MESTRADO INTEGRADO EM MEDICINA – TRABALHO FINAL

INÊS MARGARIDA SANTOS FAUSTINO

From Liver to Muscle: A Retrospective Study of Diagnostic Suspicions and Analytical Changes

Do Fígado Ao Músculo: Um Estudo Retrospetivo de Suspeitas Diagnósticas e Alterações Analíticas

ARTIGO CIENTÍFICO ORIGINAL

ÁREA CIENTÍFICA DE PEDIATRIA

Trabalho realizado sob a orientação de:

DR. FILIPE MANUEL FARTO PALAVRA

DRA. JOANA AFONSO RIBEIRO

ABRIL, 2023

From Liver to Muscle: A Retrospective Study of Diagnostic Suspicions and Analytical Changes

Do Fígado Ao Músculo: Um Estudo Retrospetivo de Suspeitas Diagnósticas e Alterações Analíticas

ARTIGO CIENTÍFICO ORIGINAL

ÁREA CIENTÍFICA DE PEDIATRIA

Inês Margarida Santos Faustino [1]

Filipe Manuel Farto Palavra [2]

Joana Afonso Ribeiro [3]

[1] imsfaustino@gmail.com, Faculty of Medicine, Coimbra University, Portugal

[2] fpalavra@fmed.uc.pt, Centre for Child Development – Neuropediatrics Unit, Hospital Pediátrico, Centro Hospitalar e Universitário de Coimbra, Avenida Afonso Romão, 3000-602 Coimbra, Portugal; Faculty of Medicine, Coimbra University, Portugal

[3] joanaaribeiro87@gmail.com, Centre for Child Development – Neuropediatrics Unit, Hospital Pediátrico, Centro Hospitalar e Universitário de Coimbra, Avenida Afonso Romão, 3000-602 Coimbra, Portugal

Table of Contents

Abbreviations List 5
Abstract 6
Resumo 8
1 - Introduction 10
2 - Materials and Methods 12
2.1 - Study Design 12
2.2 - Population 12
2.3 - Procedures 12
2.4 - Statistical analysis 13
3 - Results 14
4 - Discussion 22
5 - Conclusion 25
Acknowledgments 26
References 27

Abbreviations List

ALL – Acute Lymphocytic Leukemia

ALT – Alanine Transaminase

ASD – Autism Spectrum Disorder

AST – Aspartate Transaminase

BMD – Becker Muscular Dystrophy

CDC – Centro de Desenvolvimento da Criança

CK – Creatine Kinase

CNS – Central Nervous System

DMD – Duchenne Muscular Dystrophy

ED – Emergency Department

EHBA – Extrahepatic Biliary Atresia

IBD – Inflammatory Bowel Disease

MHD – Metabolic Hereditary Disorders

NASH – Nonalcoholic Steatohepatitis

PFIC 1 – Progressive Familial Intrahepatic Cholestasis type 1

PIMS – Pediatric Inflammatory Multisystem Syndrome

PNS – Peripheral Nervous System

PDD – Psychomotor Development Delay

SMA – Spinal Muscular Atrophy

UC – Ulcerative Colitis

UICD – Unidade de Internamento de Curta Duração

Abstract

Introduction: Serum alanine transaminase (ALT) and aspartate transaminase (AST) are commonly used as biochemical indicators of hepatocellular injury. Because transaminases are not unique to hepatocytes, other tissues should be considered as a potential origin, when high enzyme levels are found in serum. This will reduce the risk of misattributing abnormal transaminase levels to nonexistent liver pathology. Serum creatine kinase (CK) is a sensitive indicator of muscle injury and a fast and reliable mean of determining if high transaminase levels are associated with a muscle disease. Muscular dystrophies represent an important subset of neuromuscular disorders. The dystrophinopathies – Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (BMD) – are included in this group of diseases and are characterized by childhood-onset progressive weakness and variable cardiac, respiratory, and cognitive involvement. Therefore, ALT and AST levels can also detect occult muscle disease. Nevertheless, clinicians may be reluctant to attribute high transaminase levels to muscle. Our goal is to understand how this investigation is conducted in our center, and what are the main reasons to request CK concurrently with serum transaminases dosing.

Methods: A retrospective observational study was carried out, for which children aged between 0 and 18 years, with elevated levels of ALT and/or AST and normal total bilirubin, between January 2021 and December 2021, were recruited. Provenance of the analytical study, clinical context in which the analytical study was requested and management after the detection of high AST/ALT – including a concurrently request for CK and consequent neurological follow-up – were analyzed. Descriptive analysis was made.

Results: A sample of 383 children was collected, with a mean age of 9.55 ± 5.7 years, and a male:female ratio of 1.4:1. Regarding the provenance of the analytical study, 250 (65%) came from the outpatient clinic, 93 (24%) were obtained during hospitalization and 40 (11%) from the emergency department. Characterizing each, from 106 studies with high ALT/AST requested by Hepatology, 34 (32.1%) had concurrently requested CK. In Oncology, from 59 analytical studies, only 5 (8.5%) also requested CK. In the ED, there were 40 children with high ALT/AST, and 13 (32.5%) had CK requested. Nine out of 17 (53%) patients from Centre for Child Development had CK requested. And 5 out of 13 (38.5%) general Pediatrics hospitalizations had CK measured. From the total of 383 children with high ALT/AST, 18 had concurrently elevated CK, 3 (16.7%) related with neurological or metabolic disease. We found 8 children without definitive etiology for the elevation of the transaminases or CK.

Conclusion: In the absence of clinical context to justify the elevation of serum transaminases, pediatric hepatologists see elevated enzyme serum activity as possible

indicator of a muscle disorder and request CK dosing. In other specialties, CK is only measured when muscle manifestations are present or if a neurodevelopment study is in place. However, we were still able to find children followed in Hepatology and in Neurodevelopment consultations that had elevated transaminases, no definitive diagnosis and still did not have CK requested. These findings indicate a need to raise awareness when it comes to extra-hepatic sources of transaminases elevation, such as the muscle.

Keywords: dystrophinopathies, muscle, transaminases, creatine kinase.

