

# The Elastographic Aspect of Liver in Pediatric Patients with Hepatopathies and Malignities Versus Healthy Children

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**Background:** Liver injury in context of chronic diseases, including malignancies, obesity, viral hepatitis, drug-related hepatotoxicity is an important concern for practicing pediatricians. The usual (biochemical) parameters for liver assessment prove to be now insufficient. Nowadays there is a high interest for finding non-invasive methods of hepatic evaluation, as an alternative to liver biopsy. Elastography fills a gap, providing information on the degree of hepatic fibrosis.

**Objective:** Real-time elastographic assessment of liver tissue in correlation with biochemical parameters in children with hepatopathies and malignancies versus healthy children.

**Material and method:** Between September 15, 2010 and March 15, 2011 we conducted a prospective study in the 1<sup>st</sup> Pediatric Clinic Tîrgu Mureș, Romania, including a group of children with various malignancies under/after chemotherapy, a group of children with liver diseases (drug-related hepatotoxicity, obesity, hepatitis) and a control group composed of children with normal biochemical parameters. We assessed the liver tissue elasticity, expressed as SWV (shear wave velocity) on elastography, biochemical parameters, then statistical correlations were performed.

**Results:** Comparing SWV-values between the three groups, higher speeds were found in groups with liver damage after chemotherapy and those with hepatopathies ( $p=0.04$ ). Aspartate transaminase (AST, IU) and alanine transaminase (ALT, IU) levels were different in a statistically significant manner between the three groups ( $p=0.0006$  and  $0.0002$  respectively). In the after-chemotherapy group significant correlations were obtained between elasticity and AST ( $p=0.0001$ ).

**Conclusions:** In children with liver damage, SWV (which is correlated with the degree of liver fibrosis) increase in parallel with transaminases.

**Keywords:** liver diseases, elastography, children

## Introduction

In the context of chronic diseases, the liver is damaged either by direct involvement or because of complications. Chronic liver diseases – chronic hepatitis with virus B, C, D, E, autoimmune diseases, infections with cytomegalovirus (CMV), alpha-1-antitrypsin deficiency, Wilson's disease – produce asthenia, fatigue on exertion, decreased school performance, stationary weight curve, loss of appetite, and so on, as well as clinical hepatomegaly and changes in laboratory findings [1]. Tumor infiltration of organs in different malignancies often involve the liver [2]. Non-alcoholic fatty liver disease (NAFLD) is increasing due to the fact that the incidence of obesity in children has increased. It represents a broad spectrum of conditions ranging from fatty liver, which in general follows a benign non-progressive clinical course, to steatohepatitis or NASH, a more serious form of NAFLD that may progress to cirrhosis and end-stage of liver disease (liver failure) [3].

Drug hepatotoxicity is a matter of great interest in iatrogenic pathology and a great challenge to clinical practice, raising special problems of diagnosis. Damage may be hepatocellular, cholestatic or mixed. Drug-induced liver injury is an important concern for many existing drugs, as well as for new therapeutic agents [4]. Drug treatments, especially antiepileptics, antibiotics and chemotherapeutic agents, can induce toxic effects on the liver in children; the

damage is more important in malnourished or immunosuppressed children, children with chronic diseases, prolonged therapy, total parenteral nutrition, viral hepatitis [5]. The cytostatic therapy, with or without other treatments (antibiotics, anti-inflammatory, analgesics) inevitably affects the liver, which is one of the tissues with the most intense metabolic activity in the body [5,6].

The evaluation of liver damage means the clinical signs of liver damage (fatigue, anorexia, nausea, vomiting) with increased transaminases and cholestatic signs (jaundice, pruritus), associated with increased alkaline phosphatase (AF), bilirubin [7], and other laboratory investigations like albumin, prothrombin time, gamma-glutamyl transpeptidase (GGT), 5'-nucleotidase, flow on portal vein, as well as imagistic tests (abdominal ultrasonography and computerised tomography), and for the time being the greatest standard, liver biopsy. Liver biopsy is an invasive procedure, with many secondary effects, hardly accepted by children and parents [8]. There is a great interest currently in finding a non-invasive method of liver evaluation, of the degree of liver fibrosis, as an alternative to liver biopsy. A sequential approach or the combination of serum markers and transient elastography is able to significantly reduce the need for biopsy for the diagnosis of cirrhosis; serum markers also seem to provide useful prognostic information in end-stage liver disease. Newer imaging methods re-

quire further validation, but appear promising adjunctive techniques for the prediction of fibrosis [9].

