UNIVERSIDADE ESTADUAL DE CAMPINAS

FACULDADE DE CIÊNCIAS MÉDICAS

DANIEL IANNI FILHO

BIOIMPEDÂNCIA: NOVA ABORDAGEM PARA A DETECÇÃO NÃO INVASIVA DA FIBROSE HEPÁTICA – ESTUDO PILOTO

BIOIMPEDANCE: NEW APPROACH TO NON-INVASIVE DETECTION OF LIVER FIBROSIS – A PILOT STUDY

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Tese apresentada à Faculdade de Ciências Médicas da Universidade Estadual de Campinas como parte dos requisitos exigidos para a obtenção do título de Doutor em Ciências.

Thesis presented to the Faculty of Medical Sciences of the Universidade Estadual de Campinas in partial fulfillment of the requirements for the degree of Doctor in Sciences.

ORIENTADOR: Prof. Dr. Luiz Roberto Lopes

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Dedico também a todos os pesquisadores que me apoiaram no grande desafio de quebrar alguns paradigmas permitindo que a pesquisa e a evidência científica se encarregassem de comprovar ou não várias premissas e hipóteses levantadas e, deste modo, apontar novas aplicações e conceitos na prática médica cotidiana. Obrigado pelos que inicialmente desacreditaram mas, tiveram a grandeza da alma de pesquisador que não utiliza a crença, mas a evidência científica como base do conhecimento e da aceitação do desconhecido.

A grandeza de um homem se mostra pelo modo como ele trata os pequenos

Desconhecido

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Nas mãos que oferecem rosas

Nas mãos que sabem ser generosas.”

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RESUMO

Contexto: 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**: O objetivo deste estudo piloto foi o de avaliar o potencial e a acurácia da bioimpedância bipolar em identificar a fibrose hepática e alterações elétricas e fisiológicas compatíveis com a hepatite viral C utilizando a biópsia hepática como parâmetro de comparação. **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 E.S (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, sendo excluidos nesta análise específica os pacientes (F2 n=15). 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 condutancia 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). O algoritmo SI *(30-DE) que utiliza os parâmetro Delta de condutividade no caminho da corrente elétrica entre o pé direito-mão esquerda menos mão esquerda–pé direito e a fórmula SI=Stiffness Index foi possível identificar alterações hepáticas compatíveis com a Hepatite Viral C com sensibilidade de 82.9% e especificidade de 84.8% com cutoff> 201 and *P*=0.0001. 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 ES 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 parâmetro delta da condutancia advindo da bioimpedância. Foi possível identificar alterações hepáticas compatíveis com Hepatite Viral C de modo não invasivo, rápido e indolor. Existe um potencial para o uso da bioimpedância como abordagens não invasivas para o rastreamento da fibrose hepática. Este trabalho foi um estudo piloto cujos resultados devem ser confirmados em futuros estudos com maiores amostras.

Palavras-chave: Fibrose hepática; Cirrose hepática; Diagnóstico; Biomarcador; Bioimpedância Bipolar; Delta de Condutividade; Espectrofotometria.

ABSTRACT

Background –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** –The objective of this pilot study was to evaluate the potential and accuracy of bipolar bioimpedance in identifying hepatic fibrosis and electrical and physiological changes compatible with viral hepatitis C using liver biopsy as a parameter of comparison. **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 E.S (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.The SI * (30-DE) algorithm that uses Delta value for conductivity parameter in the electric current path between the left-right and left-right foot and SI = Stiffness Index was able to identify hepatic changes compatible with Hepatitis Viral C with sensitivity of 82.9% and specificity of 84.8% with cutoff> 201 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 ES 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. Bioimpedance presented good sensitivity and acceptable specificity for the detection of liver fibrosis using the delta of conductance parameter from bioimpedance.It was possible to identify hepatic alterations compatible with Viral Hepatitis C in a non-invasive, fast and painless way. There is potential for the use of bioimpedance as noninvasive approaches for the screening of liver fibrosis. This work was a pilot study whose results should be confirmed in future studies with larger samples.

Headings: Liver fibrosis; Liver cirrhosis; Diagnosis; Biomarkers; Bipolar bioimpedance; Delta of conductance; Spectrophotometry.

LISTA DE ABREVIATURAS, SIGLAS E SÍMBOLOS

µS	- Micro Siemens
ALT	-Alanina transaminase
AST	- Aspartato transaminase
AUCROC	- Area Under Receiver Operating Characteristic Curve
DC current	- Direct Current
EIS-GS	- Eletro Intersticial Scan – Resposta Galvânica da Pele
ES Complex	- Eletro Sensor Complex
HCV	- Hepatite C Vírus
NAFLD	- Non-alcoholic fatty liver disease
ROC Curve	- Receiver Operating Characteristic Curve

SUMÁRIO

1- INTRODUÇÃO

Hepatitis C virus (HCV) infection is the most common chronic blood borne infection in the United States[1]. Data from the Third National Health and Nutrition Examination Survey (NHANES III), conducted between 1988 and 1994, found that an estimated 3.9 million (1.8%) Americans are infected with HCV[2.] The majority of these individuals are chronically infected and might not be aware of their infection status because they are not clinically ill[3].

Viral hepatitis has affected around 424 million people worldwide by 2013[2,4]. According to Brazilian News Agency[5], in July 2016, the World Health Organization (WHO) estimates that - worldwide - 400 million people are infected with hepatitis B and C viruses. The estimate of the Brazilian Society of Infectious Diseases (SBI) is that in the country there are between 1.5 million and 2 million people with hepatitis, but only about 300 thousand know they have the disease.

Chronic hepatitis is a silent, asymptomatic disease with a slow and progressive evolution and therefore, many people are unaware that they are carriers of the disease, which makes early diagnosis difficult, making late diagnosis more frequent[6] with less chance of cure, greater expenses and suffering.

Infected persons serve as a source for transmission to others and are at risk for chronic liver disease or other HCV-related chronic diseases during the first two or more decades following initial infection[3].

Chronic liver disease is the tenth leading cause of death among adults in the United States; it accounts for approximately 25,000 deaths annually, or roughly 1% of all deaths [4].

HCV-associated end-stage liver disease is the most frequent indication for liver transplantation in adults[4]. Because most HCV-infected individuals are aged between 30 and 49 years the number of deaths attributable to HCV-related chronic liver disease could increase substantially during the next 10–20 years as this group reaches ages at which complications from chronic liver disease typically occur[2].