Resumo

Introdução: A alanina aminotransferase (ALT) e a aspartato aminotransferase (AST) são usadas como indicadores de lesão hepatocelular. No entanto, dado que estas enzimas não existem apenas nos hepatócitos, outros tecidos devem ser considerados quando existem níveis séricos elevados de transaminases, reduzindo o risco de atribuir esta elevação a patologia hepática não existente. A creatina cinase (CK) é um indicador sensível de lesão muscular, tornando-se assim um meio rápido e fiável de determinar se níveis elevados de transaminases se associam a possível doença do músculo. As distrofias musculares representam um subgrupo importante das doenças neuromusculares. As distrofinopatias – distrofia muscular de Duchenne e distrofia muscular de Becker – incluem-se neste grupo e são caracterizadas por fraqueza muscular progressiva, de início na infância, com envolvimento cardíaco, respiratório e cognitivo variável. Deste modo, ALT e AST podem também detetar doença muscular. Todavia, os médicos mantêm-se relutantes no que toca a atribuir a elevação da actividade sérica destas enzimas ao músculo. O nosso objetivo foi perceber a forma como esta investigação é conduzida no nosso centro e quais são as principais razões que motivam a determinação da atividade sérica de CK em concomitância com a das transaminases.

Métodos: Foi realizado um estudo observacional retrospetivo, para o qual se recrutaram crianças com idades compreendidas entre os 0 e 18 anos, com ALT e/ou AST elevadas e bilirrubina total normal, entre Janeiro 2021 e Dezembro 2021. De seguida, foi analisada a proveniência do estudo analítico, o contexto clínico que o motivou e a conduta após a deteção de transaminases elevadas – se um pedido de CK concomitante tinha sido feito e se o estudo motivou algum tipo de acompanhamento neurológico. Utilizaram-se métodos de estatística descritiva.

Resultados: Obtivemos uma amostra de 383 doentes, com uma idade média de 9.55 $\pm$ 5.7 anos e um rácio masculino:feminino de 1.41:1. Sobre a proveniência dos estudos analíticos, 250 (65%) foram pedidos em consulta externa, 93 (24%) durante uma hospitalização e 40 (11%) no serviço de urgência. Caracterizando cada um, dos 106 estudos com ALT/AST elevadas pedidos pela Hepatologia, 34 (32.1%) incluíram pedido de CK. Na Oncologia, de 59 estudos analíticos, 5 (8.5%) pediram também CK. No serviço de urgência, de 40 crianças com transaminases elevadas, em 13 (32.5%) foi incluído o pedido de CK. Em 17 consultas do Centro de Desenvolvimento da Criança com ALT/AST aumentadas, 9 (53%) incluíram pedido de CK. Em 13 hospitalizações no serviço de Pediatria Médica, 5 (38.5%) tiveram CK doseada. Dos 383 doentes, 18 tinham CK aumentada – em 3 (16.7%) em

associação a doenças neurológicas e metabólicas. Identificámos 8 crianças sem etiologia determinada para o aumento das transaminases ou da CK.

Conclusão: Na ausência de contexto clínico que justifique a elevação da atividade das transaminases séricas, os hepatologistas pediátricos consideram-na como um possível indicador de doença do músculo e pedem CK. Noutras especialidades, o pedido de CK não é feito de forma tão rotineira, sendo só efetuado na suspeita de manifestações musculares ou quando se realiza investigação complementar em distúrbios do neurodesenvolvimento. No entanto, ainda foi possível encontrar crianças seguidas pela Hepatologia e pelo Neurodesenvolvimento com transaminases elevadas, sem um diagnóstico definitivo e ainda sem doseamento de CK. Estas conclusões reforçam a necessidade de maior consciencialização para as fontes extra-hepáticas das transaminases, nomeadamente o músculo.

Palavras-Chave: distrofinopatias, músculo, transaminases, creatina cinase.

1 – Introduction

Serum alanine transaminase (ALT) and aspartate transaminase (AST) are contained within hepatocytes and are commonly used as biochemical indicators of hepatocellular injury. Because transaminases are not unique to hepatocytes, other tissues should also be considered as a potential origin when abnormally high enzyme levels in serum are found. ALT is also found in cardiac and skeletal muscle. AST is found within cardiac and skeletal muscle, kidney, brain, pancreas, lung, leukocytes, and erythrocytes. In fact, due to the larger proportion of skeletal muscle, adult males have 4 times more ALT and 26 times more AST on average in their muscle than in their liver. The AST was found to fall in parallel with CK, suggesting a direct relationship with muscle damage.[1] Considering other tissues as possible sources of high serum transaminase levels will reduce the risk of misattributing abnormal transaminase levels to nonexistent liver pathology.[2,3]

Serum creatine kinase (CK), is a sensitive indicator of skeletal and cardiac muscle injury and is a fast and reliable mean of determining if high transaminase levels are associated with an underlying muscle disease. The concentration of CK is 1000-fold higher in skeletal muscle than in liver.[2] Therefore, ALT and AST levels can also detect occult muscle disease. High concomitant CK levels can point to muscle as source of high transaminase levels. Nevertheless, clinicians may be reluctant to attribute high transaminase levels to muscle.[2]

Muscular dystrophies represent an important subset of neuromuscular disorders. The spectrum of conditions implicated range from severe and fatal congenital muscular dystrophies to mild forms of limb weakness with onset in adulthood and from minimal respiratory compromise to being cause of respiratory emergencies and cardiac involvement.[4] The dystrophinopathies are included in this group of diseases, and they comprise Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (BMD). These are a spectrum of X-linked genetic disorders characterized by childhood-onset progressive weakness and variable cardiac, respiratory and cognitive involvement.[5] DMD constitutes the most common cause of muscular dystrophy in pediatric age.[4] Early identification of neuromuscular disorders allows genetic counseling and appropriate early management.