Ultrasonography is usually the first-line investigation in the assessment of patients, due to its low-cost, non-invasive nature, repeatability and easy access; ultrasonographic elastography fills a gap, allowing the assessment of tissue elasticity, providing information on the degree of hepatic fibrosis and thus of hepatotoxicity. MRI and PET, and often CT, are restricted to selected cases, due to their limited availability and high costs. Thus, the introduction of new techniques to increase the sensitivity of US would be a major advantage [10]. Technically described since the last decade, elastography was introduced at the beginning of 2008, as an application of the latest ultrasound devices which include a wide range of analytical applications related to tissue strain or request and which allow visual qualitative and quantitative measurements of tissue stiffness/elasticity [11].

The objective of the present study is to assess the liver tissue in correlation with biochemical parameters in children with liver-damage of different causes (malignancies, hepatopathies) versus healthy children.

## Material and methods

Between September 15, 2010 and March 15, 2011 we have conducted a prospective study in the I<sup>st</sup> Pediatric Clinic Țirgu Mureș, Romania on 82 cases, including three groups: the first group – children with various malignancies (tumor infiltration of the liver), under chemotherapy or after cytostatic therapy, with hepatotoxicity during chemotherapy – 30 cases; a second group – children with hepatopathies of different causes (viral hepatitis, acute toxic hepatitis, drug hepatotoxicity, obese children), selected on the basis of clinical signs and symptoms of liver damage (abdominal discomfort, fatigue, anorexia, nausea, vomiting, sclero-tegumental jaundice, pruritus, hepatomegaly, ascites) and changes in liver tests (albumin, prothrombin time, cytolytic liver enzymes, cholestasis tests: AF, GGT, bilirubin) – 22 cases, and the third group – control, composed of 30 children with normal clinical findings related to the digestive system.

We assessed the liver tissue elasticity (SWV), biochemical and nutritional status parameters. A single examiner has examined all the patients who were placed supine with the right arm straight over the head. The elastography examination was performed with a Siemens S 2000 ultrasound machine, its software being updated in November 2010, equipped with a transducer of 4,1 MHz with the latest generation technology. The elastographic ARFI technology can be used to measure a numerical value of the wave speed SWV (shear wave velocity) by implementing the Virtual Touch tissue quantification; the software produces a qualitative gray-scale map of the relative tissue stiffness (elastogram) for a user defined ROI (region of interest). This information is calculated by examining the relative displacement of the

tissue elements due to an acoustic pulse push. Thus, on the elastogram the shining regions correspond to a more elastic (less stiff) tissue and the dark regions correspond to the stiff tissue.

For statistical analyses 10 measurements have been made and the median velocity values were calculated for that region (eliminating the extreme values of the examination). Data were processed in Microsoft Excel, and statistical analysis was performed with the program Graph Pad Prisma and Graph Pad InStat Demo. The existence of statistically significant differences among groups was tested using parametric and non-parametric tests. Student t test, Pearson - Chi square ( $\chi^2$ ), Fisher exact test, ANOVA test were used. The threshold of significance was  $p < 0.05$ . For continuous variables, mean values were expressed as mean  $\pm$  standard error of mean (SEM) or standard deviation (SD). Correlation between average SVW of different groups and study variables (transaminases and parameters of nutritional status) was assessed based on Pearson correlation coefficient ( $r$ ).

Legal tutors of each patient have signed an informed consent at the moment of hospitalization in the clinic (consent in accordance with the principles of the Helsinki declaration).

## Results

The group of children with malignancies consisted of 8 girls and 22 boys; in the group of patients with other causes of hepatotoxicity there were 8 girls and 14 boys, while the control group consisted of 13 girls and 17 boys (as presented in Figure 1).

Descriptive statistics show averages  $\pm$  standard deviations (SD) of all parameters in all three groups surveyed.

In the control group the average stiffness (SVW) was  $1.18 \pm 0.28$  m/s; in the group of children with different causes of liver diseases, stiffness was  $1.42 \pm 0.38$  m/s, and in the group of malignancies, it was  $1.37 \pm 0.45$  m/s.

Comparing liver tissue stiffness values between the three groups higher speeds were found in groups with liver diseases and post-chemotherapy, the differences being statistically significant with a CI of 95%.