Hepatitis C virus (HCV)-related cirrhosis is associated with an extremely high risk of hepatocellular carcinoma (HCC) development, with a reported annual

incidence ranging between 3% and 8%[7,8]. The prognosis of HCC is deemed poor unless the cancer is detected and treated at an early stage[9].

The risk factors for hepatic carcinogenesis in patients with chronic hepatitis have been extensively studied[9] 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.

Not all patients with viral infections are treated with antiviral therapy after diagnosis because only 20 to 30% of untreated individuals will subsequently develop cirrhosis[6]. Treatment decisions are based, in part, on the stage of liver fibrosis, which marks the progression to cirrhosis. Individuals with minimal fibrosis (i.e., a METAVIR score of F0 or F1), even those with long-standing disease, are not likely to develop advanced fibrosis in the short-term, and these patients are typically monitored every 3 to 5 years[6]. However, individuals with significant fibrosis (i.e., METAVIR scores > F2) are at an increased risk of developing cirrhosis and are generally treated[6].

The gold standard for determining the extent of fibrosis is liver biopsy[6]. However, this procedure carries a moderate risk for complications such as bleeding and a small risk of death[2,6]. For this reason, liver biopsy is not the best technique for screening purposes. Moreover, because fibrosis is not uniformly distributed in the liver and a biopsy can only sample 1/50,000th to 1/30,000th of the liver mass[10,11], cirrhosis is overlooked in an estimated 15 to 30% of liver biopsies[11.12].

Whereas in the clinical context the biochemical tests ALT-Alanine transaminase[13] and AST-Aspartate transaminase are very important but they are not highly specific, being able to vary of concentration in the different phases of disease´s evolution and also, that the liver biopsy is not easily accessible to population due to technical costs and difficulties, the development and improvement of non-invasive and viable tests in the use of large-scale screening in order to determine the degree of liver fibrosis is of great importance.

Several methods of assessing liver fibrosis are currently available, among them Hepascore[14], Fibrotest[15], APRI[16,17], Elastography[18,19] etc., all of than with advantages and disadvantages, reaches and limitations. In this context, a new method of investigating the presence and severity of hepatic fibrosis and hepatic

alterations compatible with viral C hepatitis is proposed and analyzed in this pilot study. These are Bipolar Bioimpedance and Photoelectric Plethysmography, components of the Electro Sensor (ES) Complex device[20].

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[21] and differentiating between cancerous pulmonary masses and pulmonary masses due to pneumonia[22]. Bipolar Bioimpedance (EIS-GS Galvanic Skin response module[20] of the Electro Sensor (E.S) Complex) medical device (manufacturer LD Technology Ltd, USA), uses a weak DC current, voltage 1.28V applied during 2 minutes and in bipolar mode. The EIS-GS has been investigated for improving total PSA measurements in prostate cancer screening[23], SSRI treatment responses[24] Attention deficit hyperactivity disorder (ADHD) screening in children[25]and assessing the activity of the sympathetic nervous system[26]. However, bioimpedance has not previously been applied for use in assessing liver fibrosis.

The ESO spectrophotometry module of the E.S Complex measures arterial stiffness using photoelectrical plethysmography[26] and heart rate variability[27,28,29]. In addition, hepatitis has been shown to provoke hemodynamic disorders[30].

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[22].

Although there is no other research using Bioimpedance as a tool to identify liver fibrosis, studies on prostatic tissue coming from radical prostatectomy provide subsidies to assert that the behavior of the electric current applied in the Bioimpedance is different when comparing healthy, hyperplastic and tumor tissue[23,31]. This information and the possibility of bioimpedance identify the living tissue architecture (acute, chronic inflammation, fibrosis, tumor) are promising for the development and improvement of noninvasive tests based on physiological parameters as a basis for the construction of algorithms capable of identifying innumerable pathologies including liver fibrosis.

2 - OBJETIVO

The aim of this research was to assess the accuracy of non-invasive Bipolar Bioimpedance technology associated or not to other physiological parameters obtained by Photoelectric Plethysmography for the detection of physiological hepatic changes and liver fibrosis using physiological data intra and inter groups of the healthy and chronic viral hepatitis C patients

3 - METODOLOGIA

This study was approved by the regional ethics committee (Ethics committee Unicamp–Approval number 541/2010) and adhered to the ethical principles of the Declaration of Helsinki. Each patient provided informed consent via signature, and confidentiality was maintained for all participants.

This was 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), who underwent ultrasound guided biopsy without any record of complications 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.

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 57 years, 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); 4) patients with ascites; 5) performance of intense physical exercises or sauna which could compromise the accuracy of bioimpedance examination; 6) resting blood pressure greater than 180 mm Hg systolic and/or 100 mm Hg diastolic; 7) uncontrolled cardiac arrhythmias[32].

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 EIS-GS bioimpedance module with DC current, which uses the ES Complex (Electro Sensor Complex) system medical device (LD Technology Ltd, USA)[20].

The EIS-GS module is a programmable electromedical system (Figure 1A,B) that consists of a USB plug and hardware within an interface box, disposable electrodes, reusable plates, reusable cables and software installed on a computer.

Figure 1A- Hardware within an interface box, disposable electrodes, reusable plates, reusable cables and software installed on a computer; **Figure 1B** - Reusable cables.

The EIS-GS Bioimpedance module that integrate the ES Complex medical device apply electrical Bioimpedance using a weak direct current (DC current), voltage 1,28 V which was applied for two minutes performing measures of the electrical conductivity of different pathways in the body (Figure 2).

Figure 2 – Stainless steel plates on the feet and hands (right and left) and two disposable electrodes on the forehead (right and left sides) function as cathode and anode and allow electrical conductivity of 22 pathways in the body

The system medical device uses bioimpedance in the bipolar mode with direct current and measures the electrical conductivity of 22 pathways in the body (Figure 3) which are each recorded twice from anode to cathode and then from cathode to anode.

