ABSTRACT

Background:

Cystic fibrosis (CF) is one of the most common autosomal recessive genetic diseases caused by mutations in the intramembrane conductance regulator - a protein that forms a chloride channel in the cell membrane - CFTR. CFTR protein disorders lead to a wide range of liver and biliary diseases, referred to as cystic fibrosis liver disease (CFLD). CFTR modulators are drugs that bind to the CFTR protein and increase chloride conductance in the cell membrane. The introduction of CFTR modulators has revolutionized the treatment of CF, changed the lives and prognosis of patients, contributing to improving the quality of life and slowing the progression of changes in the course of CF.

The presented scientific work uses two elastography methods: SWE (Shear Wave Elastography) and MRE (Magnetic Resonance Elastography) to assess liver damage in patients with CF before and after treatment with CFTR modulators. MRI also allows for quantitative measurement of liver steatosis (%) using the "IDEAL-IQ" technique. There are no scientific reports in the world literature using two elastography techniques (SWE and MRE) simultaneously in patients with CF after treatment with CFTR modulators.

Aim of the study:

The primary aim of the presented study is to use elastography as a key method in the non-invasive diagnosis of liver damage and fibrosis in patients with CF, primarily as an alternative to biopsy, as well as to optimize diagnosis and treatment. Specific aims:

1. Assessment of liver changes in patients with CF using the SWE, MRE and MR "IDEAL-IQ" methods and comparison of the obtained results with the control group
2. Assessment of liver changes in CF patients after treatment with CFTR modulators compared to tests performed before the initiation of treatment using SWE and MRE methods and the assessment of liver steatosis in the MR "IDEAL-IQ" study
3. Evaluation of monitoring liver damage in patients with CF using SWE and MRE non-invasive elastography methods

Material and methods:

The study included 84 participants: 41 (48,8%) patients with with genetically confirmed CF, 22 female (53,66%) and 19 male (46,34%), aged 5–39 years (average 19,95 years), who were treated and diagnosed in the years 2020–2022 at the Allergology and Cystic Fibrosis Clinic of St. Jadwiga Queen Provincial Hospital No. 2 in Rzeszów, Poland or at the Cystic

Fibrosis Hospital Outpatient Clinic. The control group consisted of 43 healthy volunteers (51,2% of the study): 28 female (65,12%) and 15 male (34,88%) in the age range of 8–39 years (average 20,93), in whom no liver diseases were diagnosed. Liver stiffness was measured by SWE (in all study participants, in the CF group before starting treatment with CFTR modulators) and MRE (in all volunteers and 27 CF patients before starting treatment with CFTR modulators).

In the MR examination, the degree of liver steatosis was measured using the "IDEAL-IQ" technique. MRE was performed up to 3 months after the SWE examination. Stiffness measurement was expressed in kPa (SWE and MRE), the degree of liver steatosis in % (MR "IDEAL-IQ".

The SWE control study included 38 of 41 initially studied CF patients (19 female – 50% and 19 male – 50%), aged 8–42 years (mean 22,39). A total of 30 patients (78,95%) of the study group were treated with CFTR modulators - treated group (14 female – 46,67%, 16 male – 53,33% aged 8–42 years, mean age 21 years), while 8 patients (21,05%) did not receive this therapy – control group, including 5 female – 62,5%, 3 male – 37,5%, aged 11–42 years, mean age 27 years). In the treatment group, the control SWE was performed between 28 and 54 months after the previous SWE (mean 36,6 months). The time since treatment was initiated to the control SWE ranged from 12 to 33 months (mean 29,9 months).

Follow-up MRE was performed in 24 CF patients out of 27 examined at baseline; however, 22 examinations were included in the analysis (12 female – 54,54% and 10 male – 46,46%, aged 11– 42 years, mean age 24 years at follow-up examination). 17 patients from the study group (77%) were included in CFTR modulator treatment (9 female – 53%, 8 male – 47%, aged 14–35 years, mean age 22 years), 5 patients (23%) were not included in CFTR modulator treatment. All patients underwent measurement of the degree of fatty liver disease (MR “IDEAL-IQ”).

Age, gender, BMI, laboratory test results (AspAt, AlAt, GGTP), spirometry, both at baseline and follow-up were used for the analysis in the CF group.

