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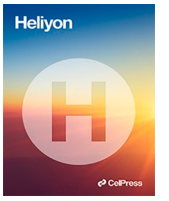

Case report

Macroenzymes as a reason for aminotransferases flare in cystic fibrosis patients on CFTR modulators therapy – Report of three cases

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ABSTRACT

It has been shown that macro-ALT/macro-AST cause false increase of ALT/AST activity in standard laboratory testing.

This short communication presents a group of cystic fibrosis subjects who developed aminotranferases flare a few months after initiation of CFTR modulators therapy. Patients did not present any clinical signs or symptoms of liver failure and differential examination did not show any underlying liver disease. All patients tested positive for macro-ALT and macro-AST. Despite increased and fluctuating ALT/AST activity in standard tests patients restarted CFTR modulators therapy with good clinical effect and do not present any other than hypertransaminasemia signs or symptoms of progressing liver disease.

Our data shows that ALT/AST flare during CFTR modulators therapy may be related to macro-ALT/macro-AST, thus patients with high ALT/AST during CFTR modulators therapy should be tested for macro-ALT/macro-AST.

1. Introduction

Cystic Fibrosis Transmembrane Regulator (CFTR) modulators became standard treatment for patients with cystic fibrosis (CF). It is recommended to monitor CFTR modulators therapy with liver function tests every three months because of the risk of increased aminotransferases [1]. In some subjects hypertransaminasemia may be associated with increase of bilirubin concentration. According to CFTR product characteristics treatment should be discontinued when aminotransferases are >5 x ULN (Upper Limit of Normal) or >3 x ULN with bilirubin >2 ULN [2]. After normalization of aminotransferases activity, the CFTR modulators therapy may be re-challenged in the reduced dose and under careful laboratory control of the liver function. There is one report describing liver failure requiring liver transplantation in patient who underwent ivacaftor + tezacaftor + elexacaftor (IVA + TEZ + ELX) combined with

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ivacaftor (IVA) therapy [3]. The patient had pretreatment liver cirrhosis and possibly autoimmune hepatitis, thus CFTR treatment should be used with care in patients with advanced CFLD (Cystic Fibrosis related Liver Disease) presenting as liver cirrhosis or portal hypertension.

CFTR modulators have spectacular effect on pulmonary function and life quality of CF patients [4]. This effect is not permanent and is reversed after discontinuation of CFTR modulators therapy. For that reason patients who started this therapy have very high treatment compliance and are afraid of disease relapse after cessation of this modern therapy [5].

Abnormal liver ultrasound (USG) and increased activity of alanine aminotransferase (ALT) and asparagine aminotransferase (AST) are not infrequent conditions in subjects with cystic fibrosis [6]. Recently published Irish study with the longitudinal observation of almost 500 children with cystic fibrosis showed that different stage CFLD affects 30 % of subjects, but only 7 % had advanced liver disease with portal hypertension. The remaining patients had ultrasound features of liver fibrosis or biochemical liver function tests abnormalities only. The incidence of CFLD was highest in children under 10 years. Those aged over 10 years, with normal hepatic function, did not develop severe CFLD [7]. In this situation the flare of aminotrasferases activity during CFTR modulators therapy can be interpreted as the evidence of medication toxicity and may trigger treatment discontinuation [8].

The aim of this short report is presentation of the group of CF subjects with sudden aminotrasferases flare during CFTR modulators treatment that were neither related to CFLD deterioration nor to medication toxicity and did not progress to liver failure.

2. Material

Data for this report was collected from three different centres caring for CF patients. All sites provide the CFTR modulators treatment within therapeutic programme for cystic fibrosis patients financed either by Polish government or within clinical trials. Patients participating in Polish governmental program must fulfil inclusion criteria specified by Polish Ministry of Health and Polish National Health Fund [9] and receive medication according product characteristics [2].

The total number of CF patients observed at these three sites at the moment of data collection was 495. 306 of subjects were treated with CFTR modulators either within governmental program or within clinical trials. All subjects treated with CFTR modulators have liver function tests every three months during the first year of treatment and once a year afterwards. Within this group of patients many continued therapy with mild fluctuation of ALT/AST activity but marked ALT/AST flare resulting in the need for CFTR modulators therapy modification or discontinuation occurred in 3 female patients only.

3. Case series presentation

Characteristics of the three patients with ALT/AST flare during CFTR modulator therapy is provided in Table 1. None of our index patients had pre CFTR treatment history of hypertransaminasemia, hyperbilirubinemia, or advanced CFLD with portal hypertension, splenomegaly or hypersplenizm. Two patients had mild ultrasound features of liver involvement (increased echogenicity) but only one of them had slightly increased liver stiffness.

