34th Annual International Conference of the IEEE EMBS
San Diego, California USA, 28 August - 1 September, 2012

Quantitative Ultrasound Visualization of Cell Death: Emerging Clinical Applications for Detection of Cancer Treatment Response*

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Abstract— **Differentiable echogeneities exhibited by living and dead cells enables the monitoring of cell death response via quantitative ultrasound techniques at high-frequencies and recently at clinical range frequencies. Such capability can be potentially employed to provide rapid and quantitative functional information in real time, and at the patient bedside for evaluating therapy response early following treatment. This paper summarizes backgrounds on quantitative ultrasound visualization of cell death and highlights its potential capabilities for monitoring cancer treatment response, where favorable results have been reported, according to a recent pilot clinical study.**

I. Introduction

Cell death introduces structural changes in the cell's nucleus including nuclear condensation and fragmentation. We have previously demonstrated that nuclear structure is closely linked to ultrasound backscatter properties of cells and tissues for high frequency ultrasound. The changes in nuclear structure associated with cell death hence results in differentiable echogenicities of living cells, necrotic cells and cells dying of programmed cell death or apoptosis. This has been confirmed through several studies conducted, *in vitro*, *in situ*, *ex vivo*, and *in vivo* [1–10].

Ultrasound (US) radiofrequency (RF) signals carry information about tissue echogenicity but until recently have not been readily accessible on commercial ultrasound systems. Since a large number of instrument parameters are involved in a typical ultrasound imaging and data acquisition session, it is difficult to establish a reasonable comparison between imaging data acquired by different standard ultrasound machines, or even by the same machine when different settings are used. Quantitative ultrasound methods have been proposed to address this shortcoming. Quantitative ultrasound analyzes the acquired raw-data before it is envelope detected, log-amplified and processed to form B-mode ultrasound image and employs calibration techniques to provide parameter estimates which are predominantly independent of instrument settings. Such estimates are frequently based on backscatter analysis of RF echoes and include the integrated backscatter, RF envelope statistics, frequency dependence of the backscatter, ultrasound tissue attenuation, and in a broader sense can include elastic properties of tissues, propagation of shear waves in tissues, and other signal classification techniques such as entropy metrics of RF ultrasonic backscatter [11], [12]. Different subsets of these parameters have been utilized in a number of clinically related applications, and particularly for tissue classification purposes, such as differentiating benign versus malignant disease [13–20].

II. Quantitative Ultrasound and Cell Death

The application of quantitative ultrasound techniques for the detection of cell death is a relatively new development [21], [22]. High-frequency (20-60 MHz) quantitative ultrasound parameters have been found in preclinical animal tumour experiments to demonstrate reproducible and statistically significant features in the ultrasound signals that are associated with cell death. The methods are robust and can be applied to detecting and determining the extent of cell death from different anticancer therapies [1], [5], [10]. This is because high-frequency ultrasound is particularly sensitive to the structural changes that cells and tissues undergo during treatment response [3], [7], [9]. Such changes including nuclear condensation and fragmentation frequently result in substantial increases in tissue echogeneity, and consequently cause a large boost in backscatter signal. Other factors such as cell shape may also contribute, but the nuclear changes associated with cell death have been demonstrated to be responsible for the contrast in quantitative ultrasound

* This research has been supported by the Terry Fox Foundation, Canadian Institutes of Health Research (CIHR), and Canadian Breast Cancer Foundation (CBCF).

