BRIEF COMMUNICATION

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Bioimpedance: new approach to non-invasive detection of liver fibrosis – a pilot study

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ABSTRACT – Background – Fibrosis are common structural hepatic change in patients with chronic hepatitis. Liver biopsy is the gold standard for determining the extent of liver fibrosis. Considering the technical difficulties and cost, improvements in non-invasive screening tools are greatly needed. Bioimpedance have been shown to be safe to evaluate tissue fibrosis. **Objective** – To assess the utility of using monofrequential bipolar bioimpedance for the detection of severity of liver fibrosis consistent with chronic viral hepatitis C infections. **Methods** – One hundred and ten patients were studied prospectively and formed two groups according to the lab tests results for the detection of HCV, ALT and AST: Group 1 Control (n=50 healthy patients with HCV negative and with ALT and AST values within the normal clinical range) and Group 2 Positive (n=60 patients positive for anti-HCV positive) which were biopsied. All patients underwent an examination with an Electro Sensor Complex, bioimpedance technology. To compare the groups 1 and 2, the ROC curves was used to determine the specificity and sensitivity of the bioimpedance to detect liver fibrosis. To identify liver fibrosis severity the Group 2 Positive was subdivided according to the liver biopsy results (Metavir fibrosis score) into: Sub Group 2A (F0-F1 n=25) - patients without or with minimal portal fibrosis and Sub Group 2B (F3-F4 n=20) patients with numerous septa/cirrhosis. A statistical analysis was conducted to analyze the bioimpedance data differences in delta of the conductance. **Results** – From the comparison between Groups 1 and 2: 1) The delta value for conductance in the pathway representing the right foot-left hand minus left hand-right foot demonstrated a sensitivity of 85% and a specificity of 78% with a cutoff value ≤5 and *P*=0.0001. 2) For the comparison between Sub Group 2A (Metavir F0+F1) and Sub Group 2B (Metavir F3+F4), the neural network for the Electro Sensor Complex data demonstrated a sensitivity of 85% and a specificity of 72% with a cutoff probability >50% and *P*=0.001. AUCROC=0.81. **Conclusion** – Bioimpedance technology had good level sensitivity and acceptable specificity for detecting liver fibrosis using delta of the conductance. There is a potential for the use of bioimpedance technology as non-invasive approaches for screening of liver fibrosis.

HEADINGS – Liver cirrhosis, diagnosis. Biopsy. Fibrosis. Liver, pathology. Chronic hepatitis C, complications.

INTRODUCTION

The risk factors for hepatic carcinogenesis in patients with chronic hepatitis have been extensively studied[1] and liver fibrosis is known to be the most significant factor involved. Treatment decisions are based, in part, on the stage of liver fibrosis, which marks the progression to cirrhosis. The Metavir score is very useful scale to gauge the fibrosis severity.

The gold standard for determining the extent of fibrosis is liver biopsy[2]. However, this procedure carries a moderate risk for complications such as bleeding and a small risk of death[2,3]. Then there is interest in the development of non-invasive testing to determine the degree of hepatic fibrosis.

Bioimpedance is an electrical property of living tissues that has been applied in many biomedical settings, such as the quantification of brain edema in neurosurgery[4] differentiating between cancerous pulmonary masses and pulmonary masses due to pneumonia[5], in in prostate cancer[6]. However, bipolar bioimpedance has, so far as we are aware, not previously been applied in assessing liver fibrosis.

Bioimpedance uses a weak direct current (DC), voltage 1.28 V applied for two minutes and in bipolar mode. In living tissues, electrical current is normally limited in living tissue by highly insulating cell membranes, although measurements of this current can reflect acid levels in tissues and can therefore be used to detect hepatic alterations provoked by viral infection or abnormal architecture resulting from tissue fibrosis. Moreover, electrical current can be impeded differently, which enables the detection of differences between normal and fibrotic tissue.