Persistent elevation of serum transaminase levels has been well documented in patients with muscular dystrophies (including the dystrophinopathies). Also in inflammatory and metabolic myopathies, such as the potentially treatable myopathy caused by acid α-1,4 glucosidase deficiency (Pompe disease), there are studies screening for asymptomatic patients with elevated CK to improve detection of the condition.[6] Failure to recognize muscle as the cause of high serum transaminase levels in these patients has resulted in their having

to undergo costly and invasive procedures (as liver biopsy) and in delayed recognition of occult or minimally symptomatic muscle disease.[2,7,8]

Our goal is to understand how this investigation is conducted in our center, and what are the main reasons to request CK concurrently with serum transaminases dosing. Lastly, we investigated if, in those patients with elevated CK and in the ones with no obvious explanation for the elevated transaminases and without CK, a muscle disorder hypothesis was thought and if a neurological follow-up was ever made.

2 – Materials and Methods

2.1 – Study Design

An observational, retrospective and unicentric study was performed. This study was approved by the local Ethics Committee (OBS_SF_144_2022) and followed the principles of the Declaration of Helsinki, national legislation for clinical research and good clinical practice (ICH-GCP).

2.2 – Population

We recruited all children and teenagers (0-17 years and 364 days of age), that went to Hospital Pediátrico, Centro Hospitalar e Universitário de Coimbra, and did an analytical study by any reason between January 2021 and December 2021. Then, we included patients with the following laboratory results:

- elevated ALT and/or AST (≥40 U/L was considered for both);
- normal total bilirrubin (≤ 1.2 mg/dL).

Therefore, the cohort would include children with high ALT and/or AST without high total bilirubin, the ones more probable to have hyperCKemia concurrently to transaminase elevation. In patients who performed several analytical studies between January 2021 and December 2021, the one with higher values of ALT and/or AST was selected.

2.3 – Procedures

Data was retrospectively collected from clinical records. The selected variables included:

1. demographic data (age, gender);

2. provenance of the analytical study by type (outpatient clinic, hospitalization, emergency department [ED]), medical specialty and department/service responsible for the request;

3. the clinical context in which the analytical study was requested, which included pharmacological follow-up; post-surgical, clinical investigation, or acute illness;

4. the management after the detection of high AST/ALT:

    4.1. Was creatinine kinase dosing requested in the same analytical study?

4.2. If yes, if there was hyperCKemia (>1.5 times the superior limit of normality, thus more than 250 U/L).

4.3. Was a neurological disorder considered (including neuromuscular conditions)?

4.4. Was the patient referred to a Neurologist or was the patient already on Neurology follow-up?

5. The final diagnosis of the patient, with focus neurological diagnoses.

A subset of patients – the ones with elevated CK – was further characterized.

2.4 – Statistical analysis

Descriptive analysis of biodemographical and clinical characteristics was performed. Moreover, the description of the variables mentioned was also conducted by descriptive analysis. Qualitative variables were displayed as absolute value (n) and percentage (%), and quantitative variables as mean ± standard deviation. Microsoft Excel® was used for the statistical analysis.

3 – Results

During the year of the study, there were 2358 requests with increased ALT and AST in our center – 496 samples (21%) had concomitant total bilirrubin elevation, and were therefore excluded from the analysis. After excluding repeated samples, we included 383 patients (Figure 1), with mean age of 9.55 ± 5.7 years, median 10 years, and interquartile range of 12 years. Concerning gender, the male:female ratio was 1.41:1, being 41.5% female participants and 58.5% male participants.

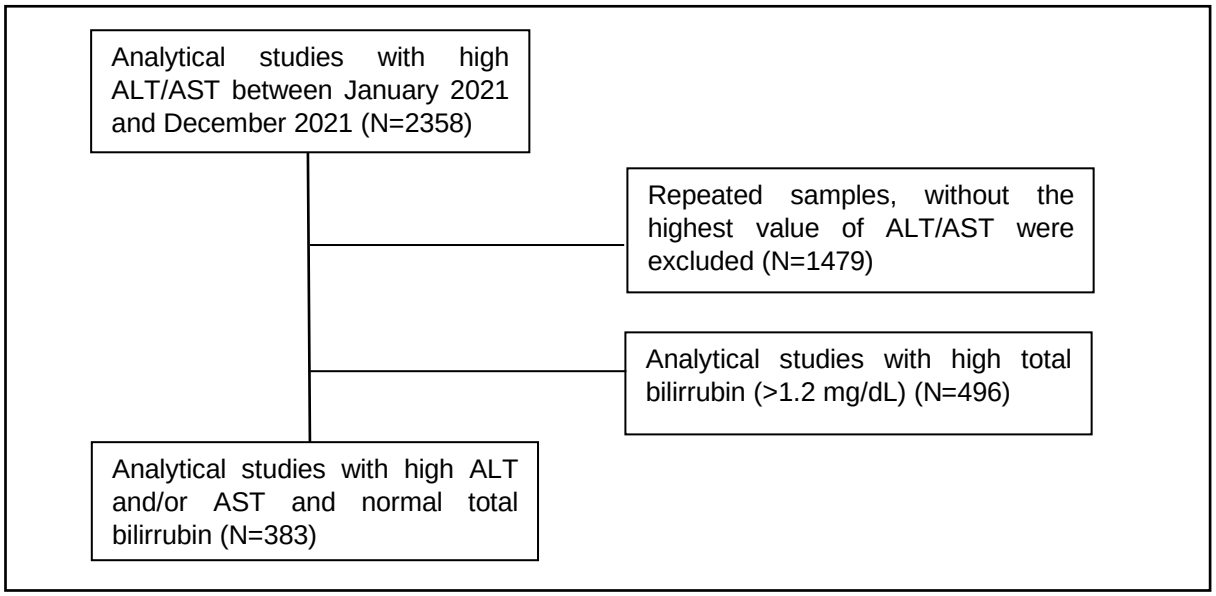

Figure 1. Flow diagram showing patient recruitment in the study.

Regarding the provenance of the analytical study request, we divided data into three groups (Figure 2):

- analytical study requested in the outpatient clinic;
- analytical study requested during hospitalization;
- analytical study requested during a visit to the ED.