	Anode		Cathode
2.	L hand	→	L forehead
10.	R forehead	→	L forehead
4.	R hand	→	R forehead
11.	L hand	→	R hand
6.	L foot	→	L hand
14.	R foot	→	L foot
7.	R hand	→	R foot
16.	L forehead	→	R hand
17.	L hand	→	R forehead
19.	R foot	→	L hand
22.	R hand	→	L foot
1.	L forehead	→	L hand
9.	L forehead	→	R forehead
3.	R forehead	→	R hand
12.	R hand	→	L hand
5.	L hand	→	L foot
13.	L foot	→	R foot
8.	R foot	→	R hand
15.	R hand	→	L forehead
18.	R forehead	→	L hand
20.	L hand	→	R foot
21.	L foot	→	R hand

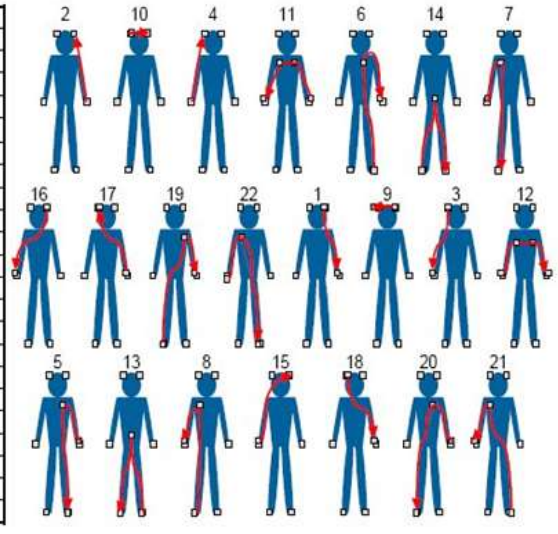


Figure 3 - Pathway for the left foot-right hand minus the right hand-left foot

The pathways are measured between four large tactile reusable electrodes (.270 cm2) placed on the palms of the hands and soles of the feet, and smaller disposable electrodes (15 cm2) placed on the left and right forehead (Figure 4A,B).

Figure 4A – Close of the stainless steel reusable plates and **Figure 4B** patient doing an examination with head electrode.

Electrode polarization does not affect the bioimpedance measurements and the transmission of the current from the electrode to the hardware is performed by chronoamperometry[33]. 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.

The chronic hepatitis group immediately prior to the ultrasound guided liver biopsy underwent a “blind” examination with electrical Bioimpedance (Figure 5 A, B) using a weak direct current (DC current), voltage 1,28 V which was applied for two minutes in bipolar mode performing measures of the electrical conductivity of different pathways in the body.

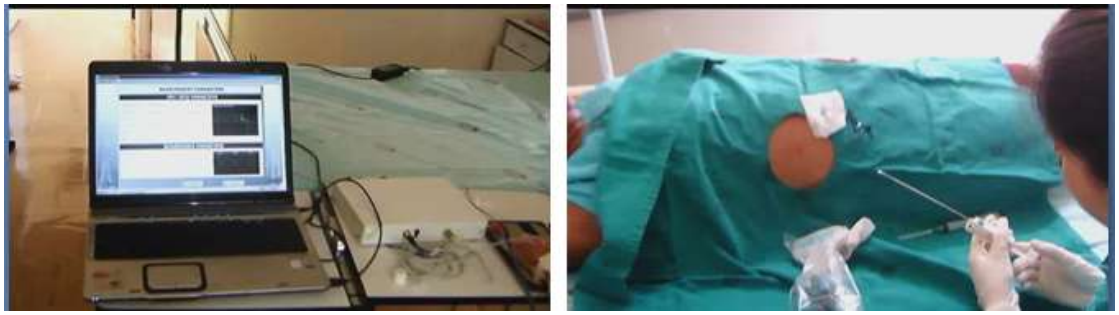


Figure 5A - ES Complex at the bedside where the bioimpedance examination was performed immediately before the liver biopsy; Figure 5B - Biopsy being guided by ultrasound

ES Complex device The chronic hepatitis group immediately prior to the ultrasound guided liver biopsy underwent a “blind” examination with electrical Bioimpedance using a weak direct current (DC current), voltage 1,28 V which was applied for two minutes in bipolar mode performing measures of the electrical conductivity of different pathways in the body.

EIS and electrical conductance/chronoamperometry

With direct current, in low frequency (<1 Khz) 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. Using high frequency (>100 Khz) the the electric current is able to invade the cell not being blocked by the bilipid layer of the cytoplasmic membrane[34,35] (Figure 6).

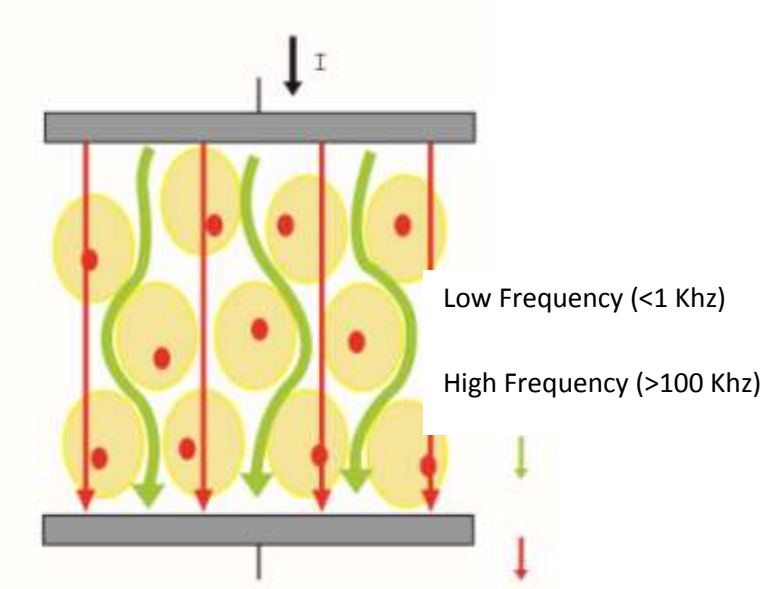


Figure 6 - Schematic representation of the action of the electric current in high and low frequency.

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)

Measurement of spectrophotometry

Spectrophotometry uses an oximeter to detect heart rate and waveform (PTG) influenced by characteristics of systemic circulation[36] (Figure 7A,B). The heart rate variability analysis provides autonomic nervous system indicators in the time (HR, SDNN, and RMSSD) and frequency domains (HF, LF and ratio LF/HF).

