

New Combined Parameter of Liver and Splenic Stiffness as Determined by Elastography in Healthy Volunteers

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ABSTRACT

Background: The diagnosis of chronic liver disease (CLD) leading to fibrosis, cirrhosis, and portal hypertension had witnessed dramatic changes after the introduction of noninvasive figure accessible tools over the past few years. Imaging techniques that are based on evaluation of the liver stiffness was particularly useful in this respect. Acoustic radiation force impulse (ARFI) emerged as an interesting figure tool with reliable repute and high precision. **Aims:** To evaluate liver stiffness measurement (LSM) and splenic stiffness measurement (SSM) in healthy volunteers as concluded by the ARFI technique and to out a numeric calculated ratio that may reflect their correlation in the otherwise healthy liver. **Patients and Methods:** A ratio (splenic stiffness/liver stiffness in kPa) was determined in 207 consenting healthy subjects and was investigated with respect to age, gender, ethnic origin, body mass index (BMI), liver and spleen sizes healthy volunteers, alanine aminotransferase (ALT), aspartate aminotransferase (AST), platelet count (PLT), APRI, and FIB-4 scores. **Results:** Data from this work led to computing an index of 4.72 (3.42–7.33) in healthy persons on an average. Females had a higher index than males 6.37 vs 4.92, $P=0.002$. There was not any significant difference of the ratio in different age groups; ethnic origins; any correlation between SSM/LSM ratio and BMI; liver and spleen sizes; or ALT, AST, PLT, APRI, and FIB-4 scores. **Conclusions:** A quantifiable numeric relationship between splenic and liver stiffness in the healthy subjects could be computed to a parameter expressed as SSM/LSM ratio. We believe that this ratio can be a useful reference tool for further researches in CLD.

Key Words: ARFI, elastography, liver stiffness, splenic stiffness

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Chronic liver disease (CLD) may lead to fibrosis, cirrhosis, and portal hypertension with its clinical sequel. A correct evaluation of liver fibrosis in these patients is very important for the treatment, assessment of prognosis, and long-term followup. Liver biopsy is considered as the “gold-standard” method of diagnosis and assessment of liver fibrosis despite the limitation of being an invasive procedure which has potential complications,^[1] leading to 0.01%–0.1% risk of mortality.^[2,3] The inadequacy of biopsy specimens obtained due to a fraction of the entire liver being studied (about

1/50,000th) besides intra- and interobserver variation are other problems encountered with this procedure.^[4]

Evaluation of portal hypertension and esophageal varices remains dependent on hepatic venous pressure gradient, and endoscopy, respectively, which are known as the gold standard techniques for these parameters; however, the invasiveness and the availability of these procedures limits their widespread use.^[5]

Alternative noninvasive methods have emerged as useful easily accessible clinical tools for evaluating liver fibrosis in every day practice. Fibrotest[®], Hépascor[®], and Fibromètre[®],

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are some of the laboratory tests that depend on evaluating a combination of direct and indirect serum markers that may be indicative, yet their role in the diagnosis of hepatic fibrosis is still being evaluated.^[6] On the other hand, some clinical calculators as APRI or FIB-4 scores showed their high value in predicting significant fibrosis and cirrhosis in HCV patients.^[7,8] Likewise, measurement of liver stiffness by non-invasive methods might help physicians to determine prognosis at earlier stages among HCV patients.^[9] LS is measured and quantified by elastometry, which evaluates the tissue deformation after applying a force and could be divided into static that includes strain elastography; strain-rate imaging and dynamic that include transient elastography (TE); acoustic radiation force impulse (ARFI); shear wave elastography; and point shear wave elastography.

Nowadays, TE and ARFI are the most widely used noninvasive methods for the evaluation of liver fibrosis.^[10] ARFI has indisputable advantages over the classical fibroscan technique such as higher rates of valid measurements in comparison to TE,^[11,12] its usage as a complement to the conventional B-mode whole-liver evaluation, and its flexibility in performing in patients with ascites and in obese patients.

