Research Article

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Sensitive and non-invasive assessment of hepatocellular iron using a novel room-temperature susceptometer

Johannes Mueller[1], Hanna Raisi[1], Vanessa Rausch[1], Teresa Peccerella[1], David Simons[2], Christian Herbert Ziener[2], Heinz-Peter Schlemmer[2], Helmut Karl Seitz[1], Nina Waldburger[3], Thomas Longerich[4], Beate Katharina Straub[5], Sebastian Mueller[1,*]

[1]*Dept. of Medicine, Salem Medical Center and Center for Alcohol Research and Liver Disease, University of Heidelberg, Germany;* [2]*Dept. of Radiology, Deutsches Krebsforschungszentrum (DKFZ), Heidelberg, Germany;* [3]*Department of Pathology, University of Heidelberg, Germany;* [4]*Department of Pathology, University of Aachen, Germany;* [5]*Department of Pathology, Universities of Mainz and Heidelberg, Germany*

Background & Aims: Liver iron accumulates in various chronic liver diseases where it is an independent factor for survival and carcinogenesis. We tested a novel room-temperature susceptometer (RTS) to non-invasively assess liver iron concentration (LIC).

Methods: Two hundred and sixty-four patients with or without signs of iron overload or liver disease were prospectively enrolled. Thirty-five patients underwent liver biopsy with semi-quantitative iron determination (Prussian Blue staining), atomic absorption spectroscopy (AAS, n = 33), or magnetic resonance imaging (MRI, n = 15).

Results: *In vitro* studies demonstrated a highly linear ($r^2 = 0.998$) association between RTS-signal and iron concentration, with a detection limit of 0.3 mM. Using an optimized algorithm, accounting for the skin-to-liver capsule distance, valid measurements could be obtained in 84% of cases. LIC-RTS showed a significant correlation with LIC-AAS ($r = 0.74$, $p < 0.001$), LIC-MRI ($r = 0.64$, $p < 0.001$) and hepatocellular iron ($r = 0.58$, $p < 0.01$), but not with macrophage iron ($r = 0.32$, $p = 0.30$). Normal LIC-RTS was 1.4 mg/g dry weight. Besides hereditary and transfusional iron overload, LIC-RTS was also significantly elevated in patients with alcoholic liver disease. The areas under the receiver operating characteristic curve (AUROC) for grade 1, 2 and 3 hepatocellular iron overload were 0.72, 0.89 and 0.97, respectively, with cut-off values of 2.0, 4.0 and 5.0 mg/g dry weight. Notably, the positive and negative predictive values, sensitivity, specificity and accuracy of severe hepatic iron overload (HIO) (grade ≥ 2) detection, were equal to AAS and superior to all serum iron markers. Depletion of hepatic iron could be efficiently monitored upon phlebotomy.

Conclusions: RTS allows for the rapid and non-invasive measurement of LIC. In comparison to MRI, it could be a cost-effective bedside method for LIC screening.

Lay summary: Novel room-temperature susceptometer (RTS) allows for the rapid, sensitive, and non-invasive measurement of liver iron concentration. In comparison to MRI, it could be a cost-effective bedside method for liver iron concentration screening.

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Keywords: Atomic absorption spectroscopy; Alcoholic liver disease; Anemia; Cirrhosis; Hemochromatosis; Iron overload; Liver iron; Liver fibrosis; Liver stiffness; Magnetic susceptibility; Susceptometry.

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* Corresponding author. Address: Department of Internal Medicine, Salem Medical Center, University of Heidelberg, Zeppelinstraße 11–33, 69121 Heidelberg, Germany. Tel.: +49 6 22 14 83 210; fax: +49 6221 483 494.

E-mail address: Sebastian.Mueller@urz.uni-heidelberg.de (S. Mueller).