The aim of this research was to assess the accuracy of non-invasive bipolar bioimpedance technology for the detection of liver fibrosis using physiological data intra and inter groups of the healthy and chronic viral hepatitis C patients

METHODS

This is a prospective study between January and December 2014. To assess the level of liver fibrosis comparing bipolar bioimpedance data from a Group 1 Control of healthy individuals, not biopsied, with the data and histopathology results of the Group 2 Positive patients with chronic hepatitis (virus C positive), biop-

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sied and subdivided according to Metavir score into Sub Group 2A (F0-F1) patients without or with minimal portal fibrosis and Sub Group 2B (F3-F4),patients with numerous septa/cirrhosis. Approval number of the Ethical Committee – Faculty of Medical Science of Campinas (Unicamp) is CEP 542/2010.

As estimated by the sample calculation with significance level of 5% with 80% power, this study included 60 patients with chronic hepatitis, of both sexes that had all previously signed consent form and had positive serology for hepatitis C virus (anti-HCV positive). These exams were performed by health centers from cities within the greater metropolitan area of Campinas, São Paulo state, southeast of Brazil, whose patients were referred to Gastrocentro, State University of Campinas – Unicamp, for biopsies to be carried out in order to determine the stage of hepatitis virus C.

A Group 1 Control composed of 50 healthy patients, aged 18 to 57years, who were without symptoms, and who were not undergoing any treatments or liver biopsies and had negative lab tests for chronic hepatitis (virus C) were included only for the purpose of comparing bioelectric data.

Patients were excluded for the following reasons: 1) if they had a neurological disorder precluding the ability to sign a consent form; 2) if they had any constraints to use the bipolar bioimpedance, that such as presence of an external defibrillator, skin lesions likely to come into contact with the electrodes, excessive perspiration, cardiac pacemaker, electronic life support, any implanted electronic device, metallic pins or prostheses in digits or joints, pregnancy from the third trimester onwards, and absence of a limb; 3) if the Metavir fibrosis score is F2 only in specific analysis of liver fibrosis in the Group 2 Positive (chronic hepatitis group).

Bipolar bioimpedance measurements, were performed in all one hundred and ten patients of an average age of 39 (20–64), 57 men and 53 women, using the Electro Interstitial Scan-Galvanic Skin (EIS-GS) bioimpedance module with DC current, which uses the ES Complex (Electro Sensor Complex) system medical device (LD Technology Ltd, USA).[7]

The chronic hepatitis group immediately prior to the liver biopsy underwent a "blind" examination with electrical bioimpedance using a weak direct current (DC current), voltage 128 V which was applied for two minutes in bipolar mode performing measures of the electrical conductivity of different pathways in the body.

The parameter analyzed in the EIS-GS module was the delta of the electrical resistance values for the pathway value for the left foot to right hand (anode to cathode) minus the pathway value for the right hand to left foot (cathode to anode). The conductance measurement values are displayed in a scale from 0 to 100 for each pathway.

EIS and electrical conductance/chronoamperometry

With direct current, the plasma membrane acts as an insulator, and the current is therefore not able to penetrate the cell. Thus, most of the current flows around the cell in the interstitial fluid[8] The analysis of the direct current at the cathode and anode in an electrolytic solution is performed at both the anode and cathode. For the analysis at the cathode, the electrochemical reaction is represented by the following:

$$2H_2O + 2^e = H_2\,(gas) + 2\,OH^-(base)$$

For the analysis at the anode, the electrochemical reaction for water is represented by the following:

$$2H_2O = O_2\,(gas) + 4H+ \; + 4^{e-}\,(acid)$$

Using as normal references the conductance values 9.2 µS (-4/19) of the Group 1 Control, was compared the groups 1 and 2 to determine the specificity and sensitivity of the bipolar bioimpedance data (delta of conductance) to detect liver fibrosis. The fibrosis severity was analyzed comparing the Sub Groups 2A and 2B. ES Complex Algorithm was made by Statistical Neural Network version 10. A part of developed algorithm was made in «C» language.

This was a triple blind study since the bioimpedance examination was done immediately before the liver biopsy without knowing the results. The liver biopsy was also performed without knowing the result of bioimpedance and liver fibrosis was graded by an independent pathologist using the Metavir classification did not know the result of bioimpedance.