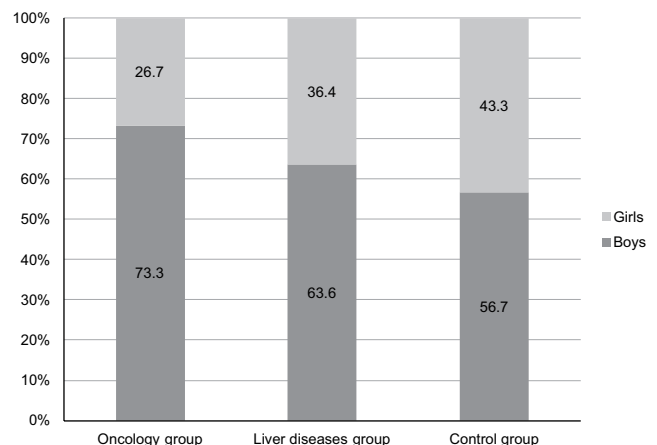


Fig. 1. The structure of the three groups by gender

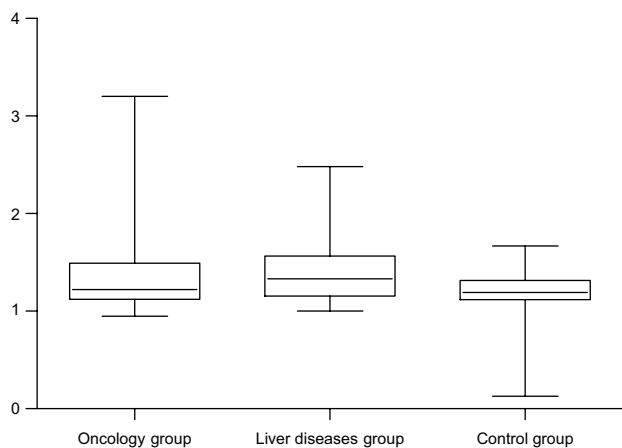


Fig. 2. Comparison between the median SVW of the three groups

By applying the ANOVA test we tried to identify differences between the averages of SVW of the three groups surveyed. We found a statistically significant difference with  $p=0.04$  (Figure 2).

Regarding alanine transaminase (ALT, IU), in the control group it was  $19.56 \pm 8.64$  SD, in the group of children with liver diseases, ALT was  $49.75 \pm 43.10$ , while in the group with oncologic diseases ALT (IU) was  $26 \pm 16.77$ .

The evaluation of ALT values between the three groups showed high values in the group with liver diseases compared with the other two groups (statistically significant difference with  $p=0.0006$ ) (Figure 3).

As far as aspartate transaminase (AST, IU) was concerned, in the control group it was  $24.88 \pm 12.62$ , in the group of children with hepatopathies AST was  $48.00 \pm 26.96$  and in the group of children with malignancies AST measured  $29.41 \pm 14.57$  (IU).

Mean AST values were significantly higher in children with liver diseases, ( $p=0.0002$ ) (Figure 4).

We subsequently tried to establish, for each group in part, correlations between global SVW and every other parameter – body mass index (BMI), medium upper-arm circumference (MUAC), triceps skin-fold (TSF) and transaminases.

