ABSTRACT – Background – Non-invasive markers are useful and practical tools for assessing non-alcoholic fatty liver disease (NAFLD), but liver biopsy remains the gold-standard method. Liver biopsy can be easily obtained on individuals undergoing bariatric surgery, but there is no ultimate evidence on the relationship between costs, risks and benefits of its systematic performance. Objective – To compare the diagnostic accuracy of non-invasive methods with liver biopsy for detection and staging of NAFLD in obese individuals undergoing bariatric surgery. Methods – This is a cross-sectional, observational and descriptive study which enrolled individuals who underwent bariatric surgery from 2018 through 2019 at a public tertiary university hospital. Ultrasound scan, hepatic steatosis index, Clinical Non-Alcoholic Steatohepatitis Score (C-NASH), hypertension, alanine aminotransferase (ALT) and insulin resistance (HAIR), aspartate aminotransferase (AST) to Platelet Ratio Index (APRI), NAFLD Fibrosis Score (NFS) and body mass index, AST/ALT ratio, and diabetes (BARD) were the methods compared with the histopathological examination of wedge liver biopsies collected during surgery. Results – Of 104 individuals analyzed, 91 (87.5%) were female. The mean age was 34.9±9.7 years. There was no biopsy-related morbidity. The respective overall accuracies of each marker analyzed were: ultrasound scan (79.81% for steatosis), hepatic steatosis index (79.81% for steatosis), HAIR (40.23% for steatohepatitis), C-NASH (22.99% for steatohepatitis), APRI (94.23% for advanced fibrosis), NFS (94.23% for advanced fibrosis), and BARD (16.35% for advanced fibrosis). Discussion – Given the high prevalence of liver disease within this population, even the most accurate markers did not present enough discretionary power to detect and/or rule out the NAFLD aspects they were designed to assess in comparison with liver biopsy, which is safe and easy to obtain in these patients. Conclusion – Wedge liver biopsy during bariatric surgery helps to diagnose and stage NAFLD, presents low risks and acceptable costs; given the limitations of non-invasive methods, it is justifiable and should be considered in bariatric routine.

Keywords – Non-alcoholic fatty hepatopathy; fatty liver; obesity; bariatric surgery; liver function tests.
This study aims at comparing the diagnostic accuracy of non-invasive methods with liver biopsy for detection and staging of NAFLD in obese individuals undergoing BS and at proposing the routine performance of liver biopsy during BS.

METHODS

Study design
This is a cross-sectional, observational, and descriptive study. Data were collected through the analysis of medical records of individuals who underwent BS (open Roux-en-Y gastric bypass) from 2018 through 2019 at a public tertiary university hospital. The study protocol was approved by the local institutional review board and all participants provided informed consent.

Work developed at Department of Surgery – Faculty of Medical Sciences – State University of Campinas (UNICAMP), Campinas (SP), Brazil.

Study population
Inclusion criteria were patients who underwent BS indicated according to the National Institutes of Health criteria (body mass index [BMI] greater than 40 kg/m² or BMI greater than 35 kg/m² with comorbidities associated with obesity), of any gender, aged between 18 and 70 years (5). Exclusion criteria were use of alcohol and hepatotoxic drugs, chronic viral hepatitis or serological abnormalities, bile duct obstruction and incomplete medical records. All individuals underwent a preoperative mandatory weight loss program and surgery was performed when a minimal 10% weight loss was achieved.

Variables
The data collected were age, gender, weight, BMI, results of abdominal ultrasound (US) scan and liver biopsy findings. The following laboratory tests were consulted: glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT), total cholesterol, high-density lipoprotein (HDL), triglycerides, insulin, alkaline phosphatase (ALK), gamma-glutamyl transferase (GGT), platelets and albumin.

Non-invasive methods
US, hepatic steatosis index (HSI), Clinical Non-Alcoholic Steatohepatitis Score (C-NASH), Hypertension, ALT and Insulin Resistance (HAIR), AST to Platelet Ratio Index (APRI), NAFLD Fibrosis Score (NFS) and BMI, AST/ALT ratio, and diabetes (BARD) were the methods evaluated. TABLE 1 summarizes their rationales and outcomes (4-13).