A meta-analysis by Friedrich-Rust *et al.*, which assessed eight studies including 518 patients, had shown that the mean diagnostic accuracy of ARFI for cirrhosis expressed as AUROC was 0.93.^[13] In addition to that some retrospective studies had shown that LS may predict the development of hepatocellular carcinoma and portal hypertension-related events.^[14,15]

The spleen plays an important role in evolution of portal hypertension-associating liver fibrosis. It is possible to measure splenic stiffness (SS) by ARFI and this was found to be variable in some liver diseases. It is thus logical to assume that there could be a correlation between the liver and splenic stiffness measurement in health and in disease. Colecchia *et al.* found that the correlation between splenic stiffness (SS) and HVPG ($r^2 = 0.78$) is higher than the correlation between liver LS and HVPG ($r^2 = 0.7$).^[16] This raises the possibility that when both are studied together one may be able to predict the onset or severity of portal hypertension. A meta-analysis however had shown that SS is not yet accurate enough to replace upper endoscopy for esophageal varices evaluation.^[17]

This work was done with a view to evaluate liver stiffness measurement (LSM) and splenic stiffness measurement (SSM) in healthy volunteers as concluded by the ARFI technique and to find out how they correlate to each other in the otherwise healthy liver. A correlation factor between them is computed as a consolidated ratio that may be taken as a reference to define in the normal population and to be used to predict the presence or severity of liver disease.

PATIENTS AND METHODS

Objective

The aim of this study was to calculate a computed ratio (SSM/LSM) that is concluded from elastography measurement of the healthy spleen and liver. Healthy volunteers and patients who were consulted for non-liver related disease were evaluated for the study. The ratio was to be studied in the different age groups, with respect to gender, ethnicity, and BMI. An attempt was also made as well to correlate the ratio with clinical, laboratory, and radioimaging studies. Our main objective was to furnish a parameter defining the normal population and that could be utilized as a possible indicator of liver health status so as to be applied as a predictor of the severity of liver pathology when diseased.

Subjects

Two hundred and seven consenting subjects were enrolled in this cohort from three medical centers over a period of 6 months (March 2015 to September 2015). Informed consent was obtained from all participants. Ethical Committee approval of the study was also secured.

Inclusion criteria

All consented subjects were above 12 and below 75 years of age. These included healthy volunteers and patients who were consulting for any disease that was not related to the liver and may not evolve or have any negative impact on the liver either because of the disease process itself or because of the medications being taken for treatment of their illness. All subjects had to be free from any complaints and/or signs related to acute or chronic present or past liver disease [Figure 1].

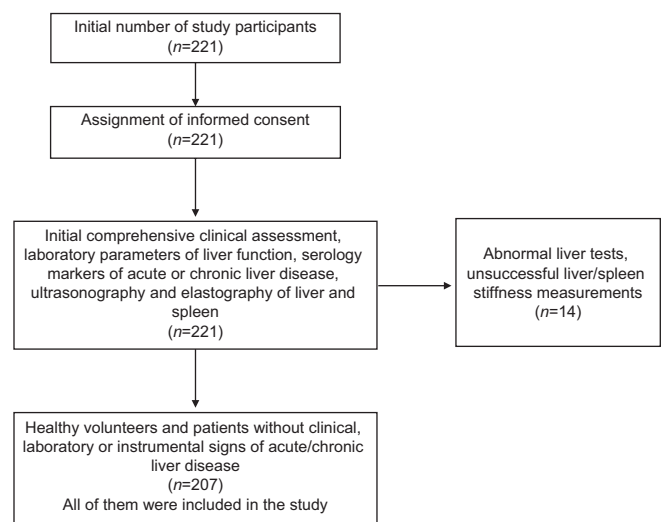


Figure 1: Flow chart of study design

Exclusion criteria

Subjects below 12 or above 75 years of age; mental disorders; status postsplenectomy; decompensated respiratory and/or renal function; hepatocellular carcinoma; immobilized patients; CVA or patients in coma; congestive cardiac failure; on medications that could have a deleterious effect on the liver; or debilitating illness: Oncogenic, immune deficiency, or genetic.

Methods

Upon recruitment, each subject had a clinical examination, and blood biochemistry to include relevant tests such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), and platelet count (PLT). APRI and FIB-4 indexes were determined according to the following published formula:^[8,18] APRI = AST (ULN)/PLT ($10^9/L$); FIB-4 = age (years) \times AST (IU/L)/(PLT ($10^9/L$) \times (ALT^{1/2} (IU/L)).

An upper abdominal echography was performed for every subject to evaluate the liver and spleen size, shape, and echotexture. Any evidence of a radiologic pathology in the liver or spleen would exclude the case from the study.

The study population was stratified according to BMI cutoffs adopted by World Health Organization criteria: Less than 18.5 (lean), 18.5–24.9 (normal), 25–29.9 (overweight), 30–34.9 (obesity 1), 35–39.9 (obesity 2), and body mass index more than 40 (obesity 3).