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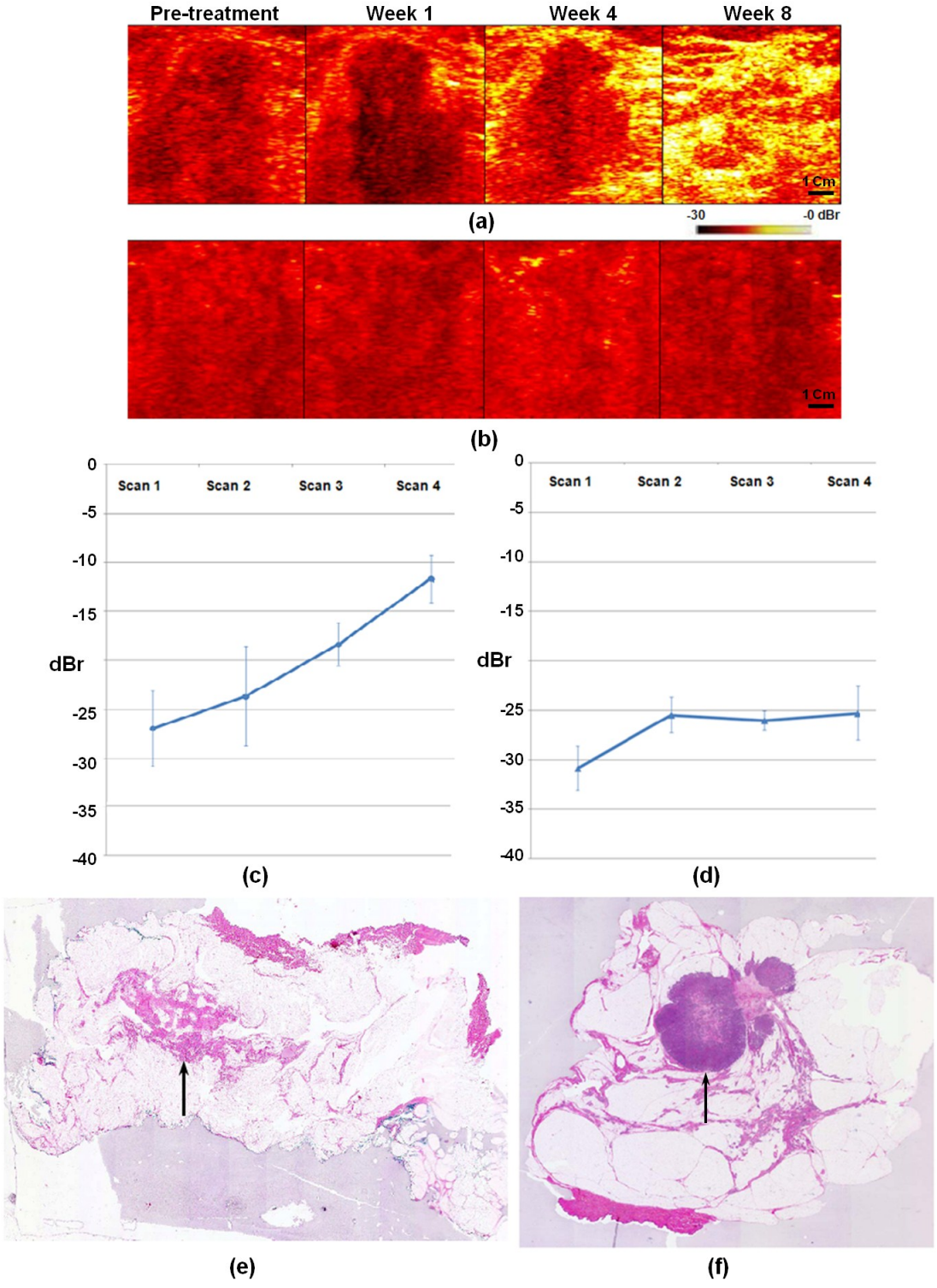

