3+ exacerbations/previous year, n (%)	6 (50)	66 (27.7)	12 (16.9)	0.010(5)
>1 hospitalization previous year	1 (0.25–2)	0 (0–1)	0 (0–0)	<0.0001(6)
N. IV antibiotic cycles/previous year	1 (0–2.75)	0 (0–1)	0 (0–0)	0.002
Extra-Respiratory involvement				
Pancreatic insufficiency, n (%)	12 (100)	153 (64.2)	24 (33.8)	0.003
CF related diabetes, n (%)	6 (50)	67 (28.1)	11 (15.5)	0.017
CF related liver disease, n (%)	3 (25)	108 (45.4)	42 (59.1)	0.035
Osteopenia/osteoporosis, n (%)	7 (70)	109 (47.2)	18 (25.7)	0.001
Cholesterolemia, mg/dL	157 (143–183)	154 (128–188)	187 (136–218)	0.007
Systemic hypertension	1 (8.3)	11 (4.6)	9 (12.7)	0.025
Outcomes				
Waiting list for lung transplantation, n (%)	2 (16.7)	7 (2.9)	1 (1.4)	0.018

(1) OW VS. NW p-value= 0.002
(2) OW VS. NW p-value= 0.055
(3) OW VS. NW p-value= 0.030
(4) OW VS. NW p-value= 0.073
(5) OW VS. NW p-value= 0.061
(6) OW VS. NW p-value= 0.003
Definitions. CBAVD: Congenital Bilateral Absence of Vasa Deferentes; MSSA: Methicillin Sensitive Staphilococcus aureus; BCC: Burkholderia Cepacia Complex.

Table 2
Multivariate logistic regression analysis of predictive factors of OW status in the study cohort.

Variable	OR (95% CI)	P
Male sex	3.520 (1.699 - 7.291)	0.001
Age at diagnosis	1.015 (0.992 - 1.039)	0.042
Pancreatic sufficiency	2.873 (1.237 - 6.676)	0.014

This study succeeded to validate previous findings about the association between OW status and clinical characteristics as male sex and pancreatic sufficiency. However, the comparison with other cohorts is limited by the fact that most of studies included children and patients with severe malnutrition, where the prevalence of OW status might have been underestimated. In 2013 Panagopoulou reported on a OW prevalence of 13.2% in 68 CF patients attending a single center in Greece; however, median age was low (19.8 years) and 54.4% of the cohort included children [13]. Then, Kastner-Cole found an OW prevalence of 9.1% in a cohort of 3000 patients (mean [range] age 16.1 [0.8–55.7] years) of the UK patient registry: OW prevalence in the adult subgroup was lower than ours, with 12.8% and 5.3% in men and in women, respectively [14]. This discrepancy can be attributed to the inclusion of only patients with *p.Phe508del* homozygosity, which is associated with PI and severe nutritional outcomes. On the other side, the Italian prevalence appears underestimated if compared with that of the USA. Harindhanavudhi found OW in 25.6% and obesity in 6.6% of CF adults [4], that might be partly explained as a reflection of a higher prevalence of overweight/obesity in the general population in the USA versus Italy (OW prevalence: 36.2% VS. 19.9%) [15].

Better lung function is associated with OW [4-6]. Harindhanavudhi reported that pulmonary exacerbations decrease with an increasing BMI [4]. We showed that OW is associated with a lower rate of hospitalizations, as well as with a lower prevalence of frequent exacerbators. The pathogenesis of this relationship is unclear but similar effects were described in other chronic respiratory diseases, such as COPD. The underlying mechanisms are likely to be multifactorial and might include difference in body composition with greater muscular mass and adiponectin-mediated modulation in the expression of pro-inflammatory mediators, as TNF-alpha and metalloproteinases [16,17]. As previously noted, we reported a higher prevalence of OW in men [4,6], in contrast with what is in the overall population where the prevalence is higher in females [18], indirectly supporting the findings of worse clinical outcomes in female CF patients [19]. PI and CFRD are less prevalent in OW. The low prevalence of CFRD in the OW group might reflect the strong correlation between CFRD and PI, which are frequently found in *p.Phe508del* patients [20]. A lower prevalence of *p.Phe508del* homozygosity was described in our OW group. The OW group show a higher frequency of cardiovascular risk factors (e.g., high total cholesterol levels and systemic hypertension); however, the prevalence of cardiovascular complications is low if compared with the general population, probably explained by the lower median age (<40 years). Liver disease is more prevalent in the OW group [21]: CFLD might depend on CFTR-mediated patho-

logical mechanism and metabolic complications of long-term hypercaloric and hyperproteic diet.

