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Time related changes of T_1, T_2, and T_2^* of human blood in vitro

Andreas Petrovic [a,b], Astrid Krauskopf [a,c], Eva Hassler [a,d], Rudolf Stollberger [b], Eva Scheurer [a,e,*]

[a] Ludwig Boltzmann Institute for Clinical-Forensic Imaging, Universitätsplatz 4/II, 8010 Graz, Austria
[b] Institute of Medical Engineering, BioTechMed, Graz University of Technology, Stremayergasse 16/3, 8010 Graz, Austria
[c] Institute of Forensic and Traffic Medicine, Voßstraße 2, Geb. 4420, 69120 Heidelberg, Germany
[d] Department of Radiology, Division of General Diagnostic Radiology, Medical University Graz, Auenbruggerplatz 12, 8036 Graz, Austria
[e] Institute of Forensic Medicine, University Basel, Pestalozzistr. 22, 4056 Basel, Switzerland

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ABSTRACT

In view of a potential future use for dating hemorrhage in forensic medicine the correlation of MR relaxation parameters with time was evaluated in blood samples. A systematic relationship could be valuable for using MRI for estimating the age of hemorrhage and soft tissue hematomas particularly in clinical forensic medicine.

Relaxation times T_1, T_2, and T_2^* of venous blood samples from 6 volunteers were measured using 3 T MRI regularly up to 30 days. The time progression of the relaxation parameters was systematically analyzed and examined for possible interrelations.

T_2 initially decreased to a minimum, and then increased again (range 24–97 ms), while T_1 started with a plateau phase followed by an almost linear decrease (range 333–2153 ms). T_2^* remained relatively constant during the entire investigation period. The higher the initial T_2 was, the lower was its minimum, and the greater was the decrease of the associated T_1. The inter- and intra-individual variability was relatively large, one reason being very likely the metabolic differences in the blood samples.

The observed characteristic changes in blood samples over time measured by quantitative MR techniques add objective information in view of an estimation of the age of hemorrhage. However, in vivo studies will be needed to verify the data with respect to influencing metabolic factors.

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1. Introduction

The appearance of blood and blood products in MRI is complex which has been shown in several studies mainly in the context of intracranial hemorrhage [1–7]. The composition of blood contained in hemorrhage changes over time which is reflected in the signal strength observed in T_1 and T_2 weighted MRI sequences [3–5,8]. In radiology and clinical medicine less attention has been paid to soft tissue, and particularly subcutaneous hemorrhage, e.g., bruises, [9–11] as these generally do not need any specific diagnostic or therapeutic measures.

However, in forensic medicine bruises are important indicators adding information for reconstructing violent events and for the determination of the time of such incidents. So far, no accurate and objective procedures for the determination of the age of bruises are known which can routinely be performed on living persons [12]. Visual assessment of the color of hematomas is highly subjective [13], and not only the person's age but also the location on the body, the amount of blood as well as the color and condition of the skin influence the external appearance. Thus, various attempts to find objective and quantitative methods, e.g., using spectrophotometry, colorimetry or reflectance spectroscopy, failed for a systematic use [14].

The idea of characterizing blood and its constituents using MRI is not new as show various studies of blood in vitro [15–23] and two case studies which investigated the changes of blood in vitro with time at 0.15 T and 0.47 T [24,25]. It was shown that the oxygenation state of hemoglobin (Hb), the amount of different Hb metabolites such as methemoglobin (metHb), and the intactness of the cell membrane of the red blood cells (RBCs) play a key role in

Abbreviations: CV, coefficient of variation; deoxyHb, deoxyhemoglobin; Hb, hemoglobin; metHb, methemoglobin; oxyHb, oxyhemoglobin; RBC, red blood cell; Y, fraction of oxyhemoglobin.

* Corresponding author. Present address: Institute of Forensic Medicine, University Basel, Pestalozzistr. 22, 4056 Basel, Switzerland.
Tel.: +41 61 267 3870; fax: +41 61 267 3907.
E-mail addresses: eva.scheurer@cfi.lbg.ac.at, eva.scheurer@bs.ch (E. Scheurer).

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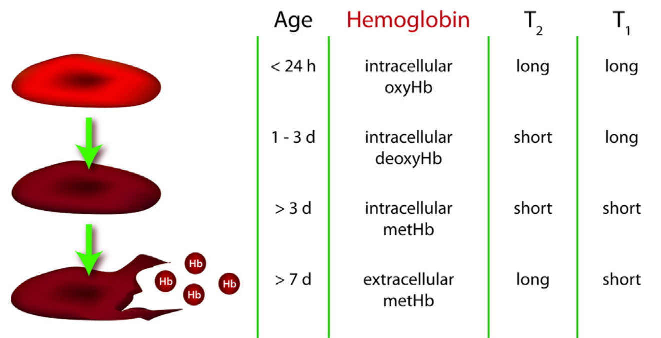

Fig. 1. Staging scheme for intracranial hematomas showing the age, dominant Hb species, and relaxation times according to Bradley et al. [3]. The corresponding stages are hyperacute (<24 h), acute (1–3 d), early subacute (>3 d), and late subacute (>7 d). The chronic stage was omitted in this illustration.