Liver biopsy technique and histopathological evaluation
Wedge liver biopsy was performed during surgery after the main surgical proceeding. A 2-cm fragment was extracted with blunt scissors from segments III or IV.

NAFLD-related features were classified into categories: 1) steatosis (absent, mild, moderate or severe); 2) fibrosis (according to the Kleiner-Brunt classification): 0- absent; 1- perisinusoidal or periportal alone; 2- periportal and perisinusoidal; 3- presence of fibrous septa (“bridging fibrosis”); 4- cirrhosis; 3) steatohepatitis (classified in grades: 0, 1+, 2+, 3+) (41).

Statistical analysis
Calculations of diagnostic accuracy tests were performed: sensitivity, specificity, positive and negative predictive values (PPV and NPV), positive and negative likelihood ratios and overall accuracy. The gold standard was the histopathological evaluation. The software SAS Release 8.2 (SAS Institute Inc., Cary, NC) was used to perform the analysis.

RESULTS
Of 104 individuals selected for study, 91 (87.5%) were female. The mean age was 34.9±9.7 years. The main comorbidity was hypertension (34.6%), followed by dyslipidemia (24%) and type 2 diabetes (21.2%). The mean BMI was 36.1±3.5 kg/m². There were no biopsy-related complications.

The main NAFLD-related histopathological aspects were steatosis (80.8%), fibrosis (82.7%) and steatohepatitis (81.7%). Most individuals presented mild macrovesicular steatosis (55.8%), whereas the commonest stages of fibrosis were 1a and 2 (both 36.5%). Most individuals with steatohepatitis presented mild acinar inflammation (55.8%) and/or mild portal inflammation (58.7%). The details of histopathological and biochemical findings are shown in TABLE 2.

The respective overall accuracies of each marker analyzed were: US (79.81% for steatosis), HSI (79.81% for steatosis), HAIR (40.23% for steatohepatitis), C-NASH (22.99% for steatohepatitis), APRI (94.23% for advanced fibrosis), NFS (94.23% for advanced fibrosis), and BARD (16.35% for advanced fibrosis). TABLE 3 presents the complete diagnostic accuracy analyses of the non-invasive markers evaluated.

DISCUSSION
The current study focused on a highly prevalent disease in the population, especially in the group of individuals with obesity, with a risk of progression to chronic liver disease and high morbidity and mortality in advanced stages, mainly liver cirrhosis, including the risk of developing hepatocellular carcinoma. Individuals with an indication for BS surgery according to the NIH criteria are likely to present metabolic abnormalities and consequent abnormal deposit of fat in the liver, thus constituting a high-risk population for NAFLD. As such, the possibility of an early diagnosis of NAFLD could lead to relevant benefits, such as receiving guidance on risks, prognosis and evolution of the disease. A previous study by our group found a prevalence of over 50% of liver fibrosis in a population undergoing BS (4).

That is, in obese individuals, in addition to the usual investigation for type 2 diabetes, dyslipidemia and other metabolic co-morbidities, they could benefit from an eventual screening for NAFLD, especially through a method with the possibility of defining the different aspects of this disease. With this aim in mind, an ideal diagnostic method with few risks and low cost, in addition to high accuracy and high availability in clinical practice is highly sought. Given the high observed prevalence of steatohepatitis, significant (≥F2) and advanced (≥F3) fibrosis within the population of the current study, an accurate assessment of NAFLD becomes even more important in this context.

US scan is a low-cost, non-invasive and operator-dependent method, which is also highly available. An important factor to be highlighted in the US of obese patients is the technical difficulty in individuals with severe obesity, due to the thick subcutaneous tissue. It presented high sensitivity (99%) and high positive predictive value (81%). Despite this, it proved to be an ineffective method to...
TABLE 1. Main characteristics of each non-invasive score assessed.