The main strength of the study is the full characterization of an Italian, large-sized, multi-center cohort of CF adults with OW. However, the retrospective nature of the study design is a limitation affecting the assessment of the risk factors for OW. Regional differences in the burden of OW and the retrospective definition of pancreatic insufficiency might affect the pooled results. The higher prevalence of RFM makes our result less generalizable.

5. Conclusions

This study focuses on a new CF population group. In view of the longer survival of CF patients, we cannot exclude that an aging population after years of exposure to abnormal inflammation and several cardiovascular risk factors could face the onset of cardiovascular complications. However, CF mortality is still secondary to progressive lung disease and the protective effect of increased BMI on FEV1 has to date been an advantage in clinical management. The advent of new CFTR modulators has the potential to change this scenario in terms of significant improvement in both respiratory and nutritional outcomes. As a consequence, an innovative clinical perspective may be needed right now based on two points: a new management of dietary interventions, which takes into account that patients are expected to live longer and be less sick; and the proactive screening and early correction of risk factors for cardiovascular diseases.

Author Contributions: Conceptualization: AG, SA, DS, FM. Data curation and formal analysis: AG, MC, MP. Methodology: GS, LS, VL, PP, FB. Writing - original draft: AG, MC. Writing - review and editing: AG, DS, FM. All authors read and approved the final manuscript.

Funding: not applicable

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by local Institutional Ethical Committee, followed by the approval of the other local ethical committees.

Credit author statement

- Andrea Gramegna: Conceptualization; Data curation and formal analysis; Writing - original draft; Writing - review and editing
- Stefano Aliberti: Conceptualization; Supervision
- Martina Contarini: Data curation and formal analysis; Writing - original draft
- Daniela Savi: Conceptualization; Writing - review and editing; Writing - review and editing
- Giovanni Sotgiu: Methodology;
- Fabio Majo: Conceptualization; Writing - review and editing
- Laura Saderi: Methodology;
- Vincenzina Lucidi: Methodology; Supervision
- Francesco Amati: Data curation and formal analysis; Writing - review and editing
- Maria Pappalettera: Data curation and formal analysis;
- Paolo Palange: Methodology; Supervision
- Francesco Blasi: Methodology; Supervision

Declaration of competing of interest

The authors declare no conflict of interest.

CRediT authorship contribution statement

Andrea Gramegna: Conceptualization, Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **Stefano Aliberti:** Conceptualization, Supervision. **Martina Contarini:** Data curation, Formal analysis, Writing – original draft. **Daniela Savi:** Conceptualization, Writing – review & editing. **Giovanni Sotgiu:** Methodology. **Fabio Majo:** Conceptualization, Writing – review & editing. **Laura Saderi:** Methodology. **Vincenzina Lucidi:** Methodology, Supervision. **Francesco Amati:** Data curation, Formal analysis, Writing – review & editing. **Maria Pappalettera:** Data curation, Formal analysis. **Paolo Palange:** Methodology, Supervision. **Francesco Blasi:** Methodology, Supervision.

Acknowledgments

Not applicable.