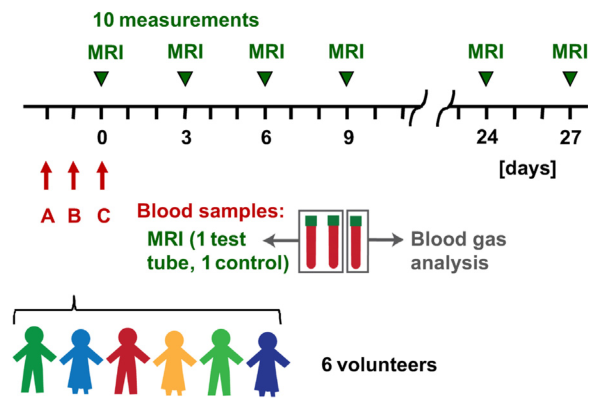

Fig. 2. Overview of the measurement setting used in this study showing measurement times, blood samples, and volunteers.

understanding hemorrhage behavior in MRI, as they affect the transversal (T_2) and longitudinal (T_1) relaxation times. According to the alteration of the relaxation times also the MRI signal is changed. These changes allow for a rough estimation of the age of intracranial hematomas. The literature largely agrees on the stages hyperacute, acute, early and late subacute, as well as chronic [3,5]. While these stages might be sufficiently determined for a use in neuroradiology, they may not be transferrable to subcutaneous hemorrhage due to the influencing factors of the surrounding tissue [10,11]. Moreover, the exact mechanism of the auto-oxidation of Hb and the associated systematic changes of the relaxation times are very complex and still not well understood [26]. Fig. 1 outlines schematically the different states of hemoglobin and the corresponding changes in relaxation times. Comprehensive reviews on this topic can be found in the literature [3,8].

In view of an increasing use of MRI in forensic medicine it could possibly also be used to date subcutaneous hematomas in living victims. As a first step the aim of this prospective study was (a) to evaluate MR relaxation changes in blood in vitro over time, and (b) to assess if MR relaxation parameters show systematic time courses which could be correlated with the age of the blood. Furthermore, the interdependence of the changing relaxation parameters was investigated.

2. Materials and methods

2.1. Sample preparation and measurement setup

A total of 54 blood samples of 9 ml were drawn from six healthy volunteers (4 m, 2 f, age: median 31 years, range 27–38 y) which had given their informed consent. Samples were placed in 9 ml heparinized plastic test tubes (S-Monovette®, Sarstedt AG & Co, Nümbrecht, Germany). Three samples of blood (one test and one control sample, and one sample for blood gas analysis) were collected daily for three consecutive days (A, B, C), so that a fresh, a one day and a two day old sample were available at day 0 when the MRI measurements started (see Fig. 2). Thereby, a complete coverage of all days was assured although measurements due to logistic reasons were only conducted every third day up to 30 days. According to Cohen et al. the samples were stored at 4 °C between the measurements to prevent degradation [24]. Prior to each measurement, the samples were warmed in a water-bath to 37 °C to simulate in vivo body temperature. During the entire measurement time this temperature was maintained for all samples. In the scanner a flow of warm air was used. A Luxtron 790 fluoroptic thermometer (LUXTRON Corporation, Santa Clara, USA) was used to measure the temperature and to trigger the attached temperature control of the heating system. Samples were rotated around their long axis between individual measurements by a mechanic axis connected with a stepper motor to prevent sedimentation of the RBCs. Blood gas analysis (pH; oxygen saturation; hematocrit; fraction of deoxyHb, $1-Y$; MetHb; fraction of oxyHb, Y; concentration of hemoglobin) was performed on the days of blood withdrawal using a ABL800 Flex blood gas analyzer (Radiometer Medical, Brønshøj, Denmark).

For T_2 and T_1 measurements six blood samples and two additional control tubes filled with H_2O, and with H_2O + Gadovist (Bayer Schering Pharma, Berlin, Germany), respectively, were placed in a box in parallel to the main magnetic field. For T_2^* measurements only one sample at a time was placed in the box to prevent mutual influence by distortion of the main magnetic field. The study was approved by the local institutional ethics committee.

2.2. MRI measurements

All measurements were conducted on a 3 T Magnetom scanner (Tim Trio, Siemens, Erlangen, Germany) with a circularly polarized one channel head coil. T_2 was determined with a multi spin-echo sequence (CPMG, fully balanced gradients, TR = 2000 ms, τ = 8.2 ms, 32 echoes). T_1 was estimated using a set of TIR measurements (TR = 6000 ms, TE = 7.8 ms, TI = 400–2800, 6 contrasts). T_2^* was measured using a spoiled GRE sequence with 12 echoes (TR = 200 ms, TE = 2.5–30 ms, $\alpha = 21^\circ$, NSA = 4). The imaging cross sections were for all measurements placed in the transversal plane in the middle of the test tube with respect to the tubes' long axis.

2.3. Data fitting

T_2 and T_2^* values were estimated from the relaxation curves by nonlinear curve fitting to a mono-exponential decay model $S = M_0 \cdot \exp(-\tau/T_2^{(*)})$ where M_0 denotes the spin density, and τ is the inter-echo spacing. As the use of a CPMG sequence for T_2 estimation usually goes along with a first echo lower than the second not fitting into the echo train, the first echo was discarded. T_1 was estimated by nonlinear fitting to a mono-exponential recovery curve $S = M_0 \cdot (1 - 2a \cdot \exp(-\mathrm{TI}/T_1))$, where "$a$" is a factor accounting for radio frequency field inhomogeneities, and TI is the inversion time. All fitting algorithms were implemented in Matlab (Natick, USA) using least squares minimization (fmincon).

2.4. Statistical analysis

Mean values and ranges for all measured relaxation times, as well as blood gas parameters, were computed. For data analysis

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