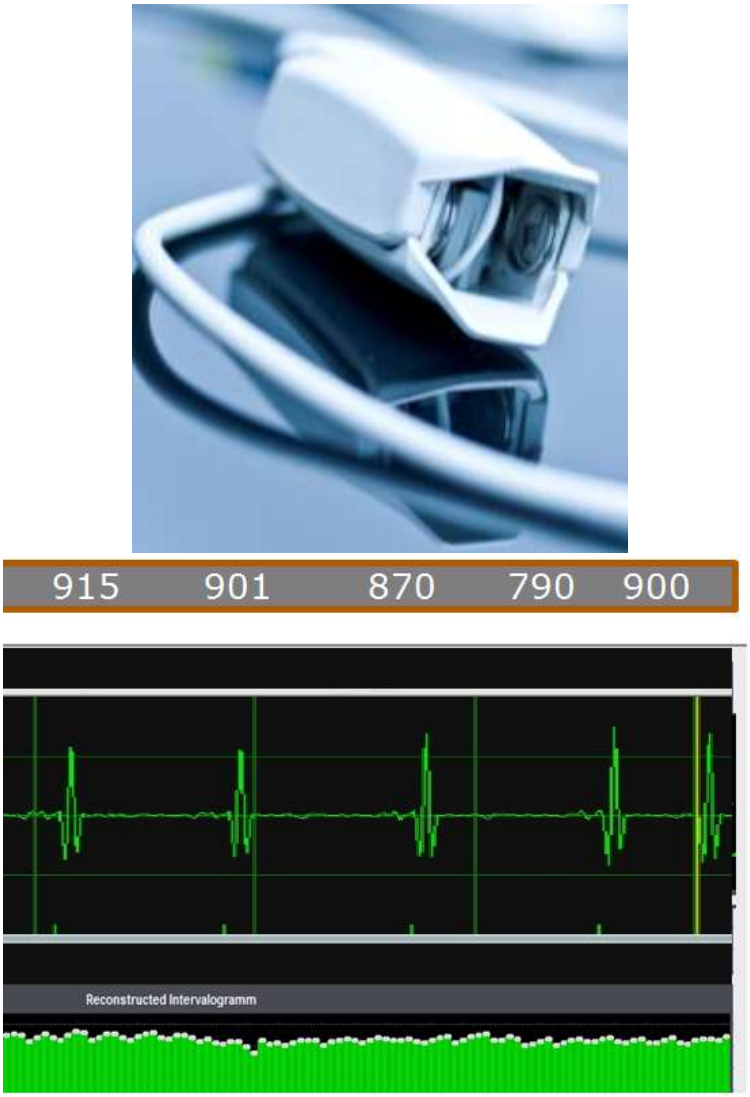


Figure 7A - Digital oximeter and **Figure 7B** Screen showing the capture and time interval of successive QRS complexes (heart rate)

The oximeter captures the arterial pulse wave. It is placed on the left index finger, and it displays in real time the plethysmographic (PTG) waveform, which represents the arterial blood volume changes during the cardiac cycle.

The Signal processing analysis of the waveform is influenced by arteriolar bed at the finger site. A normal plethysmography waveforms contains an Incisura (or notch) related to the reflection wave, in which time and height is correlated to small to medium arterial compliance[35].

A signal processing analysis of the wave form[36,37] in the time and frequency domain (figure 8A,B) provides hemodynamic indicators such as cardiac output (Q), systemic vascular resistance (SVR) and arterial stiffness (SI).

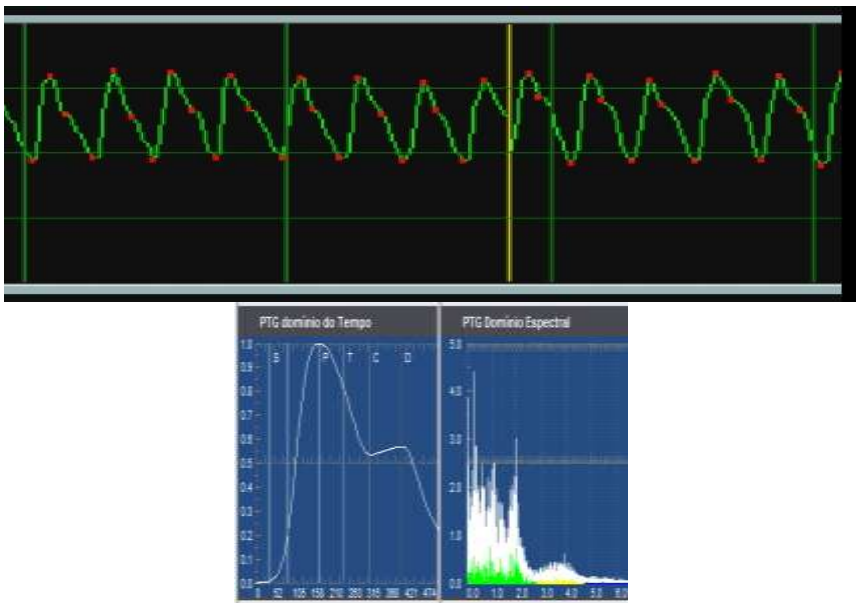


Figure 8A - Screen showing capture of arterial pulse wave and **Figure 8B** - Signal processing analysis of the wave form in the time and frequency domain.

The spectral analysis, using the Fast Fourier Transforms (FFT)[38] of the first derivative of the total record of the plethysmograph (Figure 9), provides 3 frequencies - very low frequency (from 0 to 1.46 Hz and Peak at 1.16 Hz), low frequency (from 1.47 to 2.56 Hz and peak at 2 Hz) and high frequency (From 2.57 to 5 Hz and 2 peaks at 3.2 Hz and 4.58Hz). Each frequency area is measured in millisecond square (ms2). The sum of the 3 frequency areas are the PTG. We named this parameter - Plethysmograph Total Power (PTG-TP)

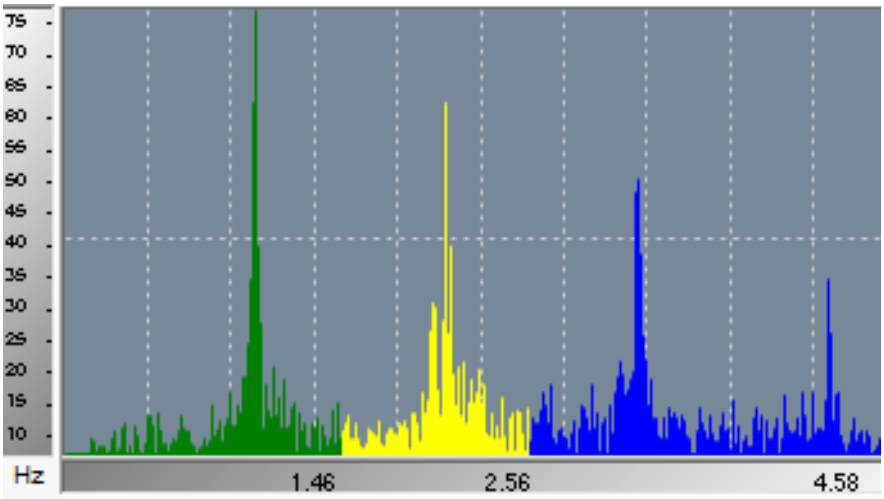


Figure 9 - Spectral analysis of the first derivative of the Photoplethysmography. Green represents the very low frequencies (from 0 to 1.46 Hz); yellow represents the low frequency (from1.47 to 2.56 Hz) and blue represents the high frequencies (from 2.57 to 5 Hz).