Acoustic radiation force impulse

Liver and spleen stiffness measurements were performed with a Siemens Acuson S2000 ultrasound system. Patients were placed in the supine position, with the right arm in maximum abduction to make the right hypochondrium accessible and to increase intercostal space (to improve the acoustic window). The probe was placed parallel to the intercostal space within the space with sufficient gel in order to minimize rib shadowing. The region of interest was positioned within the liver parenchyma under visual control in two-dimensional B-mode. The size of the region of interest was fixed at 10×5 mm. When ARFI was activated, the measurement (m/s) was displayed on the screen after a few seconds. Ten measurements were taken in the right lobe of the liver, in the intercostal space with the patient holding his/her breath gently. After taking the measurements in segments 7 and 8 of liver, the same number of measurements were taken in the spleen while the patient was in the right lateral position. The results of measurements of shear waves being expressed in m/s were converted into kilo Pascal (kPa) using the Siemens software.

Statistical analysis

The qualitative data are given in absolute figures and percentages. The numeric variables were calculated as mean (median, Q_1 , Q_3). The SSM/LSM ratio is

the quantitative relationship between spleen stiffness measurement (kPa) and liver stiffness measurement (kPa) presented as median (Q_1 – Q_3). Numeric variable SSM/LSM ratio has an asymmetrical distribution, so the differences between numerical variables were analyzed by nonparametric tests (Kruskal–Wallis and Mann–Whitney). Spearman's correlation analysis was used to assess relationships between SSM/LSM ratio and waist measurement, splenic size, laboratory tests. The cutoff for statistical significance was set at $P < 0.05$. Statistical analysis was performed using Statistical Package for the Social Sciences version 20.0 (IBM Corp, Armonk, NY, USA).

RESULTS

A cohort of 207 subjects was included in the study; 71 healthy volunteers and 136 patients presenting with other diseases that did not evolve and would not potentially affect the liver. All patients had a morphologically normal liver and spleen by the results of clinical and upper abdominal ultrasound. Transaminase profile, PLT, and fibrosis scores were normal for all patients. The main characteristics of the patients included in the study are summarized in Table 1.

The calculated SSM (kPa)/LSM (kPa) ratio for the whole cohort was 4.72 (3.42–7.33). The mean ratio across the age groups ranged from 4.98 in the group 50–59 years old to 6.64 in the 40–49 years age group with no remarkable difference between the two ratios ($P = 0.173$), thus indicating that age does not seem to have a statistically significant effect on the calculated SSM/LSM ratio. The mean ratio however was higher among females than in males being 6.37 and 4.92, respectively ($P = 0.002$), which indicated a statistically significant difference. This difference stood solid among all age groups with females having a higher ratio than males [Table 2 and Figure 2].

Ethnicity was not relevant to the stiffness ratio. The mean ratio for Arabs was 5.84, for the Europeans was 4.96, for

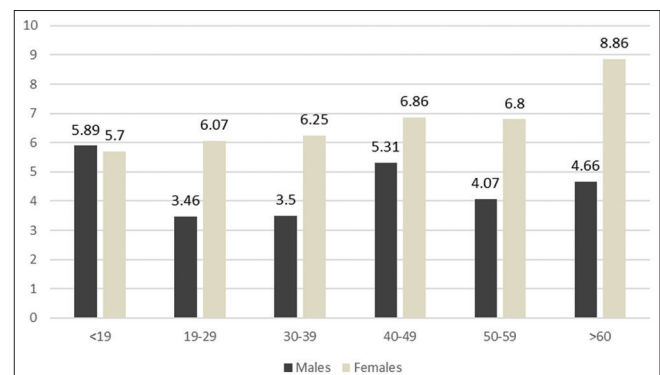


Figure 2: SSM/LSM ratio according to age and gender

Table 1: Main characteristics of study group*

Parameter	Value
Age (years)	
≤19	16 (7.7)
20-29	57 (27.5)
30-39	56 (27.1)
40-49	43 (20.8)
50-59	24 (11.6)
≥60	11 (5.3)
Gender	
Male	97 (46.9)
Female	110 (53.1)
Ethnicity	
Arabs	120 (58.0)
Asians and Indian subcontinent	46 (22.2)
Europeans	21 (10.1)
Africans	20 (9.7)
Body built	
BMI (kg/m ²)	26.36±4.61
Waist	91.67 cm (55-124)
Lean	5 (2.4)
Normal	71 (34.3)
Overweighed	82 (39.6)
Obese	49 (23.67)
Clinical	
Hepatic span (cm)	13.4 (12.2-15.5)
Splenic size (cm)	8.39 (5.7-13.3)
ALT (IU)	25.46±8.33
AST (IU)	17 (6-44)
PLT (count)	282.760 (114-436)
APRI	0.16 (0.06-0.64)
FIB-4	0.42 (0.11-1.36)

*Variables with a normal distribution are expressed as the mean±SD; Variables with a non-normal distribution are expressed as the median (range); Qualitative variable as absolute number (%). BMI: Body mass index, SD: Standard deviation, PLT: Platelet, ALT: Alanine transaminase, AST: Aspartate aminotransferase, APRI: Aspartate aminotransferase-to-platelet ratio index, FIB-4: Fibrosis index based-4

Africans was 5.16, and subjects from Asia and the Indian subcontinent had a ratio of 5.97. Although the European ethnicity seemed to have the lowest value of the ratio, yet there was no statistically significant difference between them ($P = 0.772$). Gender was also irrelevant as regards ethnicity as there was no difference between males and females in the various ethnic categories.