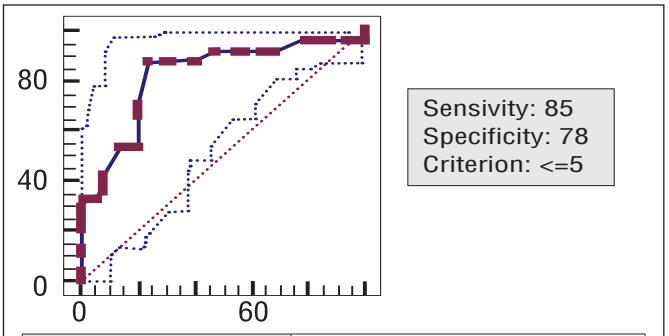
RESULTS

Demographic data can be seen in TABLE 1. From the comparison between Groups 1 and 2:

TABLE 1. Demographic data for the 4 study groups: Group 1 Control, Group 2 Positive for hepatitis C virus-infected patients, Sub Group 2A-Metavir F0+F1, and Sub Group 2B-Metavir F3+F4

	Group 1 Control	Group 2 Positive for HCV infection	Sub Group 2A Metavir F0+F1	Sub Group 2B Metavir F3+F4	ANOVA *P*-value
N	50	60	25	20	
Age	32 (18-57)	46 (28-64)	44 (28-63)	49 (29-64)	0.05
Male/female ratio	0.56	0.66	0.68	0.75	0.05
ALT	15 (12-20) U/L	77 (15-260) U/L	70 (19-260) U/L	83 (15-185) U/L	0.001
AST	26 (15-38)	72 (16-271)	44 (27-78)	75.6 (16-271)	0.001
HCV	No	60	25	20	Ns
Delta conductivity	9.2 (-4/19)	1.31 (-6/23)	2.75 (-6/12)	0.62 (-10/23)	0.001

The delta value for conductance in the pathway representing the right foot-left hand minus left hand-right foot demonstrated a sensitivity of 85% and a specificity of 78% with a cutoff value ≤5 and *P*=0.0001 (FIGURE 1).



Variable	Delta 21-22
	Delta 21-22
Classification variable	Diagnostic

Sample size	110
Positive group: Diagnostic = 1	60
Negative group: Diagnostic = 0	50

Disease prevalente (%)	32.4

Area under the ROC curve (AUC)	0.819
Standard Error	0.0570
95% Confidence Interval [a]	0.712 to 0.899
z statistic	5.594
Sighificance level P (Area=0.5)	<0.0001

[a] Binomial exact

FIGURE 1. Delta value of the conductance. The ROC curve for the Delta value of the conductance in the pathway of the right foot-left hand minus left hand-right foot for the comparison between Group 1 Control (virus-negative and within the normal range of ALT/AST levels) and Group 2 Positive (virus-positive and/or high levels ALT/AST).

From the comparison between Sub Group 2A (Metavir F0+F1) and Sub Group 2B (Metavir F3+F4) the Receiver Operating Characteristic curve (ROC curve) neural network for the ES Complex data demonstrated a sensitivity of 85% and a specificity of 72% with a cutoff probability >50% and *P*=0.001 (FIGURE 2). The area under the ROC curve (AUROC) is 0.81.

DISCUSSION

Chronic hepatitis is often closely associated with hepatic fibrosis. The response to injury consists of local inflammation followed by the recruitment and local proliferation of myofibroblast-like cells and the excessive deposition of the extracellular matrix. Therefore, within the past 20 years, hepatic fibrosis has become a common and difficult clinical challenge for gastroenterologists worldwide[9].

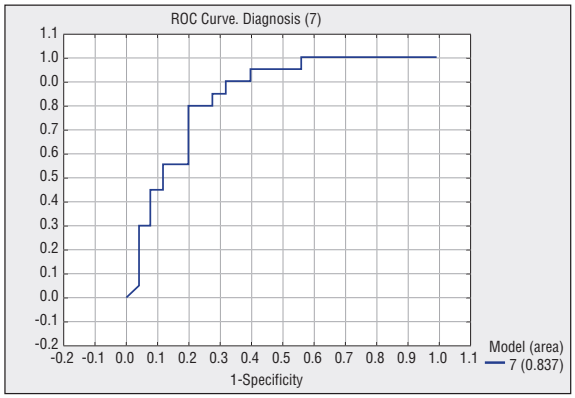
Progressive degrees of fibrosis, and ultimately cirrhosis, are reflected in alterations in blood levels of various biomarkers, and the knowledge of such alterations has led to the development of predictive models based on clinically determined algorithms that utilize the levels of selected markers[9,10].