| Score | Rationale | How to perform or calculate | Cut-off values and interpretation |
|-------|-----------|----------------------------|----------------------------------|
| US    | Used to determine the presence of liver steatosis\(^5\). | Qualitative visualization of hepatic echogenicity, measurement of the difference of the hepatic parenchyma compared to the renal parenchyma, evaluation of the penetration of the deep portion of the liver and through the determination of the hepatic structures, for example. | Subjective: normal, mild, moderate or severe. |
| HSI   | Created to predict the occurrence of steatosis in the general population\(^6\). | HSI = 8 x ALT/AST + BMI (+2 if T2DM, +2 if female) | A score >36 indicates the presence of steatosis, while a score <30 indicates absence of steatosis. |
| NFS   | Designed for prediction of advanced fibrosis in NAFLD patients\(^6\). | NFS = -1.675 + 0.037 X age (years) + 0.094 X BMI (kg/m\(^2\)) X IGT/T2D (Yes =1 OR No =0) +0.99 X AST/ALT – 0.013 X Platelet count \(10^9/\text{L}\) – 0.66 X Albumin (g/dL) | A score over 0.676 indicates advanced fibrosis, while a score below -1.455 excludes advanced fibrosis. |
| APRI  | Developed to predict advanced fibrosis and cirrhosis in patients with hepatitis C, it was then validated for detection of advanced fibrosis in NAFLD\(^9\). | APRI = [{(observed AST/AST (upper limit in iU/L)} / Platelet count \(10^9/\text{L}\)} x 100 | A score 0.98 indicates advanced fibrosis. |
| BARD  | Derived to detect advanced fibrosis in a population comprised exclusively of individuals with biopsy-proven NAFLD\(^7\). | BMI greater than or equal to 28 earns 1 point, AST/ALT ratio greater than or equal to 0.8 earns 2 points and presence of diabetes earns 1 point. | BARD Score | Risk of advanced fibrosis |
|       | | | 0–1 | Low |
|       | | | 2–4 | High |
| HAIR  | Developed to detect steatohepatitis in bariatric surgery patients\(^8\). | The variables used are the presence of hypertension, ALT greater than 40 and an insulin resistance index greater than 5. Each variable earns 1 point. | A sum of points ≥2 indicates a high probability of NASH. |
| C-NASH| Designed to predict the occurrence of NASH based in clinical characteristics\(^6\). | Clinical aspect | Points |
|       | | BMI (kg/m\(^2\)) | 40–45 | 1 |
|       | | >45 | 2 |
|       | | AST >40 iU/L | 2 |
|       | | Triglycerides >140 mg/dL | 1 |

NAFLD: non-alcoholic fatty liver disease; NASH: non-alcoholic fatty liver steatohepatitis; US: ultrasound scan; HSI: hepatic steatosis index; NFS: non-alcoholic fatty liver disease fibrosis score; APRI: AST-to-platelet ratio index; C-NASH: clinical score for non-alcoholic steatohepatitis; HAIR: hypertension, ALT and insulin resistance index; BARD: BMI, AST/ALT ratio and diabetes score; AST: aspartate aminotransferase; ALT: alanine aminotransferase; BMI: body mass index.
TABLE 2. Non-alcoholic fatty liver disease related features and biochemical examinations observed in the study population.

| Feature                          | N (%)      |
|---------------------------------|------------|
| Steatosis                       | 84 (80.8%) |
| Macrovesicular                  | 85 (79.8%) |
| Mild                            | 58 (55.8%) |
| Moderate                        | 21 (20.2%) |
| Severe                          | 3 (2.9%)   |
| Microvesicular                  | 30 (28.8%) |
| Fibrosis                        | 86 (82.7%) |
| 0                               | 18 (17.3%) |
| 1a                              | 38 (36.5%) |
| 1b                              | 4 (3.8%)   |
| 1c                              | 2 (1.9%)   |
| 2                               | 38 (36.5%) |
| 3                               | 4 (3.9%)   |
| 4                               | 0 (0%)     |
| Portal inflammation             | 84 (80.8%) |
| Mild                            | 58 (55.8%) |
| Moderate                        | 19 (18.3%) |
| Severe                          | 3 (2.9%)   |
| Steatohepatitis                 | 85 (81.7%) |
| Acinar inflammation             | 80 (76.9%) |
| Mild                            | 58 (55.8%) |
| Moderate                        | 19 (18.3%) |
| Severe                          | 3 (2.9%)   |
| Fasting glucose (mg/dL)         | 91.5±27.8  |
| Aspartate aminotransferase (U/L)| 22.9±11.1  |
| Alanine aminotransferase (U/L)  | 28±22.8    |
| Alkaline phosphatase (U/L)      | 65.2±18.2  |
| Gamma-Glutamyl Transferase (U/L)| 23.1±14.7  |
| Platelet count (x10^9/L)        | 271.7±81.6 |
| Albumin (g/dL)                  | 4.3±.3     |
| Insulin (mU/L) (N=87)           | 15.6±10.7  |
| Triglycerides (mg/dL)           | 106.9±55.9 |
| High density lipoprotein (mg/dL)| 58.2±8.3   |
| Total cholesterol (mg/dL)       | 166.8±33.5 |
| Homeostasis model assessment – insulin resistance (HOMA-IR) (N=87) | 3.5±2.6   |