The ratio of SSM/LSM varied with the body build of the subjects who were evaluated being 3.35 in the lean subjects, 5.66 in the subjects with normal BMI, 5.35 in the overweight, and 7.46 in the obese. There was no statistically significant difference between the different body build groups ($P = 0.264$).

Clinical parameters that could be related to the liver or spleen pathology such as hepatic size; splenic size; waist

Table 2: The values of splenic stiffness measurement/liver stiffness measurement ratio for age, gender, ethnicity, and body mass index groups

Parameter	SSM/LSM ratio (n=207)				P
	Mean	Median	Q1	Q3	
Age groups (years)					
<19	5.73	5.82	3.82	6.18	0.173
20-29	5.20	4.68	2.58	5.45	
30-39	5.72	4.72	3.74	7.32	
40-49	6.64	5.11	3.51	9.35	
50-59	4.98	4.29	3.42	6.35	
<60	5.80	3.60	3.60	7.43	
Gender					
Female	6.37	5.19	3.78	7.89	0.002
Male	4.92	4.22	3.23	6.37	
Ethnicity					
Arabs	5.84	4.84	3.42	7.70	0.722
Asians and ISC	5.97	4.89	3.49	7.12	
African	5.13	4.41	3.60	5.92	
Europeans	4.96	4.72	3.23	4.72	
BMI groups					
Normals	5.66	4.79	3.48	6.48	0.264
Lean	3.35	3.32	3.32	3.95	
Overweight	5.35	4.72	3.11	7.12	
Obesity 1	6.26	4.68	3.43	7.85	
Obesity 2	7.46	7.71	4.45	11.51	
Obesity 3	5.23	5.23	5.23	5.23	

BMI: Body mass index, LSM: Liver stiffness measurement, SSM: Splenic stiffness measurement

measurement; and the laboratory parameters including transaminases (ALT, AST), PLT, and fibrosis scores (APRI and FIB-4) were evaluated for any possible impact on the calculated SSM/LSM ratio under normal conditions using the Spearman's rho, which revealed that these parameters have no bearing on the ratio when the liver is healthy without any pathology [Table 3].

DISCUSSION

There is a growing popularity and reliability of liver stiffness measurement using the ARFI technique to evaluate the degree of liver fibrosis in patients with chronic liver diseases. The accuracy of elastography information obtained by ARFI can be significantly improved if splenic stiffness is also evaluated at the same time, in combination with liver stiffness.^[19] In this study we tried to demonstrate the applicability of splenic stiffness coupled with liver stiffness in conjunction as a diagnostic tool to define the status of the healthy liver without any pathology to be a reference factor when liver disease is considered. For this aim, we investigated LSM and SSM measurements in a cohort of evidently healthy subjects without a history of present acute or chronic liver disease or past medical history of liver involvement.

Table 3: Correlation between splenic stiffness measurement/liver stiffness measurement ratio and instrumental and laboratory tests

Instrumental/laboratory test	SSM/LSM ratio	
	Spearman's rho	P
Waist	0.053	0.447
Splenic size	0.074	0.290
ALT	-0.103	0.139
AST	-0.116	0.096
AST/ALT	-0.069	0.321
PLT	0.093	0.180
APRI	-0.113	0.105
FIB-4	-0.106	0.127

LSM: Liver stiffness measurement, SSM: Splenic stiffness measurement, PLT: Platelet, ALT: Alanine transaminase, AST: Aspartate aminotransferase, APRI: Aspartate aminotransferase-to-platelet ratio index, FIB-4: Fibrosis index based-4

It is known that anatomically the spleen is mainly made up of fibroelastic supporting tissue, which forms the capsule, coarse trabeculae, and a fine reticulum. Other parts of the spleen include the white pulp which consists of lymphatic nodules, arranged around an eccentric arteriole (the Malpighian corpuscle) and the red pulp formed by a collection of cells in the interstices of the reticulum, in between the sinusoids. This structure renders the spleen a relatively stiff organ with the stiffness estimated to be above 15 kPa by elastography. The liver on the other hand is a highly vascular organ with large-diameter capillaries lined by endothelial cells between rows of plates or cords of hepatocytes. The sinusoids also contain Kupffer cells of the reticuloendothelial system (RES). This structure imparts relative softness to this organ estimated to be below 4 kPa by elastography and thus it is apparent that the spleen should be stiffer than the liver when evaluated by elastography.