One such model, the Hepascore, is based on the serum levels of α2-macroglobulin, hyaluronic acid, gammaglutamyltransferase (GGT), and total bilirubin in addition to age and sex. In one study, a Hepascore ≥0.5 (possible range, 0-1.0) demonstrated a sensitivity of 63% and a specificity of 89% for the presence of significant fibrosis (Metavir score ≥F2), whereas a Hepascore <0.5 demonstrated a sensitivity of 88% and a specificity of 74% for excluding a diagnosis of advanced fibrosis[9].

Another useful model is the non-alcoholic fatty liver disease (NAFLD) fibrosis score that based on age, hyperglycemia, body mass index, platelet count, albumin, and AST/ALT ratio. In the validation study demonstrates good accuracy in to predict presence or absence of advanced fibrosis with an area under the ROC curve of 0.82, sensitivity of 82% and specificity of 88%[11].

Ultrasonic transient elastography or FibroScan (Echosens, Paris, France), is a current approach for the non-invasive evaluation of liver fibrosis and has been shown to be an accurate predictor of histological fibrosis in patients with chronic hepatitis C. The best results with FibroScan is when the patient has higher level of fibrosis with AUROC ranging from 0.79 to 0.88 for F > or = 2 and 0.95 to 0.99 for F=4), and these new trends[12,13].

In the present study, using delta of the electrical resistance values the comparison between patient from the Sub Group 2A (Metavir F0+F1) and Sub Group 2B (Metavir F3+F4) demonstrated good sensitivity (85%) and specificity (72%) to differentiation in liver fibrosis severity. The theoretical explanation is that the electrical weak DC current used in bipolar bioimpedance can be



	Diagnosis.0	Diagnosis.1
Total	25.00000	20.00000
Correct	18.00000	17.00000
Wrong	7.00000	3.00000
Unknown	0.00000	0.00000
Correct (%)	0.00000	85.00000
Wrong (%)	72.00000	15.00000
Unknown (%)	0.00000	0.00000

FIGURE 2. The neural network. The ROC curve of the neural network for the ES Complex data comparing the Sub Group 2A (Metavir score F0+F1) and Sub Group 2B (Metavir score F3+F4).

impeded differently, which enables the detection of differences between normal and fibrotic tissue.

It is not possible to discuss this result with other researchers because this is a pioneer study in liver. Prostatic research using bioimpedance offers the same explanation to understand how it could identify the variation in tissue architecture between different prostatic tissue types. This prompted, Halter RJ[6] and de Abreu DS[14,15] to suggest using the electrical properties of the prostate as a means to distinguish cancerous from non-cancerous tissue.

Bioimpedance is largely a function of a tissue's cellular morphology and the ionic concentrations of the tissue's intra- and extra-cellular fluids. Electrical current is normally limited in living tissue by highly insulating cell membranes, although measurements of this current can reflect acid levels in tissues. The second explanation for why bioimpedance is increased in hepatic fibrosis may be related to the fact that fibrotic tissue prevents the flow of the current because current flow decreases as resistance increases. A decrease in bioimpedance conductivity value (cutoff <5) is inversely proportional to the electrochemical reaction at the anode and can therefore be used to indicate an acidic tissue environment. In addition, this acidic tissue environment is likely related to tissue damage provoked by HCV infection[7].

Hence, the bipolar bioimpedance used in ES Complex equipment is a tool easy to administer, non-invasive, and has a high sensitivity and specificity would be advantageous and a great improvement for the screenings to detect liver fibrosis in asymptomatic chronic hepatitis C virus infection but not for primary diagnostic use. The value of such a tool would increase if non-clinical personnel, who assist the doctors, could also use it.