rule out the occurrence of steatosis, which would be important in a population with high prevalence. Hernaez et al., in a meta-analysis that analyzed 49 studies (4,720 individuals), observed that US is an accurate and reliable method for detecting moderate to severe NAFLD in the general population, with sensitivity and specificity greater than 80%[15]. Almeida et al., in a cross-sectional study in which 105 obese individuals were analyzed, demonstrated that US was not an accurate method for the diagnosis of NAFLD in obese individuals, with 65% sensitivity, 91% specificity, 98% positive predictive value and a negative 23%, with a limitation comparable to that observed in the current study[16]. Thus, the main strength of US, which is to detect the presence of NAFLD becomes almost dispensable in a population in which the overall prevalence of NAFLD is extremely high; discarding its occurrence and staging its characteristics would be more important, but the US did not have enough discretionary power for these purposes. Similarly to US, the results of the HSI score for the detection of steatosis demonstrated high sensitivity (99%), but low specificity. In a population with a high prevalence of hepatic steatosis at some level, a negative HSI result has low significance. Therefore, the HSI’s discretionary power to rule out steatosis in this population was not satisfactory. Lind et al., analyzing HSI in different contexts, observed that its accuracy is higher in populations at high risk compared to populations at low risk for steatosis (78% vs 74%)[17].

In regard to methods for the evaluation of steatohepatitis, the HAIR score showed an accuracy of only 40%. In clinical practice, a negative result is of little significance, while a positive result will be correct in approximately 80% of cases. On the other hand, the C-NASH score showed high specificity; despite that, a negative result has little significance, since due to the high prevalence of steatohepatitis in the obese population, there is a high chance that it is a false negative result. Thus, its accuracy is only 23%. Therefore, both tests do not present enough accuracy for widespread use in this population.

APRI demonstrated high specificity, 98%, and a negative predictive value of 96.8%. Therefore, this non-invasive score, created to assess patients with fibrosis in viral hepatitis, proved to be effective in ruling out significant fibrosis, but little accurate for its detection. Its accuracy reached 94.23% and proved effective for advanced fibrosis. De-Cleva et al. similarly observed that the APRI is highly accurate for advanced fibrosis in obese patients undergoing BS[18]. The NFS showed similar results to the APRI, presenting similarly usefulness in clinical practice to rule out advanced fibrosis, but also failing to detect incipient fibrosis, an aspect that would be of greater interest in this population. Singh et al., in a study with 1,319 individuals with biopsy-proven NAFLD, observed that the NFS showed a specificity of 93% and sensitivity of 44%, demonstrating little use in a high-risk population[19]. However, it is unusual for patients with advanced fibrosis, who present the risk of chronic liver failure, to undergo elective BS. The detection of incipient fibrosis, which would be more relevant in the context of the individual undergoing BS, is not possible with both these scores. Similarly, the BARD scoring system showed high sensitivity, but low specificity, which provides an accuracy of only 16%. In this way, it becomes a method that is difficult to interpret when assessing the presence of advanced fibrosis. De Carli et al., in a study that analyzed 323 individuals with morbid obesity, observed that the BARD showed an accuracy of 44%; the population studied in that study had a higher prevalence of advanced fibrosis than in the current study (9%), which may explain its greater accuracy[20].
TABLE 3. Diagnostic accuracy of each non-invasive method for assessment of NAFLD aspects.