Figure 1: The application of conventional frequency quantitative US for monitoring tumour cell death response. (a), (b): Representative parametric 0-MHz intercept images of a large tumour during neo-adjuvant chemotherapy for a (a): clinically responding patient, (b): clinically non-responding patient. (c), (d): Quantitative 0-MHz intercept data averaged over the tumour area for the (c): clinically responding patient, (d): clinically non-responding patient. Scans 1, 2, 3, and 4 are pre-treatment, week 1, week 4, and week 8 scans, respectively. At scan 3 (4 weeks) of the clinically responding patient an increase in intercept is apparent compared to scan 1 (pre-treatment). In the case of the clinically non-responding patient there is no striking change in the 0-Mhz intercept during the majority of therapy compared to the case of clinically responding patient. (e): The whole mount pathology corresponding to the clinically responding patient indicates a small residual mass in the mastectomy specimen (10 cm wide). (f): The whole mount pathology corresponding to the clinically non-responding patient indicates a large compact residual mass in the mastectomy specimen (8 cm wide).

parameters. However, whereas high-frequency ultrasound provides better lateral and axial resolutions (tens of microns), its clinical application is restricted due to a limited depth of ultrasound penetration [23]. Conventional (low) to mid-range ultrasound frequencies (1-20 MHz) have much deeper penetration and are hence broadly used in medicine, and very recently are being used to monitor cell death response to cancer treatment with quantitative ultrasound methods, as described in section III.

Whereas the detection of tissue changes related to necrosis using ultrasound methods were measured nearly fifty years ago, it is only very recently that quantitative methods have been applied using clinical US frequencies. In a set of recent studies, conventional ultrasound (3 to 10 MHz, -6 dB bandwidth) was used for real time detection of cell death using well controlled AML cell culture experiments. Results demonstrated an ability to detect as little as 10% apoptotic cells using ultrasound frequencies in the 10MHz range, paralleling changes observed using high-frequency ultrasound [1], [22]. Time-course experiments indicate that changes are detectable as early as 6 hours after exposure to chemotherapy drugs. These findings have been confirmed *in vivo* using prostate cancer PC3 tumour xenografts in mice [22], [24]. Here, large macroscopic areas of cell death were induced by novel anti-angiogenic agents in combination with radiation. One may argue that measurable backscatter changes from micron-sized particles are not expected at low-frequencies, mainly due to loss of scattering strength of small scattering structures. However, in the low-to-mid- frequency range, bulk changes in tissue are mostly related to ensembles of cells and nuclei smaller than the wavelength of the ultrasound being used. Such ensembles influence acoustic properties and thus ultrasound backscatter. The potential scatterers are closer in size to those that predominate in the Rayleigh scattering regime, as they are about 10 times smaller than the interrogating wavelength. In addition, when imaging cell samples, even at these low frequencies, a speckle pattern is still formed indicating that many sub-resolution scatterers contribute to the detected signals. Results based on experiments using over 50 animals assessed with high-frequency and conventional frequency ultrasound suggests that the monitoring of treatment efficacy is possible using low-frequency ultrasound.

III. Emerging clinical applications

In a pilot clinical study, quantitative ultrasound at conventional frequencies has been applied for evaluation of tumour cell death response in locally advanced breast cancer patients receiving neo-adjuvant chemotherapy [25]. Conventional 7 to 10 MHz US data were acquired prior to treatment onset and at 4 times during treatment. In each session, several scan planes with the size of 6 by 4 cm were acquired from the same nominal regions. The RF signal's power spectrums were normalized, as before [1], [4], [7], at each region of interest (ROI) using a reference's power spectrum obtained from an agar-embedded glass-bead phantom model, at the same ROI position. The results (n=10 patients) demonstrated a close association between quantitative ultrasound changes after one to two cycles of chemotherapy (weeks) and clinical response in the tumour many months later. More specifically, patients who had a significant clinical response demonstrated changes in quantitative ultrasound parameters consistent with cell death, while women with no changes in quantitative ultrasound parameters demonstrated no ultimate clinical response (Figure 1). The promising results emerging from this study pave the way for establishing protocols for the clinical applications of the conventional frequency quantitative ultrasound techniques in therapy response monitoring. As such, quantitative ultrasound at conventional frequencies is expected to provide rapid and quantitative functional information in real time for evaluating responses to a specific therapy in the near future.

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