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.

4 - RESULTADOS

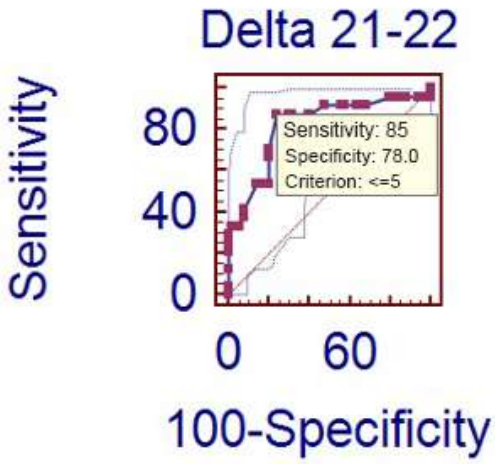
Demographic data can be seen in TABLE 1. From the comparison between Groups 1 and 2:

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

Detection of liver alterations compatible com chronic hepatitis

For the analysis of Groups 1 and 2 regarding the Delta value for the conductance in the pathway of the right foot-left hand minus left hand-right foot, Group 1 (virus-negative and within the normal range for ALT/AST) and Group 2 (virus-positive and/or high levels of ALT/AST) demonstrated a sensitivity of 87% and a specificity of 78% with a cutoff value <=5 and $P < 0.0001$, as shown in Figure 10.



Variable	Delta_21_22 Delta 21-22
Classification variable	Diagnostic

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

Disease prevalence (%)	32.4

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

[a] DeLong et al., 1988
[b] Binomial exact

Figure 10 - The receiver operating characteristic 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 (virus-negative and within the normal range of ALT/AST levels) and Group 2 (virus-positive and/or high levels ALT/AST).

For the comparison of the Stiffness Index between Groups 1 and 2, Group 1 (virus-negative and within the normal range of ALT/AST levels) and Group 2 (virus-positive and/or high levels ALT/AST) demonstrated a sensitivity of 95.8% and a specificity of 74% with a cutoff value > 7.56 m/s and P< 0.0001, as shown in Figure 11, representing the receiver-operating characteristic curve comparing the SI of Group 1 (virus-negative and within the normal range of ALT/AST levels) and Group 2 (virus-positive and/or high levels ALT/AST).

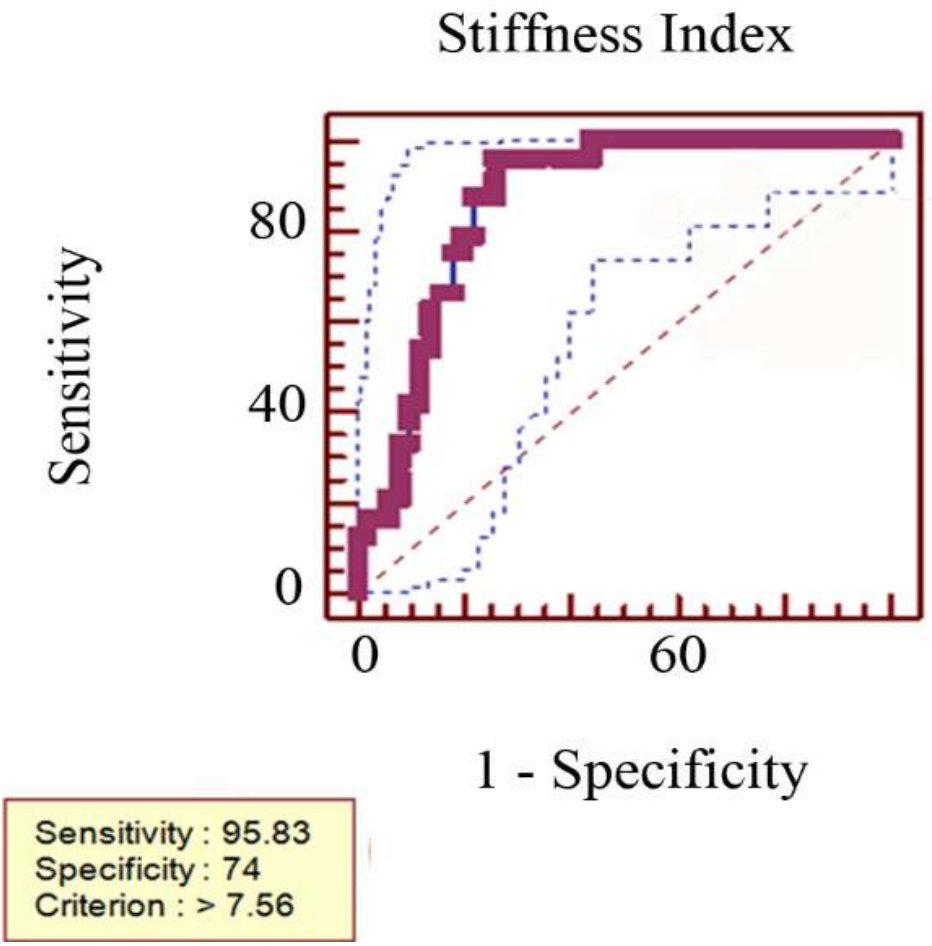


Figure 11 - The receiver-operating characteristic curve comparing the SI of Group 1 (virus-negative and within the normal range of ALT/AST levels) and Group 2 (virus-positive and/or high levels ALT/AST).