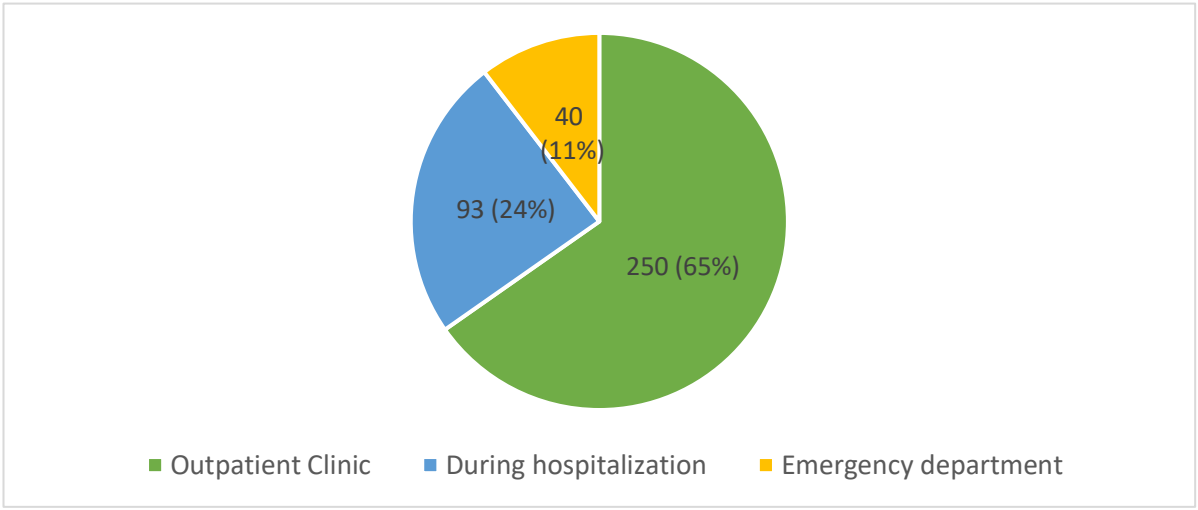

Figure 2. Origin of the request for the analytical study that led to detection of elevated serum transaminase levels

Considering the multiplicity of medical and surgical specialties and departments that exist in Hospital Pediátrico, Centro Hospitalar e Universitário de Coimbra, a more detailed examination of which specialty or service requested the analytical studies was conducted (Figure 3).

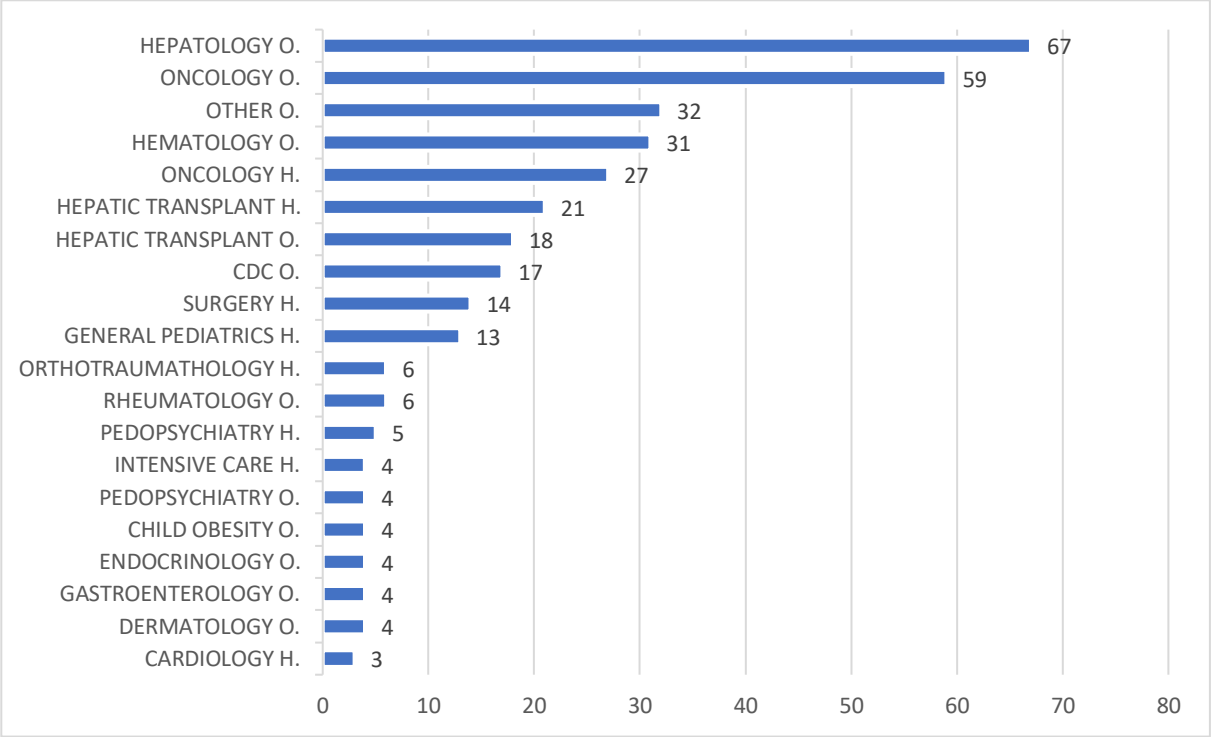

Figure 3. Listing of the specific specialty or service from Hospital Pediátrico, Centro Hospitalar e Universitário de Coimbra that requested the analytical study that led to the identification of elevated serum transaminase levels. O: outpatient clinic; H: hospitalization.

In the global sample, in around 25% of patients with high AST and/or ALT with normal total bilirrubin, a CK request was also considered. These results are further described in Table 1. In order to understand patterns of investigation, the results will be divided in: Hepatology, Oncology, ED, Center for Child Development (CCD) and miscellaneous.

Table 1. Number of analytical studies that included CK concurrently to the transaminases dosing, in each main context considered.