Results:

SWE showed higher median liver stiffness in the whole CF group (Mdn = 5,27 kPa; IQR: 4,26 kPa–6,81 kPa) compared to the control group (Mdn = 4,14 kPa; IQR: 3,43 kPa–4,86 kPa), ($p < 0.001$). The prevalence of liver stiffness >5 kPa (SWE) in the CF group was 41,46% in the control group 0%; $p<0,001$. In the group of volunteers <18 years of age higher median liver stiffness was demonstrated in SWE, Mdn = 4,66 kPa (IQR 4.11 kPa–4.99 kPa), compared

to the group of volunteers >18 years of age, Mdn = 3,78 (IQR 3.39 kPa–4.55 kPa), (p – 0.019), however, in both groups liver stiffness remained within the normal range. SWE measurements in the group of patients with CF did not show a statistically significant difference between patients <18 years of age (Mdn=4,91 kPa; IQR: 4,63–5,66 kPa) and >18 years of age (Mdn=5,42 kPa; IQR: 3,75 –7,25 kPa), (p=0,885).

MRE did not show a significant difference in liver stiffness between the entire CF group and the control group (p – 0.764). However, MRE confirmed an increased median liver stiffness in CF patients <18 years of age (Mdn = 2,27 kPa; IQR: 1,93 kPa – 2,51 kPa) compared to CF patients >18 years of age (Mdn = 1,74 kPa; IQR: 1,56 kPa – 2,06 kPa), (p = 0.017).

The median MR "IDEAL-IQ" liver steatosis in the CF group was Mdn = 5,90% (IQR: 3.00–16.25%), in the control group Mdn = 2.58% (IQR: 1,80–3,20%); (p <0.001). CF patients <18 years of age show higher values of fatty liver (%) in the MR study "IDEAL-IQ", Mdn = 14,90% (IQR: 12,30 – 24,10%), compared to patients >18 years of age, Mdn = 4.75% (IQR: 2,65 – 7,33%), (p = 0.048). The prevalence of MR "IDEAL-IQ" liver steatosis ≥ 6.5% in all CF patients was 48,15%, while in CF patients <18 years of age – 77.78%, in adults >18 years of age with CF – 33,33%; p = 0,046.

No association was found between fibrosis verified in the MRE study and steatosis (95% CI: - 0,02–0,03; p – 0.639). Only age in years and belonging to the age group <18 years of age emerge as significant predictors of the incidence of liver fibrosis (OR = 0,83; 95% CI 95%: 0,68 – 0,95; p = 0,023).

The above study shows that SWE combined with an additional assessment method such as MRE increases the chances of detecting liver abnormalities and allows for even more effective exclusion of CFLD. The best performance results were obtained for the MRE threshold >2,7 kPa with a SWE cut-off point of 11,06 kPa (sensitivity – 0.67, specificity – 0.92, and the sum of sensitivity and specificity 1,58, with the highest AUC 0,86), which indicates very good discriminatory ability of SWE in identifying fibrosis at this threshold. The accuracy of SWE in detecting liver fibrosis for MRE >2,7 kPa was 0.89. For the MRE threshold >1,9 kPa, the cut-off point was 4,86 kPa (sensitivity – 0,79, specificity – 0,77 and AUC – 0,76), which may be useful in screening.

The SWE follow-up study after CFTR modulator treatment did not show any statistically significant change in liver stiffness or significant differences between the treatment and control groups at any visit (baseline and follow-up). At the first visit, the median liver stiffness was slightly higher in the treatment group Mdn = 4,91 kPa (IQR: 4,35 kPa – 6,06 kPa) compared to the control group Mdn = 4,39 kPa (IQR: 3,43 kPa – 5,33 kPa), (p = 0,204). At the

second visit, liver stiffness values remained similar between groups, with Mdn = 4,93 kPa (IQR: 4,09 kPa–6,00 kPa) in the treatment group and Mdn = 4,87 kPa (IQR: 2,72 kPa–6,86 kPa) in the control group (p = 0,463). Similarly, within-group analysis did not show a significant difference in change in liver stiffness in SWE after treatment (baseline Mdn = 5,12 kPa, IQR: 4,35 kPa–6,45 kPa; post-treatment Mdn = 4,93 kPa, IQR 4,09 kPa–6,00 kPa), (p = 0,590).

Analysis including only treated patients showed a decrease in the number of patients with liver enlargement on ultrasound from 15 (50.0%) to 8 (26.7%) patients, p = 0,043. In the treated group, there was a significant increase in median FEV1% from Mdn = 90.50% (IQR: 71.25–100.25%) to Mdn = 98.00% (IQR: 82,50–112,25%), (p = 0,010) and an increase in median FEV1/FVC% from Mdn = 81,72% (IQR: 72,67–94,50%) to Mdn = 96.00% (IQR: 81,75–98,75%), (p = 0.001). Analysis including only the treatment group showed an increase in median BMI (kg/m²) from Mdn = 18,77 kg/m² (IQR: 17,32–21,42 kg/m²) to Mdn = 20,63 (IQR: 19,44–23,94 kg/m²), (p < 0,001).

