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Ultrasonographic Quantitative Estimation of Hepatic Steatosis in Children with Nonalcoholic Fatty Liver Disease (NAFLD)

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Abstract

Background & Aims—The diagnostic accuracy of hepatic ultrasonography for detection and grading of hepatic steatosis in children with suspected nonalcoholic fatty liver disease (NAFLD) remains poorly characterized. The aim of this study was to prospectively evaluate the clinical utility of ultrasonographic quantification of hepatic steatosis.

Methods—Our cohort consisted of 208 consecutive pediatric patients with biopsy-proven NAFLD. Hepatic ultrasonography was performed within 1-month of the liver biopsy procedure. Steatosis identified by ultrasonography was scored using a 0 to 3 scale based on echogenicity and visualization of vasculature, parenchyma and diaphragm and compared to histological features based on Brunt’s classification.

Results—The median age at time of first visit was 10.8 years and 64% were boys. Sixty-nine percent had moderate to severe steatosis on histology. Ultrasonographic steatosis score (USS) had an excellent correlation with histological grade of steatosis (with a Spearman’s coefficient of 0.80). The area under the receiver operating characteristic (ROC) curve for ultrasonographic detection of moderate to severe steatosis was 0.87. The USS did not correlate significantly with inflammatory activity or fibrosis stage; however, there was significant correlation with the NAFLD activity score (NAS) albeit this was due in large part to the strong correlation with the steatosis component of NAS. Serum ALT and AST were not associated with histological grade of steatosis and showed no correlation with USS.

Conclusions—Our results, which represent the largest prospective pediatric study evaluating the role of hepatic ultrasonography in children with biopsy-proven NAFLD, demonstrate the utility of this technique for non-invasive diagnosis and estimation of hepatic steatosis in children.

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Keywords

nonalcoholic fatty liver disease (NAFLD); steatosis; ultrasonography

Introduction

Non-alcoholic fatty liver disease (NAFLD) has become over the last decade the most common form of chronic liver disease in children and adults. It is tightly associated with obesity and threatens to become a serious public health problem in the United States and many other countries. NAFLD is estimated to affect close to 10% of the American population aged 2 to 19 years, and this figure increased to 30 - 40% among obese children.

In addition to being a highly common condition in children, several lines of evidence suggest that NAFLD is a potentially serious condition. A recent long-term longitudinal study has demonstrated that similarly to adults, NAFLD in children is a disease with the potential to progress (1). Some children in this study presented with cirrhosis at time of diagnosis, others showed progressive liver disease resulting in significant liver-related morbidity. Moreover, the presence of NAFLD in children may be a key indicator of the metabolic status and a good predictor for development of type 2 diabetes. Thus, establishing the diagnosis of NAFLD is of utmost importance and represents a major challenge as the disease is generally silent and the current gold standard for diagnosis is an invasive liver biopsy, a procedure that is not suitable for screening purposes.

Other screening measures have been employed, including monitoring liver transaminases and recently, the American Academy of Pediatrics has recommended that serum aminotransferases (ALT and AST) be performed in all overweight children starting at age 10 years if their BMI is ≥ 95 th percentile or between 85-94th percentile with risk factors. ALT and AST are to be checked in addition to fasting glucose and lipid profile. However, it has become clear that in both adults and children liver enzymes performed poorly for NAFLD diagnosis with two-thirds of NAFLD patients showing normal levels of serum ALT and AST (2).

Regarding the use of imaging for screening purposes, hepatic ultrasonography (US) is the most commonly used modality largely because it is relatively inexpensive, widely available and is user-friendly (3). Several studies in adults have demonstrated that this technique is highly sensitive and specific for detection of NAFLD. Moreover, hepatic US can provide a good estimate of the degree or extent of hepatic steatosis present based on a series of US characteristics including hepatorenal echo contrast, liver echogenicity, visualization of intrahepatic vessels, and visualization of liver parenchyma and the diaphragm (4-7). However, the diagnostic accuracy of hepatic US and the utility for quantification of the degree of hepatic steatosis in children remains unknown. Thus, we conducted this study to evaluate the utility of hepatic US for quantifying hepatic steatosis in a large well-characterized pediatric population with biopsy-proven NAFLD.