Patients 1 and 3 were treated within therapeutic programme financed by Polish government according to IVA + TEZ + ELX combined with IVA protocol. Patient 2 received CFTR modulators within clinical study.

Aminotransferases flare occurred within the first 3 months of the therapy. The possible triggering factor in patient 1 was the discontinuation of low dose of steroids administered for underlying rheumatoid arthritis. Patient 3 experienced symptoms of viral infection preceding aminotransferases flare. Patient 2 had no medical events other than CFTR modulators therapy that could possibly

Table 1
Subjects characteristics.

		Patient 1	Patient 2	Patient 3
Sex		Female	Female	Female
Age (yrs)		28	16	38
Concomitant diseases		Rheumatoid arthritis	Chronic sinusitis Glucose intolerance	Bronchial asthma, Chronic sinusitis, Diabetes mellitus
EPI[a]		YES	YES	NO
History of CFLD	LFT[b] abnormalities	NO	NO	NO
	USG liver echogenicity	Increased	Increased	Normal
	Liver stiffness (kPa)	4,8	9,6	4,05
	Portal hypertension	NO	NO	NO
Pretreatment ALT (ULN)		13 U/l (31 U/l)	48 U/l (20 U/l)	18 U/l (34 U/l)
Pretreatment AST (ULN)		13 U/l (31 U/l)	36 U/l (32 U/l)	15 U/l (31 U/l)
CFTR modulators	Type	IVA + TEZ + ELX/IVA	Clinical trial VX20-121-103[c]	IVA + TEZ + ELX/IVA
Time from start of CFTR modulators to ALT/AST flare (days)		122	83	91
Max ALT (ULN)		288 U/l (31 U/l)	211 U/l (20 U/l)	272 U/l (34 U/l)
Max AST (ULN)		201 U/l (31 U/l)	74 U/l (32 U/l)	156 U/l (31 U/l)

[a] EPI – Exocrine Pancreatic Insufficiency.
[b] LFT – Liver Function Tests.
[c] double blind treatment with elexacaftor/tezacaftor/ivacaftor or VX-121/tezacaftor/deutivacaftor.

interfere with liver function tests.

After detection of aminotansferases flare CFTR modulators therapy was stopped and all patients underwent differential work-up for other underlying liver diseases according to European Association for the Study of the Liver (EASL) guidelines for Drug Induced Liver Injury (DILI) [10]. The work-up did not reveal any underlying liver disease. Patients tested negative for hepatotropic (HAV, HBV, HCV) and nonhepatotropic (EBV, CMV, HIV) viral infections. Patient had no laboratory parameters for autoimmune hepatitis, alfa-1-antyprotease deficiency, Wilson's disease or abnormal iron storage.

Despite high aminotransferases activity, all patients were asymptomatic. They had no laboratory signs of cholestasis (normal bilirubin and gamma glutamylo transpeptidase). Ultrasound examination did not show progression of liver disease or signs of portal hypertension and liver synthetic function was well preserved (normal prothrombin time, normal protein and albumin concentrations).

At that moment patients' blood samples were tested for the presence of ALT/AST macroenzymes according to Davidson and Watson [11] and the results were positive for all subjects (Table 2). All patients restarted CFTR modulators therapy within one or two months from macroenzymes detection. Patient 2 discontinued the participation in CFTR modulators clinical study and started treatment within the Polish governmental program with IVA + TEZ + ELX combined with IVA protocol. All patients continue CFTR modulators therapy with fluctuating aminotransferases activity but without further liver function deterioration (Fig. 1). All subjects have sustained marked pulmonary function improvement.

4. Discussion

It is well known that cystic fibrosis may cause liver disease leading to increase of ALT and AST activity [7]. However it is unlikely that patients described in this report had CFLD because they had normal liver stiffness, history of normal liver function tests and no portal hypertension. Two patients had medical occurrence that potentially might affect liver function, but to our knowledge patients were not exposed to any other hepatotoxic substances except CFTR modulators treatment. ALT/AST flare occurred in relatively short time after introduction CFTR modulators therapy. This therapy is known to be potentially hepatotoxic, so DILI seemed to be the first explanation for increased ALT/AST activity in our subjects.

DILI is unpredictable and challenging liver disorder that can be related to different medication, herbs, dietary supplements with potentially hepatotoxic effect. There are no DILI specific markers and it may present with a different clinical and pathological phenotypes. This makes the diagnosis of drug-induced liver injury an uncertain process, requiring a high degree of awareness of the condition and careful exclusion of alternative aetiologies of liver disease [10]. The only treatment for DILI is a dose reduction or discontinuation of the causative medication.