Introduction

Besides hereditary iron overload diseases,[1,2] many chronic liver diseases such as hepatitis C virus (HCV), non-alcoholic fatty liver disease (NAFLD) and alcoholic liver disease (ALD) ultimately cause hepatic iron overload (HIO).[3] HIO is highly toxic and carcinogenic in animal models[4,5] due to the Fenton reaction.[6] In humans, both in hereditary hemochromatosis and ALD, HIO determines overall survival[7,8] and independently increases the risk of hepatocellular carcinoma (HCC).[9,10] While treatment of HIO *e.g.* in hemochromatosis or thalassemia efficiently improves survival, the role of phlebotomy or chelation therapy in metabolic and viral liver disease remains unsettled.[3] Notably, in a large prospective cohort of healthy individuals, continued depletion of iron also lowered general carcinogenesis.[11]

Unfortunately, the diagnosis, screening and monitoring of elevated liver iron levels is still limited. Quantitative iron analysis from liver biopsy specimens, typically done by atomic absorption spectroscopy (AAS), is considered the current gold standard.[1,2] However, liver biopsy is not indicated for follow-up studies, because it is invasive and prone to sampling error (up to 30%).[12] Serum markers such as ferritin and transferrin saturation (TSAT) are not reliable, but remain the preferred screening method for studying iron overload.[13] When screening patients with HIO, guidelines recommend appropriate cut-off levels of TSAT (>45%) and serum ferritin (>1000 ng/ml).[1] Unfortunately, serum markers can easily overestimate iron stores in the

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presence of inflammation or cancer related disease (*e.g.* anemia of chronic disease). At the same time, a significant proportion of patients may have anemia with serum measures indicating an iron depleted phenotype, despite an increased liver iron concentration (LIC).[14] In such patients, the presently established serum ferritin cut-off values underestimate HIO. Therefore serum markers are not considered ideal for iron overload disease screening.[15]

For these reasons, various technologies have been pursued in order to directly and non-invasively assess hepatic iron. A number of non-invasive imaging tools, such as dual-energy computer tomography (DE-CT)[16,17] and magnetic resonance imaging (MRI) using the T2 and T2* mode[18–20] are suitable for detection of iron. These techniques have become increasingly accurate for determining both hepatic and cardiac iron deposition.[21] However, they have specific limitations, including exposure to radiation in CT and strong magnetic fields in MRI. In addition, MRI is expensive and the physical basis of magnetic resonance of nearby water molecules is still incompletely understood,[22] with signals depending on hydration status, proton mobility and clustering of iron.[22,23] Hepatic iron concentration can also be determined non-invasively by magnetic susceptometry (MS) using a Superconducting Quantum Interference Device (SQUID).[24–26] SQUID has been in clinical use for nearly thirty years, where it has primarily been used to monitor iron stores in rather rare thalassemia patients. However, because of the complexity and expense of SQUID devices and their requirement for liquid helium cooling, only four instruments are available worldwide for clinical use. Other techniques using susceptometry-like approaches are also under investigation.[27–29]

We studied room-temperature susceptometer (RTS), which uses less expensive (50×) sensor technology than SQUID, without liquid helium, in a large cohort of patients with and without chronic liver disease or iron disorders. Our study indicates that RTS measures predominantly hepatocellular iron with the same accuracy as AAS, outscoring all other non-invasive iron markers. We suggest RTS as a cost-effective method for future liver iron screening and follow-up studies, *e.g.* in response to phlebotomy or iron chelation therapy.

Patients and methods

Patients

The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was reviewed and approved by the Ethics Committee of the University of Heidelberg (no: S064-2013 and S150-2015). All participants gave written informed consent prior to inclusion. Two hundred and sixty-four patients, mean age 48.6 ± 15.8 years, 66 females and 198 males, from Germany were consecutively enrolled from June 2013 – March 2017 (Table 1 and Fig. 1A). All participants were adults (>18 years). Most patients had ALD (n = 171, 37 with cirrhosis), NAFLD (n = 9), iron overload syndromes such as hereditary hemochromatosis (n = 18) or transfusional iron overload due to thalassemia or sickle cell disease (n = 13), and other non-iron homeostasis dependent diseases with or without increased serum ferritin values (n = 45). Eight normal volunteers were also included. In all patients, RTS measurements, abdominal ultrasound, and transient elastography (Fibroscan) were performed. In addition, morphometric data, such as body mass index (BMI), and data for routine blood and serum parameters, including serum ferritin, transferrin and TSAT, were obtained. Thirty-five patients underwent medically indicated liver biopsy with histological determination of the semiquantitative degree (0–4) and cell type-specific distribution (macrophage *vs.* hepatocyte) of iron (Prussian Blue staining). In 33 of these patients, LIC was quantified by AAS. In a further 15 patients, hepatic iron was quantified by MRI using a 1.5 Tesla MRI scanner (Siemens Aera) and LiverLab software. In two patients, MRI could not be obtained due to contraindications such as permanent make up and metal implants.