The SSM/LSM ratio is an index measured by dividing the values of SSM in kPa over LSM in the same units, which is meant to provide a relationship between the normal spleen and liver. This index when validated may be useful to define deviating chronic diseases of the liver that cause fibrosis by extrapolation of the value of the ratio obtained for these patients and comparing it with the norm.

This study indicated that there is an important relationship between the normal spleen and the liver, which ranges from 3 to 7/1. It appeared that age does not have any impact on the SSM/LSM ratio, as it stands almost similar with no significant differences across the age groups; however, this fact should be interpreted with caution due to the relatively small size of the sample. In addition to that, dedicated studies to determine the potential of a factor such as age and its effect on the liver and spleen stiffness measurements

are still lacking. Most of the relevant studies are devoted to ARFI measurements in the pediatric population.^[20-23]

There was a slight noticeable but statistically significant difference among both genders with a mean ratio of 6.37 in females and 4.92 in males ($P = 0.002$). Some studies had shown that liver stiffness values were higher in males than in females,^[24,25] so it is logical to assume that SSM/LSM ratio was higher in women.

Ethnicity did not pose any effect on the stiffness ratio, which revealed minor differences that were not statistically significant among the various ethnic groups in this cohort. It is worth mentioning here, that other authors had reported that the normal range of LSM in healthy population was lower in Asians than Europeans.^[26] This implies an expected higher ratio among the Asian population, whereas we encountered a higher ratio among Europeans. The information about the influence of BMI and hepatic steatosis on this difference is still controversial,^[27] so further studies in this field should be done. On the other hand, this study shows the versatility of calculated ratio for different ethnicities and nationalities.

The influence of BMI on SSM/LSM ratio was not found among males and females in this study. This fact also did not maintain the suggestion that hepatic steatosis could have an impact on the calculated parameter in healthy people. Palmeri *et al.* reported that no correlation was noted between BMI and liver shear stiffness in patients with NAFLD.^[28]

Laboratory tests such as the transaminases, platelets, and clinical calculators of fibrosis and cirrhosis (FIB-4 and APRI) did not reveal any correlation with the stiffness ratio in the current study. This is likely to be due to the fact that only those with normal ALT, AST, and PLT count were included. Some studies had shown that laboratory parameters were associated with increased LSM, but all of them were held in patients with CLD.^[29,30] Furthermore, serum markers of fibrosis are still insufficient to predict the evolution of chronic liver diseases. For example, a meta-analysis of 14 studies examining 10 panels of indirect blood markers in chronic hepatitis C has shown that in some cases they were not a reliable tool to differentiate stages of fibrosis.^[31]

Our study had the limitation that liver histology was not done to assure the normality of the liver in the recruited participants because liver biopsy the "gold-standard" for such information is invasive and was not justified in subjects who were apparently healthy or do not have any evidence of liver disease. On the other hand, percutaneous liver biopsy is an invasive procedure, which is not indicated for a large sample like this cohort and is prone to sampling errors and/or interobserver variability.^[4] Therefore the

“normality” of patients was concluded on volunteers’ history, physical examination, laboratory parameters, and ultrasound structure of the liver. We also acknowledge that the sample size for calculation of suggested parameters in a healthy population was small. There is a possibility that our results might be a platform for further investigations in this field. Because of these restrictions, the results of this study need to be validated in independent populations by other researchers.

CONCLUSIONS

There is an established cause and effect relationship between the liver and the spleen as understood from their physiological and pathological events. In real-life practice, this is concluded from clinical, laboratory, and radioimaging indicators. In this study, elastography using ARFI’s technique had allowed identifying a quantifiable numeric relationship between splenic stiffness and liver stiffness in the healthy subject, which could be computed to an index expressed as SSM/LSM ratio. This ratio appeared to be reasonably constant across different ages, ethnic origins and different body builds. It only appeared to be slightly higher among females. The computed ratio was 4.72 (3.42–7.33).

We provide this index hoping that it may be an acceptable tool to be used for predicting the severity of liver pathology that may lead to fibrosis and/or cirrhosis. Therefore, we consider that this parameter is useful for screening by gastroenterologists and hepatologists, using Virtual Touch techniques should it be validated by other researchers.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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