| Test/disease          | Present | Absent | Total |
|-----------------------|---------|--------|-------|
| Ultrasound scan       | True positive: 83 | False positive: 20 | 103 |
| Absent                | False negative: 1 | True negative: 0 | 1 |
| Total                 | 84      | 20     | 104   |
| Sensitivity: 98.81%; specificity: 0; positive predictive value: 80.58%; negative predictive value: 0; positive likelihood ratio: 0.99; negative likelihood ratio: 0; overall accuracy: 79.81%. |
| HSI (diagnosis of steatosis) | True positive: 83 | False positive: 20 | 103 |
| Absent                | False negative: 1 | True negative: 0 | 1 |
| Total                 | 84      | 20     | 104   |
| Sensitivity: 98.81%; specificity: 0; positive predictive value: 80.58%; negative predictive value: 0; positive likelihood ratio: 0.99; negative likelihood ratio: 0; overall accuracy: 79.81%. |
| HAIR (diagnosis of steatohepatitis) | True positive: 26 | False positive: 7 | 33 |
| Absent                | False negative: 45 | True negative: 9 | 54 |
| Total                 | 71      | 16     | 87    |
| Sensitivity: 36.62%; specificity: 56.25%; positive predictive value: 78.79%; negative predictive value: 16.67%; positive likelihood ratio: 0.84; negative likelihood ratio: 1.13; overall accuracy: 40.23%. |
| C-NASH (diagnosis of steatohepatitis) | True positive: 4 | False positive: 0 | 4 |
| Absent                | False negative: 67 | True negative: 16 | 83 |
| Total                 | 71      | 16     | 87    |
| Sensitivity: 5.63%; specificity: 100%; positive predictive value: 100%; negative predictive value: 19.28%; positive likelihood ratio: 0; negative likelihood ratio: 0.94; overall accuracy: 22.99%. |
| APRI (diagnosis of advanced fibrosis) | True positive: 0 | False positive: 2 | 2 |
| Absent                | False negative: 4 | True negative: 98 | 102 |
| Total                 | 4       | 100    | 104   |
| Sensitivity: 0; specificity: 98%; positive predictive value: 0; negative predictive value: 96.08%; positive likelihood ratio: 0; negative likelihood ratio: 1.02; overall accuracy: 94.23%. |
| NFS (diagnosis of advanced fibrosis) | True positive: 0 | False positive: 2 | 2 |
| Absent                | False negative: 4 | True negative: 98 | 102 |
| Total                 | 4       | 100    | 104   |
| Sensitivity: 0; specificity: 98%; positive predictive value: 0; negative predictive value: 96.08%; positive likelihood ratio: 0; negative likelihood ratio: 1.02; overall accuracy: 94.23%. |
| BARD (diagnosis of advanced fibrosis) | True positive: 4 | False positive: 87 | 91 |
| Absent                | False negative: 0 | True negative: 13 | 13  |
| Total                 | 4       | 100    | 104   |
| Sensitivity: 100%; specificity: 13%; positive predictive value: 4.40%; negative predictive value: 100%; positive likelihood ratio: 1.15; negative likelihood ratio: 0; overall accuracy: 16.35%. |

NAFLD: non-alcoholic fatty liver disease; HSI: hepatic steatosis index; HAIR: Hypertension, ALT, Insulin Resistance; C-NASH: Clinical Non-alcoholic Steatohepatitis; APRI: AST to platelet ratio index; NFS: NAFLD fibrosis score; BARD: Body mass index, AST/ALT ratio, diabetes.
Therefore, the scores for the detection of advanced fibrosis should be analyzed with caution, being more useful in populations with a higher prevalence of individuals with severe and already manifest forms of NAFLD. A meta-analysis conducted by Xiao et al. demonstrated that the FIB-4 score tends to present an accuracy higher than APRI and comparable to NFS; thus, it might also be considered as an option(23).

Most scores analyzed were not developed specifically to assess obese populations at high risk for NAFLD, especially those whose objective is to analyze liver fibrosis. Their initial scopes were to evaluate patients with advanced chronic liver diseases, mostly viral hepatitis. The natural history of these diseases and the evolution of non-NAFLD fibrosis are different, with a more insidious evolution in the group of patients analyzed in the current study. A recent study conducted by our group also showed that the accuracies of these markers are variable according to BMI status, pointing another difficulty in their interpretation(23).