Context of the analytical study	n (%)	N (%) in which CK dosing was requested	N1 (%) in which the CK was elevated
Total	383 (100%)	98 (25.6%)	18 (18.4%)
Hepatology investigation and follow-up	106 (27.7%)	34 (32.1%)	4 (3.8%)
Pharmacological follow-up in Oncology	59 (15.4%)	5 (8.5%)	2 (3.4%)
Emergency Department	40 (10.4%)	13 (32.5%)	2 (5%)
CCD	17 (4.44%)	9 (53.0%)	2 (22.2%)
General Pediatrics Department	13 (3.4%)	5 (38.5%)	0 (0%)
Pedopsychiatry	9 (2.3%)	4 (44.4%)	2 (22.2%)
Pharmacological follow-up in Dermatology (due to use of isotretinoin for acne treatment)	3 (0.8%)	3 (100%)	0 (0%)

Table 1. Number of requests for an analytical study that led to the identification of elevated serum transaminase levels (n), number of requests for concurrently CK dosing (N) and number of requests with elevated CK (N1), for each main specialty/service considered.

Regarding the Hepatology department, 67 consultations of hepatological clinical investigation, 18 consultations of post-liver transplant follow-up and 21 hospitalizations related with liver transplantation were included, giving a total of 106 requests of analytical studies that resulted in identification of elevated serum transaminase levels.

Of these, 34 (32.1%) requests included CK dosing. This group is characterized in Figure 4. A total of 4 (3.8%) patients had elevated CK levels. Regarding their specific diagnosis and if any neurological follow-up was done, the following was verified:

- 2 were patients with asymptomatic elevation of transaminase levels in study (one was referred to Neurology);
- 1 was a patient with alpha-1-antitrypsin deficiency (this patient was also referred to Neurology);
- 1 patient had Alagille syndrome (this patient was already followed in Neurology).

Concerning the neurological follow-up, besides the two patients mentioned above, there were three more Neurology consultations requested, in patients with normal CK or no CK dosing:

- 1 patient with high transaminase levels in study and with normal CK (221 U/L);
- 1 patient with Schwachman-Diamond syndrome, without CK dosing;
- 1 patient with extrahepatic biliary atresia (EHBA) and an intracranial hemorrhage as intercurrence, without CK dosing.

Summarizing, from 9 patients with high transaminases in study and CK request, 7 had normal CK levels and 2 had elevated CK levels. In the group with normal CK, 5 had CK < 100 U/L and were not forwarded to any neurological follow-up. One had a normal CK (221 U/L) activity and was forwarded to neurological follow-up. One had normal CK (241 U/L) with previous registration of hyperCKemia and was not forwarded to a neurological observation. In the group with elevated CK, 1 was forwarded to Neurology and 1 was not.

Three children had asymptomatic elevation of transaminases without CK dosing.

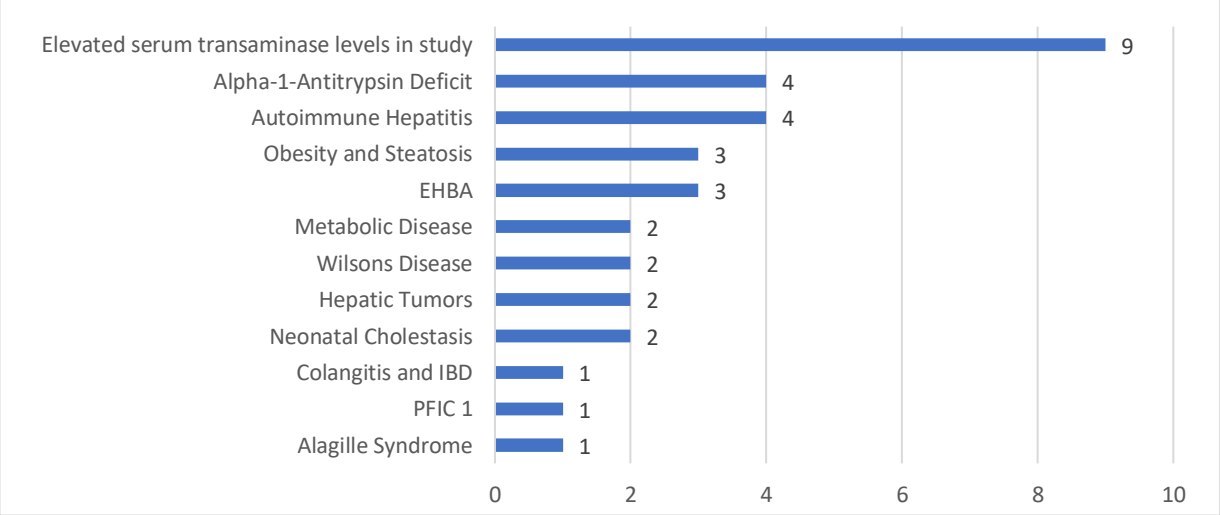

Figure 4. Diagnosis of the patients followed in Hepatology with elevated ALT and/or AST that had CK dosing included in their analytical study. EHBA: extrahepatic biliary atresia; IBD: inflammatory bowel disease; PFIC1: progressive familial intrahepatic cholestasis type 1

In Oncology, from 59 pharmacological follow-ups, only five (8.5%) requested CK, and all of them were patients diagnosed with a nervous system (CNS or PNS) tumor. From these, only 2 had elevated CK levels. Concerning the neurological follow-up, none of the 5 patients with CK dosing were forwarded to Neurology, but there were 3 Neurology consultations asked: 2 patients with acute lymphocytic leukemia (ALL) and 1 patient with Hodgkin lymphoma.

Concerning the visits to the ED, 34 visits to the ED itself and 6 short-term hospitalizations in UICD ("Unidade de Internamento de Curta Duração") were included in this group. From the total of 40 visits to the ED that resulted in analytical studies with high ALT and/or AST levels, 13 (32.5%) requested CK dosing – 2 were patients with a suspicion of pediatric inflammatory multisystem syndrome (PIMS), 1 had a suspicion of myositis, 1 was a polytraumatized child, 1 complained with asthenia, and 1 was diagnosed with migraine. The other 7 were a miscellaneous of clinical presentations, not related with neurological or muscular complaints. Only 2 out of 13 (15.4%) patients had elevated CK levels – the patient with polytrauma and 1 patient with chronic hepatitis C and HIV infections. None of these were forwarded to Neurology. The patient with a migraine diagnosis was the only one from this group redirected to Neurology.