For the comparison between groups 1 and 2 regarding the algorithm based on the Stiffness Index (SI) and Delta values for the conductance of the pathway for the left foot-right hand minus right hand-left foot, Group 1 (virus-negative and within the normal range of ALT/AST levels) and Group 2 (HBV- or HCV-positive) demonstrated a sensitivity of 82.9% and a specificity of 84.8% with a cutoff value > 201 and *P* = 0.0001, as shown in Figure 12 representing the receiver-operating characteristic curve comparing the algorithm incorporating the Stiffness Index (SI) and Delta values of conductance (DE)

*Algorithm = SI *(30-DE)

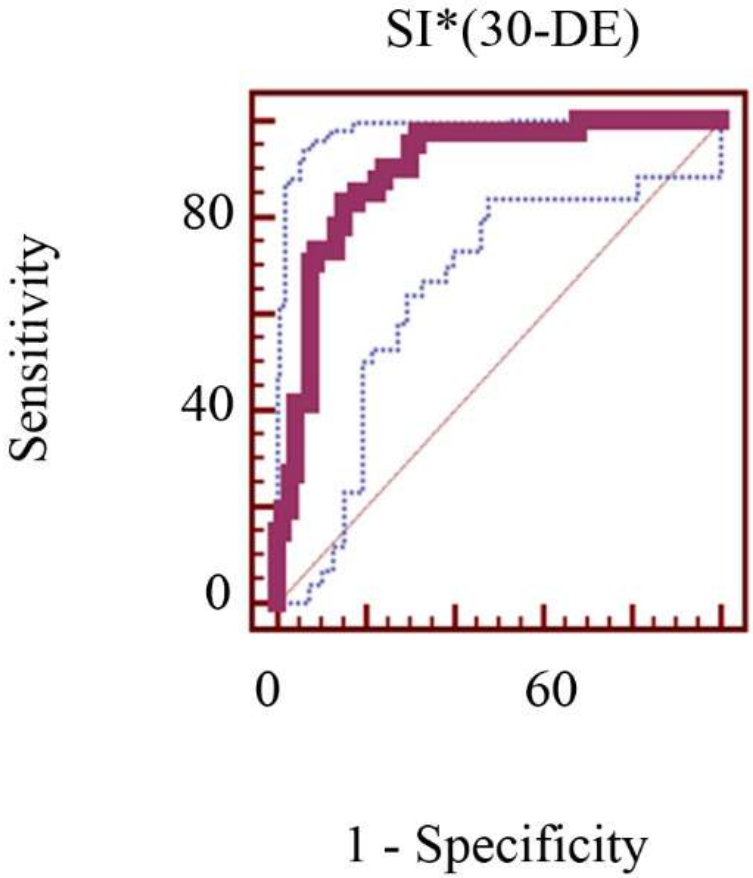
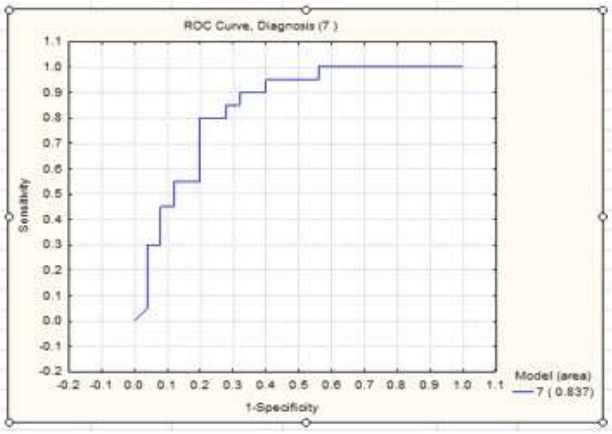


Figure 12. The receiver-operating characteristic curve comparing the algorithm incorporating the Stiffness Index (SI) and Delta values of conductance (DE) for the right foot-left hand between Group 1 (virus-negative and within the normal range of ALT/AST levels) and Group 2 (virus-positive and/or high 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 13). The area under the ROC curve (AUROC) is 0.81.



	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(%)	72.00000	85.00000
Wrong(%)	28.00000	15.00000
Unknown(%)	0.00000	0.00000

FIGURE 13 - 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).

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

BRIEF COMMUNICATION

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

Daniel **IANNI FILHO**[1], Ilka de Fatima Santana Ferreira **BOIN**[2] and Ademar **YAMANAKA**[3]

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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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Correspondence: Daniel Ianni Filho, Universidade Estadual de Campinas (UNICAMP), Gastrocentro, Cidade Universitária Zeferino Vaz – Barão Geraldo – CEP: 13083-970, Campinas, SP, Brasil. E-mail: recadodaniel@terra.com.br

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:

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

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

$$2H_2O = O_2(gas) + 4H^+ + 4e^-(acid)$$

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

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N	50	60	25	20	
Age	32 (18-57)	46 (28-64)	44 (28-63)	49 (29-64)	0.05
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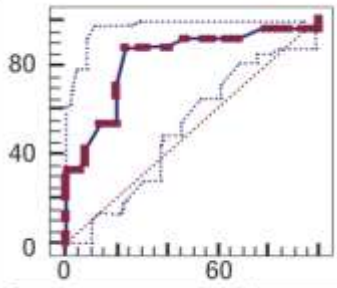
HCV	No	60	25	20	Ns
Delta conductivity	9.2 (-4/19)	1.31 (-6//23)	2.75 (-6/12)	0.62 (-10//23)	0.001

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 *	0.712 to 0.899
z statistic	5.594
Significance level P (Area=0.5)	<0.0001

*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].

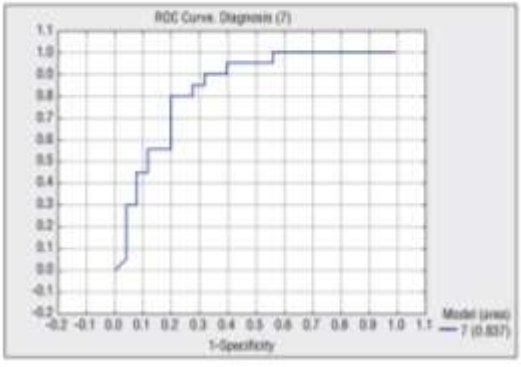
Progressive degrees of fibrosis, and ultimately cirrhosis, are reflected in alterations in blood levels of various biomarkers, and the knowledge of such alterations has led to the development of predictive models based on clinically determined algorithms that utilize the levels of selected markers[9,10].

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[8] 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.

Ianni Filho D, Boin IFSF, Yamanaka A. Bioimpedância: nova abordagem para detecção não invasiva da fibrose hepática – estudo piloto. Arq Gastroenterol. 2018;55(1):2-6.

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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5 - DISCUSSÃO GERAL

Hepatitis C is a common infection in the United States, with an estimated 2.7 to 3.9 million people living with chronic hepatitis C infection[2]. Recently, the Institute of Medicine report on Hepatitis and Liver Cancer emphasized the lack of knowledge and awareness concerning hepatitis C among healthcare providers, social-service providers, and the general public, even among communities at risk for hepatitis C[4]. Misconceptions and lack of awareness concerning this infection can lead to missed opportunities for diagnosis, prevention, and appropriate care.