Other non-invasive methods, such as ultrasound elastography or magnetic resonance imaging, are promising in the attempt to define which NAFLD patient will progress to fibrosis without the need for percutaneous biopsy. However, elastography itself is a high-cost and largely unavailable diagnostic tool in our country. In addition, its accuracy when performed by ultrasound is considerably lower in obese individuals(23).

Considering all these flaws, liver biopsy during BS assumes an important role. During BS, the patient is already undergoing an invasive procedure. The biopsy under direct vision does not add significant operative time and risk. It is not associated with prolonged hospital stay, postoperative pain and the cost is only due to histological analysis. Khorgami et al. observed that intraoperative needle liver biopsy was significantly associated with an increased cost in bariatric procedures, but this increase was an average 17% higher, which our group considers fair in relation to liver biopsy, which is safe and easy to obtain in these patients.

Wedge liver biopsy during bariatric surgery helps to diagnose and stage NAFLD, presents low risks and acceptable costs; given the limitations of non-invasive methods, it is justifiable and should be considered in bariatric routine.

CONCLUSION

Wedge liver biopsy during bariatric surgery helps to diagnose and stage NAFLD, presents low risks and acceptable costs; given the limitations of non-invasive methods, it is justifiable and should be considered in bariatric routine.

Authors’ contribution

Concon MM collected the data and wrote the first draft. Gestic MA, Utrini MP and Chaim FDM collected the data and performed the biopsies and operations. Chaim EA provided critical intellectual inserts and reviewed the final draft. Cazzo E conceived and designed the analysis, performed the analysis and wrote the final draft.

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RESUMO – Contexto – Marcadores não-invasivos são ferramentas úteis e práticas para avaliar a doença hepática gordurosa não alcoólica (DHGNA), porém, a biópsia hepática continua sendo o método padrão-ouro. A biópsia pode ser facilmente obtida em indivíduos submetidos à cirurgia bariátrica, mas não há evidências definitivas acerca da relação entre custos, riscos e benefícios de sua realização sistemática. Objetivo – Comparar a acurácia diagnóstica de métodos não-invasivos com a biópsia hepática para detecção e estadiamento da DHGNA em obesos submetidos a cirurgia bariátrica. Métodos – Tratase de um estudo transversal,observacional e descritivo que envolveu indivíduos que se submeteram à cirurgia bariátrica de 2018 a 2019 em um hospital universitário público terciário. Ultrasonografia (US), índice de esteatose hepática (HSI), Escor Clinico de Eatoe-hepate Náo-Alcoólica (C-NASH), Índice de Hipertensão, alana aminotransferase (ALT) e resistência à insulina (HAIR), Ração entre apatamento eplasetas (AST) e plaquetas (APRl), Eocre de Fibrose da DHGNA (NFS) e índice de massa corporal (ICM), relação AST/ALT e diabete (BARD) foram os métodos comparados com o exame histopatológico de biópsias hepáticas em cunha coletadas durante a cirurgia. Resultados – De 104 indivíduos analisados, 91 (87,5%) eram do sexo feminino. A média de idade foi de 34,9±9,7 anos. Não houve morbidade relacionada à biópsia. As respectivas acurácias globais de cada marcador analisado foram: US (79,8% para esteatose), HSI (79,8% para esteatose), HAIR (40,2% para esteato-hepate), C-NASH (22,9% para esteato-hepate), APRl (94,2% para fibrose avançada), NFS (94,2% para fibrose avançada) e BARD (16,3% para fibrose avançada). Discussão – Considerando a alta prevalência de doença hepática nesta população, mesmo os mais acurados destes marcadores não apresentaram poder discrimionário suficiente para detectar e/ou descartar os aspectos da DHGNA que foram desenvolvidos para avaliar em comparação com a biópsia hepática, que é segura e de fácil obtenção nestes pacientes. Conclusão – A biópsia hepática em cunha durante a cirurgia bariátrica auxilia no diagnóstico e estadiamento da DHGNA, apresenta baixo risco e custos aceitáveis, e dadas as limitações dos métodos não-invasivos, é justificável e deve ser considerada na rotina bariátrica.

Palavras-chave – Hepatopatia gordurosa não alcoólica; fígado gorduroso; obesidade; cirurgia bariátrica; testes de função hepática.

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