There were case reports on DILI during CFTR therapy [3,12,13]. However, in the long term observation, CFTR modulators may have beneficial effect on liver function [14] and it may improve markers of liver fibrosis [15]. CFTR modulators treatment may lower the rate of patients progressing to liver cirrhosis in comparison with no treatment or single UDCA therapy [16]. Continuation of CFTR therapy is essential for sustained remission of pulmonary malfunction. Thus the decision about cessation of CFTR modulators therapy due to abnormal aminotransferases must be weighed against several circumstances.

We think that the ALT/AST flare observed in our patients was not the effect of CFLD deterioration or DILI related to CFTR modulators hepatotoxicity but resulted from the induction of macro-ALT and macro-AST. For that reason the decision to restart the CFTR modulators therapy was undertaken in all patients. Within almost one year observation patients had fluctuating ALT/AST activity but none of them manifested any signs or symptoms of liver disease.

Macroenzymes are rarely detected and the prevalence varies from less than 0.1 % to 3.5 % [17]. Macroamylase was the first macroenzyme described in the literature [18]. Now it is known that other enzymes like: AST, ALT, GGTP, ALP, lipase, LDH and CPK may have the form of macroenzymes and the specific laboratory cut-of values were defined [19].

Macroenzymes are big particles resulting from polymerisation or connection of normal enzyme with serum immunoglobulins or lipoproteins. Macroenzymes typically show an increased enzyme plasma activity due to reduced clearance of the high-molecular weight complex. Routine enzyme measurements cannot distinguish macroenzymes from monomeric enzymes, what may cause diagnostic confusion [11,18].

Macroenzymes may be present in healthy subjects [20] and their presence was described in patients with chronic hepatitis C, autoimmune diseases including rheumatoid arthritis, lupus erythematosus, ulcerative colitis, coeliac disease and some neoplastic disorders [21–23].

Detection of macro-ALT or macro-AST requires two simultaneous laboratory tests: standard laboratory aminotransferases evaluation and second test with prior precipitation using 25 % polyethylene glycol (PEG) solution. Both tests are used to calculate PEG

Table 2
Aminotransferases results in standard laboratory assessment and in the test with PEG.

		Patient 1	Patient 2	Patient 3
Macro-ALT (U/l)	Standard test	146 U/l	122 U/l	108 U/l
	Test with PEG	11 U/l	37 U/l	38 U/l
	%PPA	92 %	70 %	64 %
Macro-AST (U/l)	Standard test	112 U/l	71 U/l	111 U/l
	Test with PEG	28 U/l	28 U/l	37 U/l
	%PPA	75 %	61 %	67 %