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 worldwid[14].

Progressive degrees of fibrosis, hepatocellular carcinoma 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 [14.39].

One such model, the HepaScore, is based on the serum levels of α2-macroglobulin, hyaluronic acid, gammaglutamyl transferase (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[14].

Another useful model is the 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%[40].

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[18,19].

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

When used as a screening test, the E.S Complex system was shown to meet the requirements of the World Health Organization guidelines, which state that a screening test should be acceptable for the general population, rapidly performed (requiring no more than two minutes), cost-effective and noninvasive, and that the total cost of finding a positive case should be economically balanced in relation to the medical expenditures as a whole.

One 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. However, it is also possible that the electrochemical reaction at the anode is related to chloride ion migration. The electrochemical reaction provides 4 H+ ions and therefore an acidic tissue environment. A decrease in the Delta 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[23,41].

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 R.J (2008)[31,42] and Abreu D.S. (2011)[23] 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[43].

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.

Plethysmography PTG3[36,37] is calculated from the change of arterial blood volume during the cardiac cycle. These changes are related to the small to arterial medium compliance. Ikeda in 2007[30], shows hemodynamic change during the progression of chronic hepatitis C which is in agreement with the findings of this study. For the comparison of the Stiffness Index between Groups 1 (healthy virus-negative) and Group 2 (virus-positive and / or high levels ALT / AST) the sensitivity was 95.8% and the specificity was 74% with a cutoff value> 7.56 m/s. When the comparison between groups 1 and 2 was made using the algorithm based on the Stiffness Index (SI) coming from Plethysmography and Delta values for the conductance of the pathway for the left foot- right hand minus right hand-left foot coming from Bioimpedance , the sensitivity was 82.9% and a specificity 84.8% with a cutoff value>201. This results show the potential of combining different non-invasive technologies that provide physiological parameters that are related to liver diseases and therefore should be considered in future studies as a possible strategy to increase accuracy in identifying liver diseases as well as severity of liver fibrosis.

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.

FINAL CONSIDERATIONS

This was a pilot study but it envisions innumerable powers of technological development and future clinical applications.

Despite the current limited number of publications on the subject, research has shown the potential of Bioimpedance in identifying the physiological state and the anatomical architecture of living tissues allowing to differentiate healthy tissues from physiologically altered tissues either by acute, chronic inflammation, hypoxia or neoplasms.

In this pilot study, the Monofrequential Bipolar Bioimpedance with a frequency of 0.7 Khz was used. In this frequency, electricity is driven by water and ions from the interstitium since the bilipid cytoplasmic membrane acts as a capacitor preventing the electric current from entering the cell. Nowadays, the Multifrequency Tetrapolar Bioimpedance devices whose frequency can range from 1 to 500 Htz are available, in which the electrical conductivity is not restricted to the interstitial environment, allowing the evaluation of the intracellular environment since the increase in frequency allows the electric current to win the resistance of the cytoplasmic membrane and within the cell.

Thus, not only the physiological conditions of the interstice but also the intracellular environment may be known. Increasing the number of information increases the potential to develop algorithms with increasing accuracy and breadth of

clinical applications The current study was a pilot study that could not draw conclusive results, although it may serve as the first step for further investigations into whether bioimpedance in vivo can be used to help clinicians detect (although not at the diagnostic level) liver fibrosis during screening procedures.

What we know as a disease is either functional or anatomical can be the expression of alterations and dysfunctions of various origins with the possible involvement of genetic, biochemical, energetic, electrical, vascular, anatomical, etc. variables. If this premise is true, on the use of several technologies that provide different sources and forms of information, they can be beneficial in the construction of increasingly accurate, sensitive and specific algorithms in the screening and diagnosis of diseases. The ES Complex device used in this research combines four technologies: 1) Monofrequential Bipolar Bioimpedance[44,45,46,47,48,49,50,51,52,53], 2) Photoelectric Plethysmography[,54.55,56,57,58,59], 3) Heart Rate Variability[60,61,62] (gold standard to access Autonomic Nervous System), 4) Galvanic Skin Response (which evaluates the function of cholinergic sympathetic postganglionic fibers that innervate the sweat glands and therefore evaluates the sweating). By using not only the Bioimpedance but the data coming from the combination of these technologies more information can be acquired such as pH, concentration of chemical elements inside and outside the cell, micro and macro vascular changes, physiological changes in waveform vascular, repercussions on ANS, etc. With more physiological information checked, new algorithms can be developed to identify early many diseases and dysfunctions in their early stages of development. Thus, this work was intended to be a drop of water in the ocean of possibilities of science and an incentive to use non-invasive technologies in the service of the health sciences.

6 - CONCLUSÃO

The Bioimpedance demonstrate good to high levels of sensitivity and specificity to identify structural liver alterations like liver fibrosis 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.

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ANEXO 1 – PARECER DO COMITÊ DE ÉTICA

Approval number of the Ethical Committee – Faculty of Medical Science of Campinas (Unicamp) is CEP 34541/2010

FACULDADE DE CIÊNCIAS MÉDICAS
COMITÊ DE ÉTICA EM PESQUISA

www.fcm.unicamp.br/pesquisa/etica/index.html

CEP, 30/08/10
(Grupo III)

PARECER CEP: Nº 542/2010 (Este nº deve ser citado nas correspondências referente a este projeto)
CAAE: 0417.0.146.000-10

I - IDENTIFICAÇÃO:

PROJETO: **"SISTEMA ES TECK COMPLEX COMO ADJUNTO NO SCREENING DE DESORDENS DIGESTIVAS COM TÉCNICAS CONVENCIONAIS".**
PESQUISADOR RESPONSÁVEL: Daniel Ianni Filho
INSTITUIÇÃO: Gastrocentro/UNICAMP
APRESENTAÇÃO AO CEP: 11/06/2010
APRESENTAR RELATÓRIO EM: 30/08/11 (O formulário encontra-se no site acima).

II - OBJETIVOS

Avaliar a especificidade e sensibilidade dos métodos tradicionais de avaliação das desordens digestivas (imagem, ultrasom, biopsias, exames laboratoriais e marcadores tumorais) contra os parâmetros do sistema EIS ES TECK COMPLEX obtidos de modo não invasivo.