Regarding CCD (Metabolic Hereditary Disorders [MHD], Neuropediatrics and Neurodevelopment), 17 patients were included in the study, from which 9 (53%) had CK requested. MHD represented 10 of these patients, 4 of which (40%) had CK included in the analytical study, from which only 1 patient with a congenital disorder of glycosylation had an elevated CK level (1937 U/L). Three out of 5 children with elevated transaminases followed by Neuropediatrics had CK dosing, and only 1 patient already diagnosed with Becker muscular dystrophy (BMD) had elevated CK (7979 U/L). Lastly, in two children observed in a Neurodevelopment consultation, a study with CK was requested (both normal).

With respect to the General Pediatrics department, from a total of 13 hospitalizations in this service that led to identification of high serum transaminase levels, only 5 (38.5%) included CK dosing in the same analytical study, and it was always normal: 2 patients with PIMS, 2 patients with encephalitis, and 1 patient with ulcerative colitis. Only the 2 children with encephalitis were forwarded to Neurology.

Lastly, regarding Child Psychiatry, in 9 patients included in our study, 4 (44.4%) had CK dosing made, and all of them were under treatment with neuroleptics. None of these patients were redirected to Neurology.

Patients with Psychomotor Development Delay (PDD) and/or language delay background

In our study, we discovered 15 patients who had an analytical study with elevated serum transaminase levels requested by different specialties, but also had a background of PDD and/or language delay. For these 15 children we went back and studied if they were also followed in the CCD and if they had any past CK request. Four profiles were established:

- 6 children with previous normal CK and an already established neurological diagnosis;
- 4 children with previous normal CK and without a specific neurological diagnosis;
- 4 children without previous CK dosing and without a specific neurological diagnosis;
- 1 child without previous CK dosing, but with an established neurological diagnosis.

Creatine kinase requests analysis

Only 18 patients from the total of 383 had elevated CK serum levels. Their final diagnosis and the context of the analytical study is depicted in Table 2.

Table 2. Characterization of patients with elevated CK

Patient	***Diagnosis***	***ALT/AST (U/L)***	***CK (U/L)***	***Request of the analytical study***
A	Chronic hepatitis C and HIV infection	39/47	632	Emergency department
B	Sarcoma	52/40	1269	Oncology follow-up
C	Congenital Disorder of Glycosylation	40/100	1937	Metabolic Hereditary Disease follow-up
D	Dyslipidemia with high ALT and AST – suspected MDR3 deficiency	66/55	1006	Hepatology

E	Alagille syndrome	54/48	252	Hepatology follow-up
F	Fall with thoraco-abdominal trauma	27/44	1194	Emergency department
G	Idiophatic scoliosis	36/116	2877	Orthotraumathology hospitalization after surgical correction of the scoliosis
H	Congenital scoliosis	28/69	1051	Orthotraumathology hospitalization after surgical correction of the scoliosis
I	Chiasmatic Hypothalamic Glioma	361/295	376	Oncology follow-up
J	Astrocytoma	119/59	769	Othorrinolaringology follow-up
K	HIV infection	65/70	276	Infectious Diseases follow-up
L	Becker muscular distrophy	183/116	7979	Neuromuscular follow-up
M	Alpha-1-antitrypsin deficiency	87/57	386	Hepatology follow-up
N	Fallot Tetralogy	72/311	2034	Hospitalization after cardiac surgery

O	Medico-legal evaluation due to suspected drug abuse	54/34	357	Child Psychiatry first appointment
P	Psychotic break investigation	173/56	430	Child Psychiatry first appointment
Q	Assymptomatic ALT and AST elevation, hepatic steatosis	137/135	10426	Hepatology
R	Hit and run	48/109	1205	Intensive care hospitalization

Table 2. Characterization of the 18 patients with elevated CK from the 383 children included in the study – context of the analytical study request, value of ALT, AST and CK and final diagnosis. MDR3, Multidrug Resistance Protein 3; HIV, human immunodeficiency virus.

Eight children had elevated transaminases without a definitive etiology for it, 7 without CK request: 3 children without a definitive diagnosis followed by Hepatology and 4 children with PDD/language delay already followed by the Neurodevelopment team, but still without a definitive neurological diagnosis. One child with high ALT/AST and concurrently high CK (10426 U/L) was followed by Hepatology.

4 – Discussion

In this study, we described 383 children with high serum transaminase levels and compared their clinical diagnosis, if a request for CK dosing was made concurrently to the transaminases analysis, which was the context of the analytical study and if a follow-up by Neurology was requested as consequence of the analytical findings. First of all, it is important to recognize that our hospital, as a tertiary center, is composed by a very heterogeneous subset of medical and surgical specialties, and there is a myriad of clinical pictures that prompt the request of analytical studies. Even so, only one quarter of the samples with an AST/ALT increase was accompanied by a request of a CK analysis, with some percentages being important to mention, namely 53% in the CCD.

Regarding the Hepatology service, we can conclude that it is common to include the CK dosing in their analytical studies if no established hepatic disease diagnosis exists, which is important to avoid unnecessary and invasive hepatic studies (as liver biopsies). The majority of patients to whom CK was requested had an elevation of serum transaminases in study or systemic diseases with possible neuromuscular manifestations – alpha-1-antitrypsin deficiency, autoimmune hepatitis type 1 and Wilson disease.[9-11] The only patient with asymptomatic elevation of transaminases plus concurrently elevated CK who was not forwarded to Neurology had already a possible diagnosis in consideration, MDR3 deficiency, a disorder affecting predominantly the liver. There are, however, 3 patients with elevated transaminases, without an established diagnosis and no CK measurement in the current analytical study, that should be more profoundly studied. It is important to understand if past CK request was made to exclude muscle involvement and if it is opportune to redirect these patients to Neurology.

