Detection and Classification of Leukemia using MRI and NMR Techniques

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Introduction: Leukemia is a form of cancer affecting the blood-forming cells of the bone marrow. Currently, diagnosis is achieved by cytogenetic analysis where the sample is retrieved either as a fluid aspirate, typically taken from the cerebrospinal fluid in the vertebrae, or as a bone biopsy. Both techniques are highly invasive and cause extreme discomfort to the patient. The goal herein is to develop a non-invasive protocol to detect and classify early onset of leukemia prior to resorting to these painful and highly invasive methods. Two approaches will be investigated: contrast enhanced MRI and MR spectroscopy. To this end, normal volunteers were recruited to explore the potential of contrast-enhanced MRI for the characterization of bone marrow. In order to first understand the nature of *in vivo* MR spectroscopy, a second component of the study adapts previous NMR work for differentiating grades of fibrosarcoma [1] for use in discriminating between two subclasses of acute myelogenous leukemia (AML).

Methodology: [A] ***Dynamic Contrast Enhanced MRI*****:** All MR imaging was performed in a Siemens Symphony 1.5T scanner. Localization of the L3 vertebral body was achieved using a T1-weighted short tau inversion recovery (STIR) sequence in 3 healthy male volunteers, ages 21, 26 and 31. The dynamic contrast-MRI protocol was adapted from previous work by Montazel et al. [2]. A bolus of 0.2 mmol/kg Gadodiamide (Omniscan, GE/Amersham) was administered and enhancement followed with serial imaging, once every 3 s for up to 3 min post-bolus (saturation recovery turboFLASH, 'srTFL,' TR/TE = 2.69/1.22 ms, α=15°, 78 x 128 matrix, 162.5 mm x 200 mm FOV, slice thickness=7.5 mm). All image analysis was performed using ImageJ software (public domain, NIH). Two regions of interest were selected for each volunteer: (1) in L3 and (2) in the abdominal aorta. For each time point post-bolus, contrast enhancement in L3 was expressed as the ratio of the change in signal-intensity (SI) in L3 ([pre-post] contrast) to the change in SI in the aorta, yielding an estimate of the partition coefficient of Gadodiamide (PC). **[B]** ***NMR Spectroscopy*****:** Two subclasses of AML, U937 and NB4, were grown in MEM_{α} medium, with 10% FBS, 1% P/S and 0.1% Fungizone, and incubated at 37°C for one week. The final cell count of 10^6 cells/mL was achieved in a 20 mL solution, to which 1 mL of PBS-D_2O was added and subjected to pulse-acquire ^{1}H-NMR spectroscopy using a Bruker AV600 instrument. The dwell time and number of points was chosen such that the Fourier Transform of the time domain signal yielded a spectrum approximately 50kHz wide, enabling the visualization of water, fat, and various metabolites, such as choline.

Results: The average PC values for all volunteers are shown with respect to time post-bolus in Fig. 1. When PC was examined according to age, two important differences were observed: (1) contrast wash-in appeared to depend on age, with younger patients showing increased uptake compared with their elders; and (2) contrast wash-out, measured as the slope of PC vs. time after 1 min post-bolus, appeared to increase with age, as evidenced by the increase in the slope. Fig. 2 depicts the NMR spectra obtained for each cell line: clearly methylene (CH_2; 1.3 ppm) containing compounds were in higher concentration in the NB4 line, in approximately two times the concentration of such metabolites in U937. This, in turn, suggests a higher concentration of membrane lipids (e.g. the fatty acyl groups common in cell membranes) indicating morphological differences in the cells themselves.

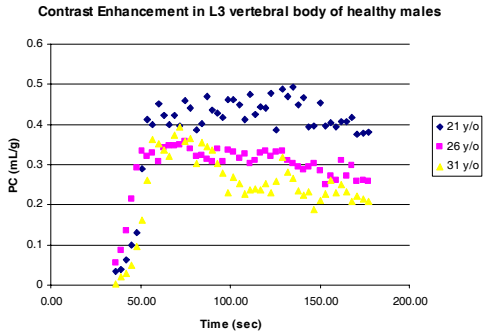

Fig 1: *(above)* The PC of Gadodiamide with respect to time post-bolus for three healthy male volunteers, ages 21, 26 and 31, respectively.

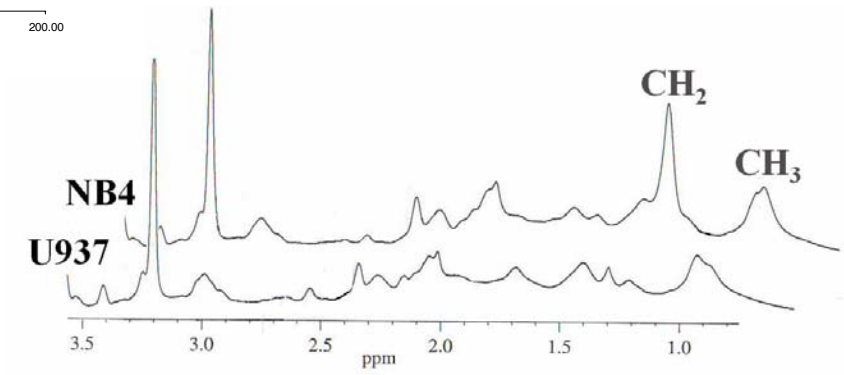

Fig 2: *(right)* ^{1}H-NMR spectra for U937 and NB4 cell lines. The relative peak intensity of CH_2 containing molecules was found to be higher in the NB4 cell line.

Conclusion: The variations in the relative contrast enhancement across different age groups of healthy males may be useful in the diagnosis of leukemia with MRI. There exist two hypotheses: one suggests that as the marrow of leukemia patients becomes increasingly dense due to the overproliferation of undifferentiated cancer cells, the uptake of the gadodiamide and the partition coefficient would decrease with respect to the "normal" standard defined above. The second hypothesis suggests that increased vascularity due to angiogenesis within the marrow will increase contrast leakage and, thus, increase the rate of wash-out. The NMR spectra of the two different cell lines showed evidence of morphological differences in the two subclasses of AML, suggesting that spectroscopy may yield a novel form of classification. Ultimately, the *in vitro*, NMR characterization of leukemic cell lines will be correlated with those obtained *in vivo* via MRS and a fully non-invasive protocol will be developed.

[1] Jayashree *et al.Current Science* (Indian Academy of Sciences) 77(4): 587 (1999).
[2] Montazel JL *et al. Radiology* 229: 703-709 (2003).