III - SUMÁRIO

Trata-se de um estudo controlado onde os pacientes realizarão a medicação com o sistema EIS ES Teck Complex como parte da avaliação inicial de primeira consulta, sem alterar qualquer conduta de avaliação clínica protocolada pelo Gastrocentro. Serão comparados os achados de diversos parâmetros deste exame com os achados dos métodos tradicionais (exames laboratoriais, tomográficos, endoscópicos, biópsias, colonoscopia, etc.) analisando a sensibilidade e a especificidade do sistema EIS TECK Complex com os diagnósticos realizados a partir do padrão ouro aceitos pela literatura medica mundial. Serão selecionados pacientes que procurem por diagnóstico e tratamento nos ambulatórios do Gastrocentro do Hospital das Clínicas da Faculdade de Ciências Médicas. A duração do estudo será de aproximadamente 1 ano tempo necessário para obter amostra de indivíduos avaliados em primeira consulta. A participação dos indivíduos não ultrapassará 10 minutos, uma vez que o tempo necessário para realizar um exame com o sistema EIS ES Teck é de apenas 4 minutos. Serão incluídos em 3 amostras separadas os pacientes que apresentarem sintomas de patologias dos órgãos intestino, estomago e fígado ou aqueles que apresentarem riscos de desenvolvimento desta patologia seja por fatores hereditários, síndromes genéticas ou problemas comportamentais como o alcoolismo, exposição a fatores de risco. O pesquisador não receberá nenhuma remuneração para desenvolver a pesquisa. Cada exame utilizará dois eletrodos descartáveis a um custo de R$4,00 por exame. A previsão é do gasto de R$ 3.000,00 em eletrodos descartáveis. Tendo em vista que o equipamento e os eletrodos descartáveis serão oferecidos pelo fabricante, não havendo nenhum custo para instituição.

IV - COMENTÁRIOS DOS RELATORES

Após analisar as respostas às pendências encaminhadas em 13/08/10, todas as questões solicitadas pelo Comitê de Ética em Pesquisa foram respondidas, com isso o projeto de pesquisa

encontra-se adequadamente redigido e de acordo com a Resolução CNS/MS 196/96 e suas complementares, bem como o Termo de Consentimento Livre e Esclarecido.

V - PARECER DO CEP

O Comitê de Ética em Pesquisa da Faculdade de Ciências Médicas da UNICAMP, após acatar os pareceres dos membros-relatores previamente designados para o presente caso e atendendo todos os dispositivos das Resoluções 196/96 e complementares, resolve aprovar sem restrições o Protocolo de Pesquisa, o Termo do Consentimento Livre e Esclarecido, bem como todos os anexos incluídos na pesquisa supracitada.

O conteúdo e as conclusões aqui apresentados são de responsabilidade exclusiva do CEP/FCM/UNICAMP e não representam a opinião da Universidade Estadual de Campinas nem a comprometem.

VI - INFORMAÇÕES COMPLEMENTARES

O sujeito da pesquisa tem a liberdade de recusar-se a participar ou de retirar seu consentimento em qualquer fase da pesquisa, sem penalização alguma e sem prejuízo ao seu cuidado (Res. CNS 196/96 – Item IV.1.f) e deve receber uma cópia do Termo de Consentimento Livre e Esclarecido, na íntegra, por ele assinado (Item IV.2.d).

Pesquisador deve desenvolver a pesquisa conforme delineada no protocolo aprovado e descontinuar o estudo somente após análise das razões da descontinuidade pelo CEP que o aprovou (Res. CNS Item III.1.z), exceto quando perceber risco ou dano não previsto ao sujeito participante ou quando constatar a superioridade do regime oferecido a um dos grupos de pesquisa (Item V.3.).

O CEP deve ser informado de todos os efeitos adversos ou fatos relevantes que alterem o curso normal do estudo (Res. CNS Item V.4.). É papel do pesquisador assegurar medidas imediatas adequadas frente a evento adverso grave ocorrido (mesmo que tenha sido em outro centro) e enviar notificação ao CEP e à Agência Nacional de Vigilância Sanitária – ANVISA – junto com seu posicionamento.

Eventuais modificações ou emendas ao protocolo devem ser apresentadas ao CEP de forma clara e sucinta, identificando a parte do protocolo a ser modificada e suas justificativas. Em caso de projeto do Grupo I ou II apresentados anteriormente à ANVISA, o pesquisador ou patrocinador deve enviá-las também à mesma junto com o parecer aprovatório do CEP, para serem juntadas ao protocolo inicial (Res. 251/97, Item III.2.e).

Relatórios parciais e final devem ser apresentados ao CEP, de acordo com os prazos estabelecidos na Resolução CNS-MS 196/96.

III – DATA DA REUNIÃO.

Homologado na VI Reunião Ordinária do CEP/FCM, em 22 de junho de 2010.

Prof. Dr. Carlos Eduardo Steiner
PRESIDENTE do COMITÊ DE ÉTICA EM PESQUISA
FCM/UNICAMP

ANEXO 2 – CARTA DA EDITORA DE ACEITE PUBLICAÇÃO

ARQUIVOS de GASTROENTEROLOGIA
- Fundada em 1964 -
Órgão oficial de:
INSTITUTO BRASILEIRO de ESTUDOS e PESQUISAS de GASTROENTEROLOGIA e OUTRAS ESPECIALIDADES – IBEPEGE
COLÉGIO BRASILEIRO de CIRURGIA DIGESTIVA – CBCD
SOCIEDADE BRASILEIRA de MOTILIDADE DIGESTIVA e NEUROGASTROENTEROLOGIA – SBMDN
FEDERAÇÃO BRASILEIRA de GASTROENTEROLOGIA – FBG
SOCIEDADE BRASILEIRA de HEPATOLOGIA – SBH
SOCIEDADE BRASILEIRA de ENDOSCOPIA DIGESTIVA – SOBED
SOCIEDADE BRASILEIRA de NUTRIÇÃO PARENTERAL e ENTERAL – SBNPE

São Paulo, 18 de janeiro de 2018

Prezado Dr. Daniel Ianni Filho

O artigo intitulado "Bioimpedance: new approach to non-invasive detection of liver fibrosis – a pilot study" (Reg. AG-2017-101) de sua autoria com a Dra. Ilka de Fatima Santana Ferreira Boin e o Dr. Ademar Yamanaka, foi aprovado pela Comissão Editorial da ARQUIVOS de GASTROENTEROLOGIA e deverá ser publicado no número 1 do volume 55, ano 2018.

Autorizamos a inclusão do artigo na dissertação ou tese, o que não infringi o direito autoral transferido à editora.

Renovamos os agradecimentos e nos subscrevemos.

Atenciosamente,

Dr. Ricardo Guilherme Viebig
- Editor Executivo -