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Commentary

Commentary on 'Detection of Tumors with Nuclear Magnetic Resonance Spectroscopy of Plasma' by S. Berger, K.-H. Pflüger, W.A. Etzel, J. Fischer

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SINCE the publication of our first paper [1], a number of groups have attempted to replicate our results. The recent paper of Berger *et al.* [2] is an example of such an attempt. Like several other groups [3–10], Berger *et al.* clearly separate a control group from an untreated cancer group ($p < 0.0017$), despite the fact that they used less than optimal field strength. They obtain an even better separation ($p < 0.000002$) using a more substantial modification of our method. The purpose of this Commentary is to point out the importance of certain aspects of the methodology which many fail to appreciate and to make clear, as we attempted to do in our initial publication, that the method required further evaluation before it could be applied to population screening [1*].

The paper of Berger *et al.* deals directly with one important experimental variable, field strength. Our studies are done at 360 MHz. In Table 1, I summarize results we obtained studying the same 22 samples (11 from persons with non-cancer disease, and 11 from persons with cancer) at five different field strengths. At 250 and 300 MHz the means of the groups are closer together than at 360 MHz and overlap is substantial. At 500 and 600 MHz the means of the groups are farther apart than at 360 MHz and, as at 360 MHz, there is no overlap. Berger *et al.* demonstrated a similar difference comparing a subset of samples at 300 and 600 MHz. However, the majority of their study was based on results at 300 MHz where it is clear substantial overlap occurs due to insufficient field strength.

In addition to field strength, there are several other experimental parameters which are important. An observation temperature of 20–21°C is used in all of our studies. Higher temperatures, even 25°C, reduce the separation between cancer and non-cancer groups and increase overlap. The lipoprotein lipids appear to undergo a phase transition around 20°C [11]. This transition temperature is lowered in the plasma of untreated cancer patients. By making the observation at a temperature between 18 and 21°C maximal differences between groups can be achieved. Table 2 shows the dependence of average linewidth on temperature for a typical normal control and a typical untreated cancer patient.

Other important parameters include good magnetic field homogeneity. Water linewidths before suppression must be 4 Hz or less (at 360 MHz). This may require detuning the receiver coil in order to avoid radiation damping. A well shimmed sample will result in a linewidth of the EDTA resonances in plasma of 2.2 Hz or less. This can be used as an internal quality control standard. The sample itself is of course of great importance. It should be plasma which exhibits no evidence of hemolysis and it should never be frozen. Freezing causes physical changes in lipoprotein structure which are irreversible [12]. Finally, careful review of the patient's medical record is required. Our cancer group (Group II, ref. [1]) are patients with untreated, biopsy proven cancer on whom a blood sample was obtained prior to biopsy. Other cancer patients are

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*p.1377, line 24. 'Large prospective clinical trials including greater numbers of patients in whom cancer is not suspected will be required to determine the value of proton NMR measurements of lipoprotein–lipid line widths for population screening.'

Table 1

Proton frequency	11 untreated cancer (Mean ± SD)	11 non-cancer disease (Mean ± SD)	Δ (Non-cancer–cancer)
250 MHz	25.4 ± 2.2 (Hz)	28.3 ± 2.0 (Hz)	2.9 Hz
300 MHz	26.8 ± 1.8	30.7 ± 1.6	3.9 Hz
360 MHz	30.4 ± 2.3	39.8 ± 2.7	9.4 Hz
500 MHz	36.1 ± 2.9	50.4 ± 6.6	14.3 Hz
600 MHz	41.6 ± 3.7	61.7 ± 7.9	20.1 Hz

Table 2. *Typical temperature dependence of linewidth in an untreated and control sample*

Temperature (°C)	Average linewidth (Hz) Control	Cancer	Δ (Control–cancer)
39	28.5	30.1	−1.6
36	31.3	29.5	1.8
33	32.6	29.7	2.9
30	33.1	29.9	3.2
27	33.5	30.0	3.5
24	34.0	30.3	3.7
21	41.2	31.3	9.9
18	50.5	35.1	15.4
15	53.5	46.0	8.5
5	55.0	52.5	2.5

assigned to Group VI in our study as once treatment is initiated, status is more difficult to evaluate with certainty.

It has become clear from the results of several studies [13, 14] that hypertriglyceridemia is a source of false positive results. It is also clear that levels of hypertriglyceridemia can be significantly different in different populations. While it was not a problem in our control group of hospital personnel [1], it is a substantial problem in other settings. Application of this method in a population with a high level of hypertriglyceridemia clearly requires a method for distinguishing true positives from false positives due to hypertriglyceridemia. We have proposed such a method based on C-13 NMR spectroscopy of plasma [15].

Whether or not NMR spectroscopy of plasma will become a screening method for cancer awaits further extensive studies. The statistical accuracy requirements of a screening test for cancer are even a subject of disagreement. In other settings in medicine, however, a screening test is a test which is less accurate than a diagnostic test. That is, the screening test simply identifies a population which require further attention. Mammography is an appropriate example. An abnormal mammogram often leads to a biopsy. The pathologic result is then diagnostic. In the case of a positive proton NMR blood test in an asymptomatic person, further evaluation would be indicated probably beginning with a plasma triglyceride measurement and perhaps a C-13 spectra to determine the validity of the initial positive result. Before such applications are considered, however, extensive clinical studies will be required.

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