References

[1] Elborn JS. Cystic fibrosis. Lancet 2016;388(10059):2519–31. doi:10.1016/S0140-6736(16)00576-6.
[2] Stephenson AL, Mannik LA, Walsh S, et al. Longitudinal trends in nutritional status and the relation between lung function and BMI in cystic fibrosis: a population-based cohort study. Am J Clin Nutr 2013;97(4):872–7. doi:10.3945/ajcn.112.051409.
[3] Hanna RM, Weiner DJ. Overweight and obesity in patients with cystic fibrosis: a center-based analysis. Pediatr Pulmonol 2015;50(1):35–41. doi:10.1002/ppul.23033.
[4] Harindhanavudhi T, Wang Q, Dunitz J, Moran A, Moheet A. Prevalence and factors associated with overweight and obesity in adults with cystic fibrosis: a single-center analysis. J Cyst Fibros 2020;19(1):139–45. doi:10.1016/j.jcf.2019.10.004.
[5] McCarthy C, Dimitrov BD, Meurling IJ, Gunaratnam C, McElvaney NG. The CF-ABLE score: a novel clinical prediction rule for prognosis in patients with cystic fibrosis. Chest 2013;143(5):1358–64. doi:10.1378/chest.12-2022.
[6] Nkam L, Lambert J, Latouche A, Bellis G, Burgel PR, Hocine MN. A 3-year prognostic score for adults with cystic fibrosis. J Cyst Fibros 2017;16(6):702–8. doi:10.1016/j.jcf.2017.03.004.
[7] Bell SC, Mall MA, Gutierrez H, et al. The future of cystic fibrosis care: a global perspective. Lancet Respir Med 2020;8(1):65–124. doi:10.1016/S2213-2600(19)30337-6.
[8] https://www.who.int/data/gho/data/themes/theme-details/GHO/body-mass-index-(bmi)?introPage=intro_3.html
[9] Farrell PM, White TB, Ren CL, et al. Diagnosis of Cystic Fibrosis: consensus Guidelines from the Cystic Fibrosis Foundation [published correction appears in J Pediatr. 2017 May;184:243]. J Pediatr. 2017 181S:S4-S15.e1. doi:10.1016/j.jpeds.2016.09.064.
[10] Lee TW, Brownlee KG, Conway SP, Denton M, Littlewood JM. Evaluation of a new definition for chronic Pseudomonas aeruginosa infection in cystic fibrosis patients. J Cyst Fibros 2003;2(1):29–34. doi:10.1016/S1569-1993(02)00141-8.
[11] Fuchs HJ, Borowitz DS, Christiansen DH, et al. Effect of aerosolized recombinant human DNase on exacerbations of respiratory symptoms and on pulmonary function in patients with cystic fibrosis. The Pulmozyme Study Group. N Engl J Med 1994;331(10):637–42. doi:10.1056/NEJM199409083311003.
[12] Rowe SM, Daines C, Ringshausen FC, et al. Tezacaftor-Ivacaftor in Residual-Function Heterozygotes with Cystic Fibrosis. N Engl J Med 2017;377(21):2024–35. doi:10.1056/NEJMoa1709847.
[13] Panagopoulou P, Fotoulaki M, Nikolaou A, Nousia-Arvanitakis S. Prevalence of malnutrition and obesity among cystic fibrosis patients. Pediatr Int 2014;56(1):89–94. doi:10.1111/ped.12214.
[14] Kastner-Cole D, Palmer CN, Ogston SA, Mehta A, Mukhopadhyay S. Overweight and obesity in deltaF508 homozygous cystic fibrosis. J Pediatr 2005;147(3):402–4. doi:10.1016/j.jpeds.2005.06.003.
[15] WHO Global health observatory; 2016. https://ourworldindata.org/obesity .
[16] Goto T, Hirayama A, Faridi MK, Camargo CA Jr, Hasegawa K. Obesity and severity of acute exacerbation of chronic obstructive pulmonary disease, 15. Ann Am Thorac Soc; 2018. p. 184–91. PMID: 29053337. doi:10.1513/AnnalsATS.201706-485OC.
[17] Hanson C, Rutten EP, Wouters EF, Rennard S. Influence of diet and obesity on COPD development and outcomes. Int J Chron Obstruct Pulmon Dis 2014 Aug 5;9:723–33 PMID: 25125974; PMCID: PMC4130708. doi:10.2147/COPD.S50111.
[18] Kanter R, Caballero B. Global gender disparities in obesity: a review. Adv Nutr 2012;3(4):491–8 Published 2012 Jul 1. doi:10.3945/an.112.002063.
[19] O'Connor GT, Quinton HB, Kahn R, et al. Case-mix adjustment for evaluation of mortality in cystic fibrosis. Pediatr Pulmonol 2002;33(2):99–105. doi:10.1002/ppul.10042.
[20] Granados A, Chan CL, Ode KL, Moheet A, Moran A, Holl R. Cystic fibrosis related diabetes: pathophysiology, screening and diagnosis. J Cyst Fibros 2019;18(Suppl 2) S3-S9. doi:10.1016/j.jcf.2019.08.016.
[21] McKone EF, Emerson SS, Edwards KL, Aitken ML. Effect of genotype on phenotype and mortality in cystic fibrosis: a retrospective cohort study. Lancet 2003;361(9370):1671–6. doi:10.1016/S0140-6736(03)13368-5.