Table 1. Patient characteristics.

Demographic and morphological data	Mean (SD)
Male	198
Female	66
Age (years)	48.6 ± 15.8
Size (m)	1.7 ± 0.1
Weight (kg)	78.3 ± 21.3
Surface (m^2)	2 ± 1.5
BMI (kg/m^2)	25.7 ± 6.3
Hip (cm)	101.6 ± 13.4
Waist (cm)	98.1 ± 15.1
H/W ratio	1.05 ± 0.09
Serum parameters	
GOT (U/L)	87.9 ± 120.2
GPT (U/L)	73.3 ± 130.1
GGT (U/L)	319.7 ± 508.6
AP (U/L)	118.6 ± 97.3
Bilirubin total (mg/dl)	1.4 ± 2.4
Quick (%)	98.7 ± 22.9
INR	1.3 ± 5
Creatinine	0.7 ± 0.3
Lipase (U/L)	61.6 ± 110.6
PTT (sec)	32.5 ± 6.1
Hemoglobin (g/dl)	13.8 ± 2.9
Hematocrite (%)	38.5 ± 7.3
MCV (fl)	91.3 ± 9.3
Erythrocytes (/pl)	4.4 ± 2.2
Leukocytes (/nl)	7.4 ± 3
Sodium (mmol/L)	138.2 ± 4
Platelets (/nl)	216 ± 103.6
Ferritin (ng/ml)	721.7 ± 824.3
CRP (mg/L)	9.1 ± 19.8
Cholesterine (mg/dl)	210.7 ± 61.3
Albumin (g/dl)	6.2 ± 9.9
Protein total (g/dl)	10.5 ± 13.9
Transferrin (g/L)	2.3 ± 0.7
TSAT (%)	45 ± 34.1
Serum iron (μg/dl)	144.7 ± 141.4
LDH (U/L)	231.7 ± 79.3
Ultrasound and Fibroscan	
Liver size (cm)	16.1 ± 3.1
Hepatic steatosis (0–3)	1.6 ± 0.9
Spleen size (cm)	10.6 ± 2.5
Signs of cirrhosis (0–1)	28
Liver stiffness (kPa)	14.8 ± 20.1
CAP (dB/m)	270.6 ± 62.7

AP, alkaline phosphatase; BMI, body mass index; CAP, controlled attenuation parameter; CRP, C-reactive protein; H/W, height/weight; GOT, glutamic oxaloacetic transaminase; GPT, glutamic pyruvic transaminase; GGT, gamma glutamyl transpeptidase; INR, international normalized ratio; LDH, lactate dehydrogenase; MCV, mean corpuscular volume; PTT, partial thromboplastin time; TSAT, transferrin saturation.

Abdominal ultrasound and patient positioning for RTS

Before RTS, abdominal ultrasound was performed in each subject to describe liver pathologies, such as degree of steatosis (0–3), signs of liver cirrhosis, and liver size (measured in the median axillar line), as well as to select an appropriate point for the RTS liver iron measurement. For each subject, we selected a location at least 5 cm from the lung, with at least 3 cm of liver depth, in all directions, from the point on the liver capsule that lay directly below the center of the sensor unit (Fig. 2B). Patients for whom the minimal distance to lung could not be established were excluded from the study (n = 8). Skin-liver-capsule distance (SLC) was measured to calculate the LIC as described below. Two Patients with an ascites layer of 10 mm or more were also excluded. For RTS measurements, the patient lay on a horizontal bed, positioned with the liver facing upward. The patient was centered

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