Most pharmacological follow-ups in Oncology lead to analysis of transaminase levels without adding CK analysis, as expected – patients have a diagnosis of a neoplastic condition and analytical studies are made in this context. From 59 pharmacological follow-ups in Oncology, the 5 that requested CK were already diagnosed with a nervous system tumor. Other reasons that can lead to requests of CK in this context are the muscular side effects of drugs used in oncological treatments.[12]

In the ED, that here represents the acute illness, CK was asked for several motives, in almost 1/3 of the patients. The majority were patients with a suspected muscle disease or systemic disease with muscular manifestations, such as myositis and PIMS.

Regarding the CCD, roundly a half of the requests (53%) had CK analysis concomitant with transaminase dosing. We found interesting that, in a total of 11 085 appointments in the Neurodevelopment unit, 5396 of Neuropediatrics, and 1445 of Metabolic Diseases[14] there were only 17 patients with high transaminases, and 2 patients with hyperCKemia (indicating involvement of muscle disease – 1 with BMD and 1 with a congenital glycosylation defect). Although it is impossible to understand the number of patients who are investigated with analytical studies in the context of a neurodevelopment, neurological or metabolic disorder, this seems a low number. We can interpret this as a low prevalence of neuromuscular disorders or, eventually, a high threshold for requesting AST/ALT and CK.

Considering General Pediatrics, CK was only requested if the patient was hospitalized with a neurological diagnosis or with PIMS, which can have muscular symptoms[13], and in some other patients with systemic diseases with possible muscular manifestations (as 1 patient with ulcerative colitis). General pediatricians usually work in the ED and in the ward, and this could be both good opportunities to find patients with unknown muscle disease manifesting hyperCKemia, as children do not usually perform routine laboratory evaluations. More awareness should be created in these environments to add CK dosing in a young child with high AST and ALT without any reasonable explanation, even in the context of acute disease.

Concerning Child Psychiatry, we believe CK measurement is mainly requested due to the risk of neuroleptic malignant syndrome, a neurologic emergency associated with the use of antipsychotic agents that causes concurrently hyperCKemia.[15,16] Every patient with CK requested was treated with neuroleptics due to their psychopathology. In the pharmacological follow-up of patients with acne treated with isotretinoin, performed by dermatologists, CK was always requested, but was also normal in all of them. We believe these results come from the fact that isotretinoin has well documented musculoskeletal side effects.[17,18]

In order to understand more deeply the study of CK in the context of neurodevelopmental disorders, we further characterized 15 patients with a PDD and/or language retardation, as those can be manifestations of dystrophinopathies.[19,20] We wanted to investigate if these patients, included in this study due to an analytical study with origin in other specialty and showing elevated transaminases, were already being followed in Neurology consultations and if they had ever gotten a CK dosing made to exclude the possibility of a muscular disease. From the group of 15, 7 patients had a neurological diagnosis well established, 6 of which had previous normal CK and 1 that did not have CK dosing before. The other 8 had no conclusive diagnosis – 4 had previously normal CK and 4 did not have CK measurements. All of them were already followed by a Neurodevelopment pediatrician. We consider that these last four patients, especially the 3 male patients, with no previous CK

record and no well stablished neurological diagnosis, should be analyzed in detail, to exclude a muscle disease – 3 of them followed in Neurodevelopment or Neuropediatrics consultations between 2018-2023 without records of analytical studies requested by these specialties and without CK requests registered in this period; and 1 with a Neurodevelopment consultation in 2008, but we did not have access to the analytical studies done at that time, so we cannot be sure if CK was ever requested.

In addition to these 4 children, we were able to identify 1 additional patient that should be called back to exclude a possible muscle disorder, a male with asymptomatic elevation of transaminases and a current normal CK, but previous hyperCKemia. Collaboration of MHD was requested by Hepatology after finding hyperCKemia, but there is still no definitive diagnosis established.

The 18 patients with hyperCKemia had very heterogeneous diagnoses, from chronic to acute medical and surgical conditions. Once again, this reflects the heterogeneity of a tertiary hospital. We highlight the patient with an already diagnosed Becker Muscular Dystrophy – although a lot rarer than DMD, the fact that we only analyzed the year of 2021 may be responsible for this bias. We also highlight the patient with high CK without definitive diagnosis, who we consider should be evaluated by the neuromuscular disorders' team.

Lastly, we tried to compare our study with similar ones from other Hospitals and Universities, to juxtapose our investigation with others. However, we were not able to find any published study that also had as starting point the elevation of transaminases and analyzed if any concurrently CK was asked and what was the semiology or diagnosis that motivated the requests. However, we were able to find some studies with a starting point on a group of children already diagnosed with DMD that did a retrospective analysis of CK and transaminase elevation. Although with different methodologies, all of these studies arrived to the same conclusions as us, enhancing the importance of maintaining a high index of suspicion for extrahepatic sources of ALT and AST elevation, to prevent delays in diagnosis, as well as unnecessary invasive testing.[21–24]

5 – Conclusion

In the absence of clinical context and semiology to justify the elevation of serum transaminases, pediatric hepatologists see elevated transaminases as possible indicators of a muscle disorders and request CK dosing. In other specialties, CK is only measured when muscle manifestations are present or if a neurodevelopment study is in place. However, we were still able to find some patients followed in Hepatology and in Neurodevelopment outpatient clinic that had elevated transaminases, no definitive diagnosis and still did not have CK requested.

The findings of this study indicate a need to raise awareness when it comes to extra-hepatic sources of transaminases elevation, such as the muscle. We can correspondingly conclude there is a need for greater consciousness regarding the importance of CK investigation as an opportunistic evaluation in children, especially in those with neurodevelopmental issues.

Acknowledgments

I would like to thank to Dr. Filipe Palavra and Dr. Joana Ribeiro for their willingness, assistance and scientific contribution, which allowed in great scale the enrichment of this thesis.

To my family – my mom, dad and brother – for always being there for me, with enormous patience and love.

To Inês and Inês, who are one of my biggest supports, even when we don't talk for months, for always having good advice and encouragement.