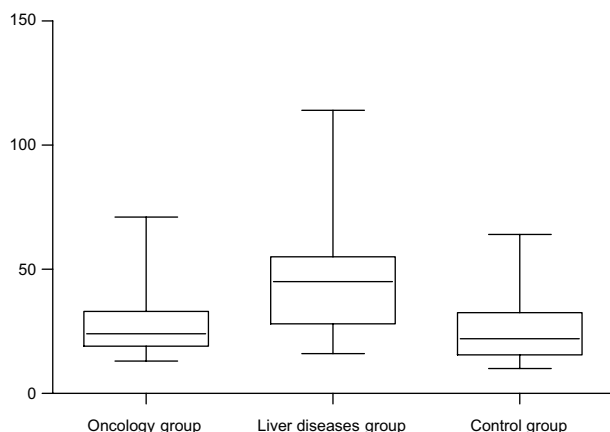


Fig. 4. Comparison between the median AST of the three groups

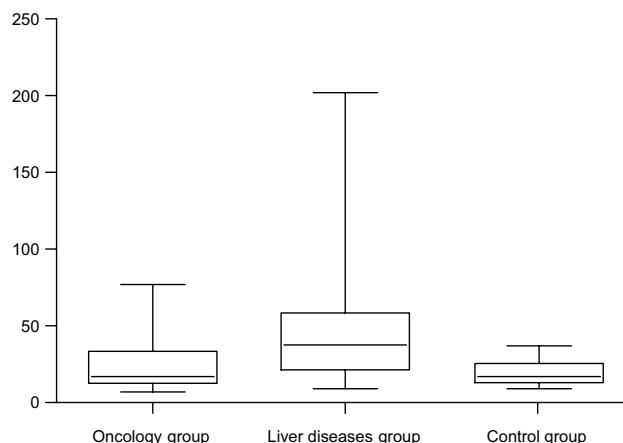


Fig. 3. Comparison between the median ALT of the three groups

We obtained no statistically significant correlations between the assessed parameters, except between SVW and AST, in the group of children with malignancies ( $r=0.57$  and  $p=0.001$ ) (Figure 5).

### Discussions

Elastography, as a new method of diagnosis, gives information related to the elasticity/stiffness of the examined tissue and the degree of tissue fibrosis. The method allows the quantification of the shear wave velocity in strong correlation with the fibrosis stage. According to some previous studies, steatosis does not influence SWV; the maximum strength of the method consists in the prediction in severe fibrosis and cirrhosis; the diagnostic accuracy is strongly comparable to transient elastography (Transient elastography, Fibroscan, EchoSens) [8,12,13].

The studies made on adults' groups with chronic hepatitis found out for different stages of hepatic fibrosis the following values of the shear wave velocity: F 0 (no fibrosis):  $1.31 \pm 0.48$  m/s; F 1 (fibrosis degree I):  $1.52 \pm 1.02$  m/s; F 2 (fibrosis degree II):  $1.61 \pm 0.68$  m/s; F 3 (fibrosis degree III):  $1.76 \pm 0.76$  m/s; F 4 (fibrosis degree IV):  $2.81 \pm 0.71$  m/s [14]. From the cases studied we found 17 patients with high values of SWV, 11 in the hepatopathies group

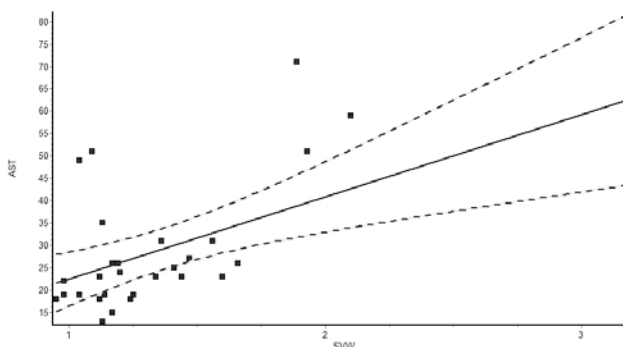


Fig. 5. The correlation between the values of SVW and AST average in group of children with malignancy

and 6 in the oncology group. Speeds should correspond to a degree I fibrosis in 8 patients (9.75%), grade II in 2 cases (2.43%), grade III in 5 cases (6.09%) and grade IV in 2 children (2.43%). We should mention that we did not perform liver biopsy to objectively confirm these changes.

Lupusor et al. correlate data obtained from elastography with FibroScan and transaminases levels [8]. For the current study this method was not available, we have cross-analyzed the transaminases levels (and got statistically significant correlations between SWV and AST in the group of children with malignant affections); also, we tried linking the parameters of nutritional status with elasticity, without obtaining in this first phase significant results (it is worth continuing our research on a larger number of patients, considering on one hand the malnutrition secondary to malignancies and chronic liver diseases, and on the other hand the increasing incidence of obesity).

After chemotherapy, AST transaminase levels increased in parallel with speed-elasticity, which shows the fact that higher AST levels correlate with a higher degree of hepatic fibrosis. So, after chemotherapy, due to the liver mobilization of cytostatics, a degree I to III liver fibrosis appears, according to data provided by Sporea et al. [15].

In our oncological group 19 or 30 patients (23.17% of all children) had an association of two to five chemotherapeutics with high toxicity (Busulfan, Carboplatin, Dacarbazine, mercaptopurine, 6-thioguanine, dactinomycin, asparaginase, daunorubicin, methotrexate [5]) and in these patients we observed higher values of SWV.