This study has certain limitations, such as the sample size and the exclusion of 19 patients with positive Metavir score F2 (portal fibrosis with few septa) although one of the challenges of the liver fibrosis evaluation method is to identify the Metavir F2.

But the purpose of this pilot study that could not draw conclusive results since it is not a validation study. The intention is to present preliminary findings and to evaluate the potential of the use of bioimpedance as a supporting parameter that in the future can be included to the currently methods of the liver fibrosis identification may be to increase yours theirs sensitivity and specificity. Thus, bioimpedance further studies with a larger patient population and the use of multifrequential tetrapolar bioimpedance may provide more information not only on the interstitium but also on the intracellular environment thus contributed to the increased accuracy of the clinicians detect chronic liver fibrosis during screening procedures.

CONCLUSION

The bioimpedance demonstrate good to high levels of sensitivity and specificity to identify structural liver alterations like liver fibrosis severity consistent with chronic viral hepatitis C infections showing that there is a potential for the use of bioimpedance lie a non-invasive technology in the approaches for low-cost and rapid screening of liver fibrosis.

Authors' contributions

Ianni Filho D: wrote the manuscript. Boin IFSF: reviewed the manuscript. Yamanaka A: participated in the sequence alignment.

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RESUMO – **Contexto** – A fibrose é uma alteração hepática estrutural comum em pacientes com hepatite crônica. A biópsia hepática é o padrão ouro para determinar a extensão da fibrose hepática. Considerando as dificuldades técnicas e os custos, melhorias em ferramentas de rastreio não-invasivas são bastante necessárias. A tecnologia bioimpedância tem se mostrado ser segura para avaliar fibrose tecidual. **Objetivo** – Avaliar a utilidade do uso da bioimpedância bipolar para detectar a severidade da fibrose hepática compatível com a hepatite viral B e C. **Métodos** – Cento e dez pacientes foram estudados, prospectivamente e dois grupos foram formados de acordo com os resultados dos testes laboratoriais para a detecção de HCV, ALT e AST: Grupo 1 Controle (n=50 pacientes saudáveis com HCV negativos e com valores de ALT e AST dentro do padrão de normalidade) e Grupo 2 Positivo (n=60 pacientes positivos para a infecção viral anti-VHC ou HBsAg positiva) que foram biopsiados. Todos os pacientes foram submetidos a um exame com o Electro Sensor Complex, que utiliza a bioimpedância bipolar. Para comparar os Grupos 1 e 2, a curva ROC foi utilizada para determinar a especificidade e sensibilidade da bioimpedância em detectar a fibrose hepática. Para identificar a severidade da fibrose hepática, o Grupo 2 Positivo foi subdividido de acordo com os resultados da biópsia (escore Metavir) em: Sub Grupo 2A (F0-F1 n=25) – pacientes sem ou com fibrose portal mínima e Sub Grupo 2B (F3-F4 n=20) pacientes com numerosos septos/cirrose. A análise estatística foi realizada para analisar as diferenças dos valores delta de condutância da bioimpedância. **Resultados** – A comparação entre os Grupos 1 e 2 mostrou: 1) O valor delta de condutância na via do pé direito à mão esquerda menos o valor do delta da mão esquerda ao pé direito demonstrou uma sensibilidade de 85% e uma especificidade de 78%, com um valor de corte ≤5 e *P*=0,0001. 2). Na comparação entre o Sub Grupo 2A (Metavir F0+F1) e o Sub Grupo 2B (Metavir F3 + F4), a rede neural para os dados aferidos pelo Electro Sensor Complex demonstrou uma sensibilidade de 85% e uma especificidade de 72%, com um corte de probabilidade >50% *P*=0,001 e AUCROC=0,81. **Conclusão** – Bioimpedância apresentou boa sensibilidade e aceitável especificidade para a detecção da fibrose hepática utilizando o delta da condutância da bioimpedância. Existe um potencial para o uso da bioimpedância como abordagens não-invasivas para o rastreamento da fibrose hepática.

DESCRITORES – Cirrose hepática, diagnóstico. Biópsia. Fibrose. Fígado, patologia. Hepatite C crônica, complicações.

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