Methods

Patients

A total of 208 consecutive patients diagnosed with NAFLD seen at Bambino Gesù Children's Hospital from January 2005 to January 2010 were included in the study. The study was approved by the Ethics Committee of the Bambino Gesù Children's Hospital and Research Institute, Rome, Italy. Informed consent was obtained from each patient or responsible guardian.

Inclusion criteria were liver biopsy consistent with the diagnosis of NAFLD (8, 9). Exclusion criteria were the presence of hepatic virus infections, alcohol consumption (≥ 140 g/week), history of parenteral nutrition, and use of drugs known to induce steatosis (e.g. valproate, amiodarone or prednisone) or to affect body weight and carbohydrate metabolism. Autoimmune liver disease, metabolic liver disease, Wilson's disease, and α -1-antitrypsin-associated liver disease were ruled out using standard clinical, laboratory and histological criteria.

The body mass index (BMI) and BMI Z-score were calculated (10, 11). Metabolic syndrome (MS) was defined as the presence of ≥ 3 of the following 5 criteria (12): abdominal obesity (defined by waist circumference $\geq 90^{\text{th}}$ percentile for age) (13); hypertriglyceridemia as TG $> 95^{\text{th}}$ percentile for age, gender and race (14); low HDL cholesterol as concentrations $< 5^{\text{th}}$ percentile for age and sex (14); elevated blood pressure (BP) as systolic or diastolic BP $> 95^{\text{th}}$ percentile for age and sex (15); and impaired fasting glucose or known type 2 diabetes mellitus (16, 17). The degrees of insulin resistance (IR) and sensitivity were determined by the homeostasis model assessment for insulin resistance (HOMA-IR) using the formula: insulin resistance = (insulin $\times$ glucose)/22.5, by the insulin sensitivity index (ISI) derived from oral glucose tolerance test using the formula: ISI = $[10,000/\text{square root of (fasting glucose} \times \text{fasting insulin)} \times (\text{mean glucose} \times \text{mean insulin during OGTT})]$ and by the quantitative insulin sensitivity check index (QUICKI) using the formula: insulin sensitivity = $1/(\log \text{ of fasting insulin} + \log \text{ of fasting glucose})$ (18-20).

Liver Histology

The clinical indication for biopsy was either to assess the presence of nonalcoholic steatohepatitis (NASH) and degree of fibrosis or other likely independent or competing liver diseases. Liver biopsy was performed in all children, after an overnight fast, using an automatic core biopsy 18 Gauge needle (Biopince, Amedic, Sweden) under general anaesthesia and ultrasound guidance. A Sonoline Omnia Ultrasound machine (Siemens, Germany) with a 5-MHz probe (5.0 C 50, Siemens) with a biopsy adaptor was employed. Two biopsy passes within different liver segments were performed for each subject. The length of liver specimen (in millimetres) was recorded. Only samples with a length ≥ 15 mm and including at least 5-6 complete portal tracts were considered adequate for the purpose of the study (9). Biopsies were evaluated by a single liver pathologist. Biopsies were routinely processed (*i.e.*, formalin-fixed and paraffin-embedded). Sections of liver tissue, 5 μ m thick, were stained with Hematoxylin-Eosin, Van Gieson, PAS-D, and Prussian blue stain. Immunohistochemical staining with antibodies to alpha-1-antitrypsin was used to exclude alpha-1-antitrypsin deficiency-associated liver disease. Liver biopsy features were graded according to the NAFLD activity score (NAS) system proposed by Kleiner et al (21). Briefly, grade of steatosis was scored as 0 = $< 5\%$; 1 = 5% - 33% ; 2 = $> 33\%$ - 66% ; 3 = $> 66\%$; grade of lobular inflammation was scored as 0 = no foci; 1 = < 2 foci/200xfield; 2 = 2-4 foci/200xfield; 3 = > 4 foci/200xfield; and grade of ballooning was scored as 0 = none; 1 = few ballooning cells; 2 = many cells/prominent ballooning. The grade of steatosis (0-3), lobular inflammation (0-3), and ballooning (0-2) were then combined to determine the NAFLD activity score (0-8) as proposed. Fibrosis was scored as 0 = none; 1 = periportal or perisinusoidal fibrosis; 2 = perisinusoidal and portal/periportal fibrosis; 3 = bridging fibrosis; and 4 = cirrhosis (10). The liver biopsy samples were then classified as either definitive NASH (unequivocally fulfills previously described criteria for steatohepatitis), borderline diagnosis (some but not all histologic features of steatohepatitis), simple steatosis (isolated fat deposition in hepatocytes).