Related to hepatotoxicity after chemotherapy, the guidelines on dose modification in hepatic disease are largely empiric. Clinical judgment and a high index of suspicion remain critical tools in preventing and treating hepatic manifestations of cancer chemotherapy [5].

## Conclusions

In children with liver damage, compared to the control group, high transaminase levels and SWV correlate with the degree of fibrosis. Also, in oncologic patients, due to the liver metabolism of cytostatics, the higher the AST levels were, the higher the stiffness (SVW) and the degree of hepatic fibrosis was.

Elastography can measure liver tissue fibrosis better than other biological parameters, and it could be a parameter for accurate assessing of liver damage; thus, it can be a helpful tool in preventing and treating liver alterations in various pathological conditions.

Maybe the results will be more conclusive in the future and more correlations will be established when the study groups will be bigger.

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## References

1. Wyllie R, Hyams JS – Pediatric Gastrointestinal Disease: Pathophysiology, Diagnosis and Management, 3<sup>rd</sup> ed., Elsevier Inc 2006
2. Lanzkowsky P (ed) – Manual of Pediatric Hematology and Oncology, 4<sup>th</sup> ed., Elsevier 2005
3. Wieckowska A, Feldstein AE – Diagnosis of nonalcoholic fatty liver disease: invasive versus noninvasive. *Semin Liver Dis* 2008, 28(4): 386–95. Epub 2008 Oct 27.
4. Arundel C, Lewis JH – Drug-induced liver disease in 2006. *Curr Opin Gastroenterol* 2007, 23: 244–254.
5. King PD, Perry MC – Hepatotoxicity of Chemotherapy. *The Oncologist* 2001;6:162–176, available at [www.TheOncologist.com](http://www.TheOncologist.com), accessed on September 28, 2010
6. Adamson PC, Balis FM, Berg S, Blaney SM – General Principles of Chemotherapy. In Pizzo PA, Poplack DG (Eds). *Principles and Practice of Pediatric Oncology*. 5<sup>th</sup> ed. Philadelphia: Lippincott, Williams & Wilkins, 2006, 290–404.
7. Roberts EA – Drug-induced liver disease. In Suchy FJ, Sokol RJ, Balistreri WF, (Eds). *Liver Disease In Children*, Third Edition. Cambridge University Press, Cambridge, New York 2007, 478–512.
8. Lupusor M, Badea R, Stefanescu H, Sparchez Z, Branda H, Șerban A, Maniu A – Performance of a new elastographic method (ARFI technology) compared to unidimensional transient elastography in the noninvasive assessment of chronic hepatitis C. Preliminary results. *J Gastrointest Liver Dis* 2009, 18(3): 303–10.
9. Keyur Patel – Noninvasive tools to assess liver disease. *Current Opinion in Gastroenterology* 2010, 26: 227–233.
10. Piscaglia F, Corradi F, Mancini M, Giangregorio F, Tamperi S, Ugolini G, et al. – Real time contrast enhanced ultrasonography in detection of liver metastases from gastrointestinal cancer. *BMC Cancer* 2007, 7: 171 doi:10.1186/1471-2407-7-171
11. Lazebnik RS – Ultrasound. Mountain View, CA USA Tissue Strain Analytic, Virtual Touch Tissue Imaging and Quantification. Siemens Medical Solutions, USA, Inc., 2008
12. Frizzell LA, Carstensen EL – Shear properties of mammalian tissues at low megahertz frequencies. *J Acoust Soc Am* 1976, 60: 1409–1411.
13. Madsen EL, Sathoff HJ, Zagzebski JA – Ultrasonic shear wave properties of soft tissues and tissue-like materials. *J Acoust Soc Am* 1983, 74: 1346–1355.
14. Popescu A, Sporea I, Sirli R, Bota S, Focșa M, Dănilă M, et al. – Care sunt valorile medii ale elasticității hepatice prin tehnica ARFI (Acoustic Radiation Force Impulse Elastography) la subiecții sănătoși? *Medical Ultrasonography* 2010, 12, Suppl.1: 178–179.
15. Sporea I, Sirli R, Popescu A, Dănilă M – Acoustic Radiation Force Impulse (ARFI) – a new modality for the evaluation of liver fibrosis. *Medical Ultrasonography* 2010, 12(1): 26–31.