To my friends, for being there since day one and for sharing the best and worst moments of this adventure with me. I hope we can still continue to make memories years from now.

To Tuna Feminina de Medicina da Universidade de Coimbra, for being a second family, always bringing joy to my years in Coimbra and for giving me the sense of responsibility and time management while showing it is possible to reconcile work with other passions.

References

1. Lash, T., & Kraemer, R. R. (2014). Elevated liver enzymes indicating a diagnosis of limb-girdle muscular dystrophy. *Journal of General Internal Medicine*, *29*(5), 813–815.

2. McMillan, H. J., Gregas, M., Darras, B. T., & Kang, P. B. (2011). Serum transaminase levels in boys with Duchenne and Becker muscular dystrophy. *Pediatrics*, *127*(1), e132-6.

3. Pratt, D. S., & Kaplan, M. M. (2000). Evaluation of abnormal liver-enzyme results in asymptomatic patients. *The New England Journal of Medicine*, *342*(17), 1266–1271.

4. Carter, J. C., Sheehan, D. W., Prochoroff, A., & Birnkrant, D. J. (2018). Muscular Dystrophies. *Clinics in Chest Medicine*, *39*(2), 377–389.

5. Lee, B. H. (2022). The Dystrophinopathies. *Continuum (Minneapolis, Minn.)*, 28(6), 1678–1697.

6. Reynolds, T. M., Tylee, K., Booth, K., & Wierzbicki, A. S. (2019). Identification of patients with Pompé disease using routine pathology results: PATHFINDER (creatine kinase) study. *Journal of Clinical Pathology*, *72*(12), 805–809.

7. Korones, D. N., Brown, M. R., & Palis, J. (2001). "Liver function tests" are not always tests of liver function. *American Journal of Hematology*, *66*(1), 46–48.

8. Morse, R. P., & Rosman, N. P. (1993). Diagnosis of occult muscular dystrophy: importance of the "chance" finding of elevated serum aminotransferase activities. *The Journal of Pediatrics*, *122*(2), 254–256.

9. Dusek, P., Litwin, T., & Członkowska, A. (2019). Neurologic impairment in Wilson disease. *Annals of Translational Medicine*, *7*(Suppl 2), S64.

10. Duckers, J. M., Shale, D. J., Stockley, R. A., Gale, N. S., Evans, B. A. J., Cockcroft, J. R., & Bolton, C. E. (2010). Cardiovascular and musculskeletal co-morbidities in patients with alpha 1 antitrypsin deficiency. *Respiratory Research*, *11*, 1–7.

11. Catherine D. Linzay1; Bashar Sharma2; Sudha Pandit3. (2022). Autoimmune Hepatitis. *StatPearls Publishing*.

12. Gawade, P. L., Hudson, M. M., Kaste, S. C., Neglia, J. P., Wasilewski-Masker, K., Constine, L. S., Robison, L. L., & Ness, K. K. (2014). A systematic review of selected musculoskeletal late effects in survivors of childhood cancer. *Current Pediatric Reviews*, *10*(4), 249–262.

13. Ahmed, M., Advani, S., Moreira, A., Zoretic, S., Martinez, J., Chorath, K., Acosta, S., Naqvi, R., Burmeister-Morton, F., Burmeister, F., Tarriela, A., Petershack, M., Evans, M., Hoang, A., Rajasekaran, K., Ahuja, S., & Moreira, A. (2020). Multisystem inflammatory syndrome in children: A systematic review. *EClinicalMedicine*, *26*, 100527.

14. *Relatório Atividades CDC 2021.pdf*. (n.d.).

15. Ware, M. R., Feller, D. B., & Hall, K. L. (2018). Neuroleptic Malignant Syndrome: Diagnosis and Management. *The Primary Care Companion for CNS Disorders*, *20*(1).

16. Masi, G., Milone, A., Viglione, V., Mancini, A., & Pisano, S. (2014). Massive asymptomatic creatine kinase elevation in youth during antipsychotic drug treatment: case reports and critical review of the literature. *Journal of Child and Adolescent Psychopharmacology*, *24*(10), 536–542.

17. Webster, G. F., Webster, T. G., & Grimes, L. R. (2017). Laboratory tests in patients treated with isotretinoin: occurrence of liver and muscle abnormalities and failure of AST and ALT to predict liver abnormality. *Dermatology Online Journal*, *23*(5).

18. Karaosmanoğlu, N., & Mülkoğlu, C. (2020). Analysis of musculoskeletal side effects of oral Isotretinoin treatment: A cross-sectional study. *BMC Musculoskeletal Disorders*, *21*(1), 1–10.

19. Cyrulnik, S. E., Fee, R. J., De Vivo, D. C., Goldstein, E., & Hinton, V. J. (2007). Delayed developmental language milestones in children with Duchenne's muscular dystrophy. *The Journal of Pediatrics*, *150*(5), 474–478.

20. Nardes, F., Araújo, A. P. Q. C., & Ribeiro, M. G. (2012). Mental retardation in Duchenne muscular dystrophy. *Jornal de Pediatria*, *88*(1), 6–16.

21. Zamora, S., Adams, C., Butzner, J. D., Machida, H., & Scott, R. B. (1996). Elevated aminotransferase activity as an indication of muscular dystrophy: case reports and review of the literature. *Canadian Journal of Gastroenterology = Journal Canadien de Gastroenterologie*, *10*(6), 389–393.

22. Urganci, N., Arapoğlu, M., Serdaroğlu, P., & Nuhoğlu, A. (2006). Incidental raised transaminases: a clue to muscle disease. *Annals of Tropical Paediatrics*, *26*(4), 345–348.

23. Tay, S. K., Ong, H. T., & Low, P. S. (2000). Transaminitis in Duchenne's muscular dystrophy. *Annals of the Academy of Medicine, Singapore*, *29*(6), 719–722.

24. Wright, M. A., Yang, M. L., Parsons, J. A., Westfall, J. M., & Yee, A. S. (2012). Consider

muscle disease in children with elevated transaminase. *Journal of the American Board of Family Medicine : JABFM*, *25*(4), 536–540.