Liver ultrasonography

Conventional hepatic US was performed within 1 month of the liver biopsy by a single radiologist using an Acuson S2000 ultrasound system (Siemens, Germany) with linear and convex transducers (frequency bandwidth 4-14MHz). The radiologist was blinded to the clinical, laboratory and histological data of the patients. The ultrasonographic steatosis score (USS) was calculated as follows: absent (score 0) steatosis was defined as normal liver echotexture; mild (score 1) steatosis as slight and diffuse increase in fine parenchymal echoes with normal visualization of diaphragm and portal vein borders; moderate (score 2) steatosis as moderate and diffuse increase in fine echoes with slightly impaired visualization of portal vein borders and diaphragm; and severe (score 3) steatosis as fine echoes with poor or no visualization of portal vein borders, diaphragm, and posterior portion of the right lobe (22).

Statistical Analysis

Descriptive statistics were computed for all factors. These included means, standard deviations and percentiles for continuous variables and frequencies for categorical factors. Univariable analysis was done to compare subjects with moderate/advanced steatosis to those with mild steatosis. Student's t-tests and Wilcoxon rank sum tests were used to compare continuous variables, Pearson's chi-square were used for categorical variables and Mantel-Haenszel chi-square tests were used for ordinal factors. Spearman's correlation coefficients were used to evaluate correlations between USS and clinical factors of interest. In addition, a multivariable logistic regression analysis was performed to assess factors associated with presence of moderate/advanced steatosis. USS was forced into the model and an automated stepwise variable selection was performed on 1,000 bootstrap samples; factors that appeared in $\geq 40\%$ of replications were kept in the final model. A $p < 0.05$ was considered statistically significant. SAS version 9.2 software (The SAS Institute, Cary, NC) and R version 2.10.1 software (The R Foundation for Statistical Computing, Vienna, Austria) were used to perform all analyses.

Results

Patient Characteristics

The main demographic and clinical features of the subjects included in the analysis are presented in Table 1. Out of the 208 participating patients, 132 (64%) were male. Overall, the median age at the time of first visit was 10.8 years (3.25-14.1 years). Children who had higher USS were significantly older at the time of first visit than those who had lower USS. Eighty-eight percent of the patients were obese. BMI percentile and waist circumference tended to increase with higher USS when compared between groups with USS 0-1 and those with USS 2-3. This was statistically significant with p value 0.046 (BMI percentile difference) and 0.014 (waist circumference difference). When comparing the same two groups, there was no significant difference in serum ALT or AST levels. Serum alkaline phosphatase and GGT were higher in the group with USS 2-3 as compared to those with USS 0-1.

Comparison of US determinations with histological features on liver biopsy

The histological findings and USS of the subjects included in the analysis are presented in Table 2. Fifty-six patients (27%) had severe steatosis on biopsy, 87 patients (42%) had moderate steatosis, 63 patients (30%) had mild steatosis and only 2 patients (1%) had no or minimal steatosis ($<5\%$ steatosis). On ultrasonographic examination, 46 patients (22%) had severe steatosis, 77 (37%) had moderate steatosis, 73 (35%) had mild steatosis, and 12 (6%) had no evidence of steatosis. Examining other individual histological features showed that

56% of the patients had stage 1 fibrosis with only 12% having stage 2 or 3 fibrosis. Regarding inflammation grade, most of the patients (73%) had grade 1 inflammatory activity. There was no inflammation on biopsy in 11.5% of the patients. Fifty-three percent showed no ballooning (grade 0), 23% had grade 1 and 24% had grade 2. Sixty-six patients (32%) had a diagnosis of NASH based on NAS and 56 patients (27%) had isolated hepatic steatosis.