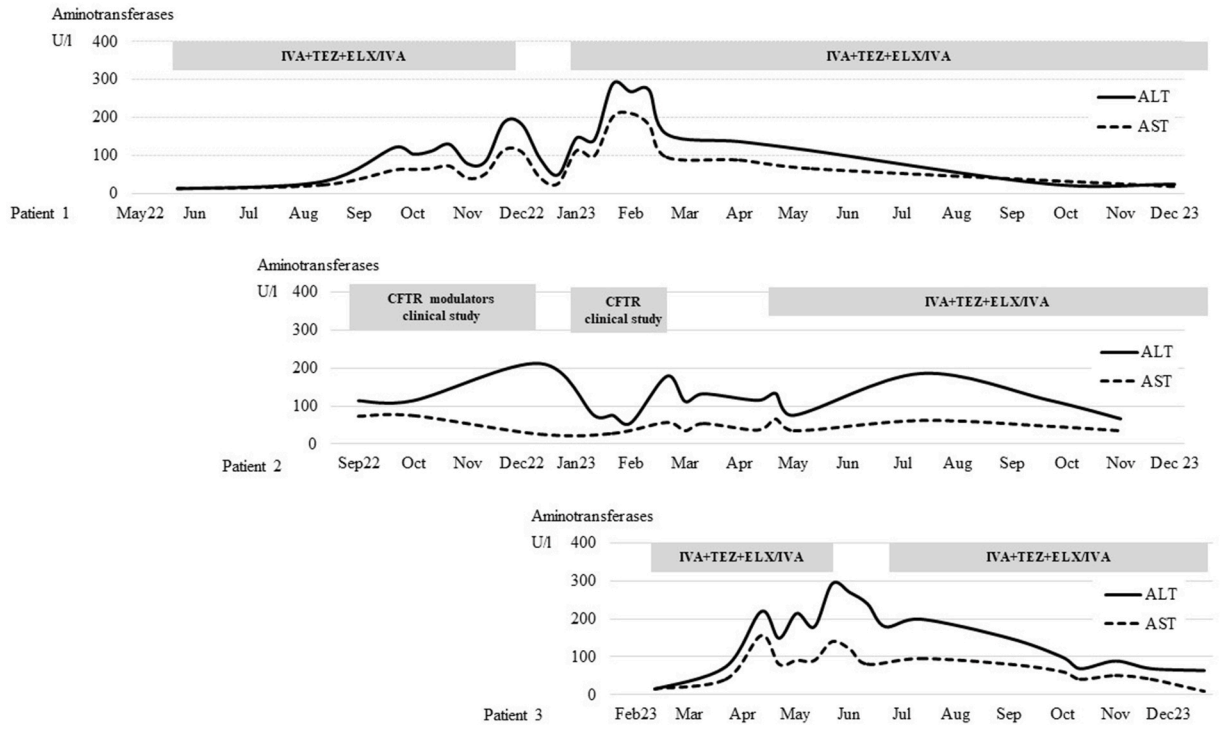

Fig. 1. Fluctuation of aminotransferases in patients 1, 2 and 3.

precipitated activity (%PPA) according to the following formula:

$$\%PPA = 100 \text{ x } \frac{(ALT/AST \text{ in standard test}) - (ALT/AST \text{ in PEG test})}{(ALT/AST \text{ in standard test})}$$

The cut-off values for %PPA confirming the presence of macroenzymes are 74 % for macro-ALT and 55 % for macro-AST according to Davidson and Watson [11] or 61 % for macro-ALT and 48 % for macro-AST according to Dedeene and Stockman [19]. The confirmation of the presence of macro-AST and macro-ALT can be done with electrophoretic separation because macroenzymes have characteristic mobility due to the electrical charge of the subunits [24,25].

Periodical ALT/AST flare is frequent in CF subjects. It is usually explained by CFLD, nonhepatotropic viruses infections or hepatotoxic effect of multiple medication used in these patients [7,26]. Macroenzymes have not yet been considered as a possible cause for liver function tests abnormality. Until now, the only case of CF patients with ALT/AST flare and presence of macroenzymes during CFTR therapy described in the literature [27] is our patient no 1 who is included into this report as well. The detection of macroenzymes in this patients triggered us to look for the same explanation for liver function abnormalities in other patients experiencing ALT/AST flare during CFTR modulators treatment. We concentrated on subjects with high ALT/AST increase with the risk of the need for CFTR therapy discontinuation.

In the treated population we had no other cases of important ALT/AST flare. Our three index patients had macroenzymes detected by two methods (test with PEG and electrophoresis). We do not know if these macroenzymes were induced by CFTR modulators therapy or whether they are related to some other abnormalities present in CF patient's blood. To answer this question we plan to test our population of CF patients with at least mild elevation of ALT/AST for the presence of macroenzymes.

5. Conclusion

To our best knowledge, the presence of macroenzymes has not been studied in patients with cystic fibrosis. Data presented in this short report shows that macroenzymes may be responsible for ALT/AST flare during CFTR therapy and they may mimic liver toxicity of CFTR modulators leading to cessation of the therapy with loss of beneficial effect on pulmonary function and life quality. For that reason it seems reasonable to test patients with the flare of aminotransferases during CFTR modulators treatment for the presence of macroenzymes.

Ethics statement

Informed consent was obtained from all patient(s) (or relative/guardian) for the publication of all clinical data and other data

included in the manuscript.

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Data availability statement

Patient's data associated with this report has not been deposited into a publicly available repository and is only available in this article.

CRediT authorship contribution statement

Marek Woynarowski: Writing – original draft, Validation, Supervision, Methodology, Formal analysis, Data curation, Conceptualization. **Ewa Sapiejka:** Writing – review & editing, Investigation, Data curation. **Maria Jóźwiak:** Writing – review & editing, Investigation, Data curation. **Justyna Milczewska:** Writing – review & editing, Investigation, Data curation. **Katarzyna Zybert:** Writing – review & editing, Investigation, Data curation. **Adam Krusiński:** Writing – review & editing, Investigation, Data curation. **Jan Siwiec:** Writing – review & editing, Investigation, Data curation. **Aldona Wierzbicka-Rucińska:** Writing – review & editing, Methodology, Investigation, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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