There were no significant changes in the frequency of hepatic enlargement or steatosis and splenomegaly verified by routine ultrasound examination before and after treatment between the treatment and control groups. There were no significant differences in the change of liver markers AspAt and AlAt between the study and control groups comparing the baseline examination and the follow-up examination.

Analysis of changes in liver stiffness using a multivariate approach using the RLM model (linear regression model) did not show any influence on the change in stiffness of factors such as mutation type, age, gender, BMI, CFRD, fatty liver in the baseline US, liver fibrosis or splenomegaly in the baseline US. Also, the time interval between the baseline and follow-up examination and the time interval between the start of treatment and the follow-up SWE examination did not significantly influence the change in liver stiffness in the SWE examination.

Only the presence of hepatomegaly at the first visit proved to be a borderline protective factor (B = -1.45 kPa; 95% CI: -2.90 – 0.00; p = 0.049), indicating that patients with hepatomegaly at baseline were more likely to experience a decrease (or a smaller increase) in liver stiffness over time compared to patients without hepatomegaly.

Analysis of changes in clinical parameters before and after treatment in a group of 17 patients treated with CFTR modulators included in the control MRE study showed a significant change in liver stiffness MRE, where the median value increased significantly from Mdn = 1,88 kPa (IQR: 1,59 kPa–2,28 kPa) to Mdn = 2,54 kPa (IQR: 2,28 kPa–2,72 kPa), (p = 0.001). Based on the regression analysis, no influence of factors such as gender, time since the initiation of

treatment and time between the baseline and control MRE examination on liver stiffness and steatosis (MRE and MR "IDEAL-IQ") was demonstrated.

In the control spirometry after treatment, an increase in the FEV1% parameter was noted, from Mdn = 82,00% (IQR: 70,00–96,00%) to Mdn = 99,00% (IQR: 87,00–114,00%), (p = 0,007). A significant improvement was demonstrated in the change of body mass index (BMI), which increased from Mdn = 18,16 kg/m² (IQR: 17,41–19,36 kg/m²) to Mdn = 21,55 kg/m² (IQR: 19,43–23,74 kg/m²), (p = 0.001), the percentage of patients with malnutrition (BMI<18,5 kg/m2) decreased, falling from 52,94% to 5,88% (p = 0,013). The remaining assessed parameters, including splenomegaly, hepatomegaly, hepatic steatosis and fibrosis assessed by imaging methods (US, SWE, MR "IDEAL-IQ"), liver enzyme levels (AspAT, AlAT) and the FEV1/FVC% ratio, did not show statistically significant changes after treatment (p >0,05).

Conclusions:

1. Assessment of liver stiffness based on SWE and MRE results allows differentiation between CF patients with liver damage and those with a healthy liver. The incidence of liver stiffness >5 kPa in SWE is statistically significantly higher in the CF group compared to the group of healthy volunteers. CF patients <18 years of age have an increased risk of liver fibrosis based on MRE results and greater liver steatosis in the MR "IDEAL-IQ" study compared to the group of CF patients >18 years of age and the group of healthy volunteers.
2. Effective CFTR modulator therapy in patients with CF increases the need for non-invasive methods to detect gradual changes in the severity of CFLD. In the context of CF-related liver disease, each patient effectively serves as their own control, and changes in liver stiffness over time are used to assess treatment efficacy or disease progression. The SWE study did not demonstrate statistically significant changes in the liver that occurred after treatment with CFTR modulators. The results of the MRE analysis showed a statistically significant increase in liver stiffness at follow-up after treatment with CFTR modulators and may impact clinical practice by suggesting the need for further studies. In addition, the study showed that the presence of liver enlargement at baseline before initiation of causative therapy can be considered as a factor indicating an improvement in liver image after treatment with CFTR modulators, as patients with liver enlargement at baseline were more likely to have

a decrease (or a smaller increase) in liver stiffness after treatment compared with patients without liver enlargement.

3. Non-invasive SWE and MRE methods enable the assessment of the liver in patients with CF. Analysis using SWE and MRE measurements offers valuable information on liver damage in the course of CF. Combining SWE with an additional method such as MRE increases the possibility of effectively excluding CFLD. Quantitative measurement of liver stiffness in kPa (SWE and MRE) and steatosis expressed in % (MR "IDEAL-IQ") allows for more precise observation of changes that occur over time, and the patient becomes his own control and reference point.
4. The study confirmed previous reports, a statistically significant increase in BMI after treatment and improvement in respiratory parameters FEV1% and FEV1/FVC% were noted.