When dividing the patients into two groups based on USS (one group with USS 0-1 and one group with USS 2-3), we found that there was a statistically significant difference between the two groups regarding steatosis on liver biopsy. Ninety-two percent of the patients with USS 2-3 also had moderate to severe steatosis on biopsy and a majority of the patients with USS 0-1 had mild or no steatosis on biopsy ($p < 0.001$). When comparing the other histological features in the two groups of patients based on USS, we found that there was no significant difference between the two groups regarding extent of fibrosis and inflammation. Patients with an USS 2-3 had more ballooning on biopsy than those patients with USS 0-1.

Utility of USS for detection of hepatic steatosis

Table 3 outlines the correlations between USS and clinical and histological features evaluated in the study. There was excellent correlation between USS and steatosis on biopsy with a Spearman's coefficient of 0.80 [CI (0.71, 0.88), p value < 0.001] (Figure 1). Using a cut-off value of 2 for the USS, the area under the receiver operating characteristics (ROC) curve for ultrasonographic detection of moderate to severe steatosis was 0.87 (Figure 2). Sensitivity and specificity for diagnosing moderate to severe steatosis with $USS \geq 2$ were 79.7% and 86.2%, respectively. With a cut-off value of 3 for the USS, specificity for diagnosing moderate to severe steatosis on biopsy increased to 100%. Using multivariable logistic regression, we found that for each 1 unit increase in USS, there was a 27-fold increase in the odds ratio for having moderate to severe steatosis on biopsy. Correlation between USS and ballooning was statistically significant, though positive correlation was only fair [$\rho = 0.4$, CI (0.28, 0.53), $p < 0.001$]. The USS did not correlate significantly with inflammation or fibrosis. There was significant correlation between the USS and NAFLD activity score (NAS); however, this correlation was due primarily to the steatosis component of the NAS. A significant association was found between USS and BMI (p value < 0.006), waist circumference (p value < 0.001), and measurements of insulin resistance and sensitivity. Interestingly, serum ALT and AST levels were not associated with histological grade of steatosis and had no correlation with USS (Figure 3).

Discussion

The principal findings of this study relate to the utility of hepatic ultrasonography (US) for detection and quantification of hepatic steatosis in children with NAFLD. The results of this study, that included one of the largest cohorts of children with biopsy-proven NAFLD reported up to date, demonstrate that ultrasonographic steatosis score (USS) correlates tightly with severity of steatosis on liver biopsy. These results suggest that ultrasonographic exam is an excellent screening test for NAFLD in children and a useful non-invasive modality for quantifying hepatic steatosis. Moreover, we found that serum aminotransferases had poor predictive value regarding the presence or severity of fatty liver disease; thus, serum ALT and AST are not helpful in dictating whether further work-up should be done in obese children in whom fatty liver is suspected.

US has long been recognized as a useful screening tool for NAFLD and is the most frequently used radiological modality in fatty liver evaluation (3). Most of the currently available data on the use of US for the diagnosis of steatosis has been retrospective and in adult populations (5-7). A recent study by Chiloiro et al (4) studied the relationship among

ultrasonographic diagnosis of fatty liver, adipose tissue distribution and metabolic profile in 94 moderately obese children with no available liver biopsy. Fatty liver on US positively related to anthropometric measurements as well as insulin resistance and other metabolic abnormalities associated with obesity, while no statistically significant association was found between fatty liver on US and increased serum ALT and AST levels. Our current study extends these observations and demonstrates a strong positive correlation between USS and degree of steatosis on liver biopsy while confirming the lack of association of serum transaminases with both fatty liver determination by US and liver biopsy. These findings are of utmost importance as current screening guidelines for identifying and performing further work up of children at risk of NAFLD is solely based on serum transaminase levels. Our current data in conjunction with the growing literature addressing the limitations of serum liver enzymes(23, 24) in the evaluation of NAFLD suggest hepatic US as a more valid alternative for screening at risk pediatric population.

We found that hepatic US was unable to make the distinction between NAFLD and NASH, a finding that is similar to previous reports in adult populations studies(5, 7). One prospective adult study looking at the use of US in detecting hepatic steatosis found that none of the US findings could distinguish between steatosis and NASH, with no significant association found between US findings and stage of fibrosis or grade of inflammation. Furthermore, the lowest amount of steatosis that could be detected via US with the greatest correlation to histological findings was $\geq 20\%$ (5). Our study adds further strength to the finding that US sensitivity and specificity in quantifying steatosis are improved when steatosis is moderate to severe. An USS of 2 in our study had a sensitivity of almost 80% in diagnosing moderate to severe steatosis. Similar to what has been reported in the adult population, determination of USS in children was not able to distinguish between steatosis and NASH and was not useful for quantitation of inflammatory activity and stage of fibrosis. These results further support the need for development of novel reliable biomarkers for risk stratification, and monitoring response to therapeutic intervention in children with NAFLD.

The main strengths of our study are the inclusion of a large group of consecutively recruited children with liver biopsy-proven NAFLD with the full spectrum of disease in whom US was performed within a month of liver biopsy procedure. Limitations of our study include the fact that the patients were seen at a large referral tertiary care medical center so it is possible that results may not be extrapolated to children with NAFLD from the community. Second, US exams were read once by a single radiologist; therefore intra- and inter-observer variability could not be evaluated. Finally, in the current study we were unable to evaluate potential causes of US failure in detecting steatosis as only a small group of patients ($n = 12$) had an USS of zero. Further studies evaluating US as a tool for longitudinal follow-up are warranted.

In summary, our results indicate that hepatic US is a useful tool for quantifying steatosis in pediatric patients who have suspected NAFLD, with USS strongly correlating with grade of steatosis on liver biopsy. Ultrasonographic exam of the liver should be an initial screening tool, even in the face of normal liver enzymes as these are poor predictors of fatty liver disease.

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Figure 1.
Scatter plot demonstrating correlation between USS and grade of steatosis

Figure 2.

ROC curve demonstrates correlation between USS and grade of steatosis. Sensitivity and specificity of ultrasonography for detecting moderate to severe steatosis using a cutoff value for $USS \geq 2$ are 79.7% and 86.2%, respectively.

Figure 3.
Scatter plot demonstrating no correlation between ALT/AST and steatosis on biopsy.

Table 1**Demographic and Clinical Characteristics of Subjects**

Factor	All (N=208)	Ultrasound 2-3 (N=123)	Ultrasound 0-1 (N=85)	p-value
Male [n (%)]	132 (63.5)	85 (69.1)	47 (55.3)	0.042
Age at first visit (yr)	10.8 ± 2.5	11.2 ± 2.3	10.2 ± 2.6	0.005
Weight (kg)	55.4 ± 16.3	59.1 ± 16.8	50.0 ± 14.0	<0.001
Height (cm)	145.5 ± 16.0	148.5 ± 15.8	141.2 ± 15.3	<0.001
BMI (kg/m ²)	25.3 ± 3.9	26.0 ± 3.8	24.3 ± 3.7	0.002
BMI Percentile	94.3 ± 6.6	95.1 ± 5.3	93.1 ± 7.9	0.046
WC (cm)	86.5 ± 10.0	88.6 ± 9.3	83.6 ± 10.3	<0.001
WC Percentile	93.8 ± 3.7	94.3 ± 3.6	93.0 ± 3.8	0.014
Systolic BP (mmHg)	111.6 ± 12.9	111.8 ± 12.7	111.4 ± 13.3	0.83
Diastolic BP (mmHg)	68.5 ± 8.5	68.9 ± 8.6	67.8 ± 8.4	0.39
Cholesterol (mg/dL)	162.8 ± 34.2	165.4 ± 35.4	159 ± 32.3	0.17
Triglycerides (mg/dL)	90.0 (71, 127)	90 (75, 130)	90 (69, 120)	0.37
ALT (U/L)	69.5 (50.0, 87.0)	69.0 (51.0, 93.0)	71.0 (49.0, 84.0)	0.5
AST (U/L)	48.5 (39.0, 62.0)	49.0 (39.0, 65.0)	47.0 (39.0, 58.0)	0.41
GGT (U/L)	25.0 (18.0, 38.0)	27.0 (20.0, 41.0)	22.0 (17.0, 32.0)	0.01
Total bilirubin (mg/dL)	0.6 ± 0.2	0.6 ± 0.2	0.7 ± 0.2	0.09
Albumin (g/dL)	4.5 ± 0.5	4.5 ± 0.5	4.6 ± 0.6	0.58
Alkaline phosphatase (U/L)	720.9 ± 190.9	772.4 ± 205.0	652.2 ± 145.5	<0.001
ISI (M/mU/L)	4.0 ± 2.2	3.8 ± 2.1	4.2 ± 2.2	0.15
QUICKI	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.06

Values presented as Mean ± SD or Median (25th, 75th percentiles) for continuous factors and N (%) for categorical variables.

p-values correspond to t-tests or Wilcoxon rank sum tests for continuous factors, Pearson's chi-square tests for categorical variables and Mantel-Haenszel chi-square tests for histology and ultrasound scores.

Table 2**Histological Features**

Histology n (%)	All (N=208)	Ultrasound 2-3 (N=123)	Ultrasound 0-1 (N=85)	p-value
Fibrosis				0.15
0	68 (32.7)	34 (27.6)	34 (40.0)	
1	116 (55.8)	74 (60.2)	42 (49.4)	
2	12 (5.8)	7 (5.7)	5 (5.9)	
3	12 (5.8)	8 (6.5)	4 (4.7)	
Steatosis				<0.001
<5%	2 (1.0)	0 (0.0)	2 (2.4)	
5-33%	63 (30.3)	9 (7.3)	54 (63.5)	
34-65%	87 (41.8)	58 (47.2)	29 (34.1)	
>=66%	56 (26.9)	56 (45.5)	0 (0.0)	
Inflammation				0.10
None	24 (11.5)	13 (10.6)	11 (12.9)	
<2 under 20x	152 (73.1)	87 (70.7)	65 (76.5)	
2-4 under 20	29 (13.9)	20 (16.3)	9 (10.6)	
>4 under 20x	3 (1.4)	3 (2.4)	0 (0.0)	
Ballooning (n=206)				<0.001
None	109 (52.9)	51 (42.2)	58 (68.2)	
Few	48 (23.3)	29 (24.0)	19 (22.4)	
Many	49 (23.8)	41 (33.9)	8 (9.4)	
NAS	3.7(1.6)	4.4(1.4)	2.7(1.2)	<0.001
NAFLD				<0.001
Steatosis	56 (26.9)	13 (10.6)	43 (50.6)	
Borderline	86 (41.4)	52 (42.3)	34 (40.0)	
NASH	66 (31.7)	58 (47.2)	8 (9.4)	
ULTRASOUND				
None	12 (5.8)	0 (0.0)	12 (14.1)	
Mild	73 (35.1)	0 (0.0)	73 (85.9)	
Moderate	77 (37.0)	77 (62.6)	0 (0.0)	
Severe	46 (22.1)	46 (37.4)	0 (0.0)	

Table 3

Correlations between Ultrasound Score and Clinical/Histological Factors

Factor	rho	95% CI	P value
Steatosis	0.80	(0.71,0.88)	<0.001
Ballooning	0.4	(0.28,0.53)	<0.001
Inflammation	0.1	(−0.03,0.24)	0.14
NAS	0.65	(0.54,0.75)	<0.001
Fibrosis	0.19	(0.06,0.33)	0.006
MS Components	−0.04	(−0.18,0.09)	0.52
Age I° visit	0.26	(0.13,0.39)	<0.001
BMI Percentile	0.03	(−0.11,0.17)	0.69
WC	0.3	(0.17,0.43)	<0.001
WC Percentile	0.15	(0.01,0.28)	0.034
BMI	0.19	(0.05,0.32)	0.006
Systolic BP	0.04	(−0.10,0.17)	0.59
Diastolic BP	0.09	(−0.04,0.23)	0.18
Cholesterol	0.08	(−0.06,0.21)	0.28
Triglycerides	0.06	(−0.08,0.20)	0.38
ALT	0.02	(−0.11,0.16)	0.75
AST	0.01	(−0.13,0.14)	0.94
GGT	0.15	(0.01,0.29)	0.031
Bilirubin	−0.16	(−0.30,−0.03)	0.019
Albumin	−0.05	(−0.18,0.09)	0.5
HOMA-IR	0.16	(0.03,0.30)	0.019
ISI	−0.1	(−0.24,0.04)	0.15
QUICKI	−0.15	(−0.29,−0.02)	0.026

rho: Spearman's correlation coefficient

CI: confidence interval