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Commentary

Detection of Malignant Tumors by ^{1}H Nuclear Magnetic Resonance Spectroscopy of Plasma

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(A COMMENT ON: Fossel, ET. Commentary on 'Detection of tumors with nuclear magnetic resonance spectroscopy of plasma' by S. Berger, K.-H. Pflüger, W.A. Etzel, J. Fischer. *Eur J Cancer Clin Oncol* 1989, **25**, 925–927.)

THE Commentary by Fossel [1] describes certain aspects of methodology, including use of optimal magnetic field strength, methods for magnet shimming and careful control of sample temperature, that are considered critical for the detection of cancer by ^{1}H NMR spectroscopic analysis of plasma [2]. According to Fossel [1], reports of difficulties in detecting untreated cancer are due to the failure to use optimal conditions. This is contradicted by the large database of our own consisting of NMR and clinical measurements on plasma from several hundred human volunteers and parallel studies on several dozen rats. We conducted these studies to further investigate methodology and clinical applications of NMR spectroscopy [3] and to address the important health questions raised by Dr Fossel's research. Here, we present for the first time results on one phase of our investigation in which we compare line widths of healthy controls with untreated cancer patients, treated cancer patients and patients with other diseases.

There are several practical difficulties to conducting such investigations. Unlike most other studies in this area, we have gone to considerable effort to duplicate experimental conditions and refine our methodology in order to replicate procedures used by Dr Fossel. This we believe has been achieved since our NMR methodologies were initially refined and tested in a collaborative study in which we evaluated plasma samples selected by Dr Fossel from his patient population at the Beth Israel Hospital in Boston [4]. We had apparent success in observing (on these selected samples) that line widths of 43 Hz or less at 500 MHz separated cancer samples from all others. This compared to a line width of 33 Hz or less for these same cancer samples when measured by Dr Fossel at 360 MHz [1, 2]. The results are unique in that they can serve as a confirmation of our NMR methodology and they enable accurate inter-laboratory comparisons of apparent lipoprotein–lipid line widths to be made. Otherwise, inter-laboratory comparisons of line widths would be unreliable, even at the same field strength, because there are many variables associated with the NMR measurements [5].

The line widths of plasma lipoprotein–lipids are presented as a function of triglyceride concentration (Fig. 1) for samples from the four groups of subjects. The measurements show that there is little distinction between untreated cancer samples and other samples once triglyceride levels are taken into account, even though we used the favorable conditions of 500 MHz and 20°C. Our procedure for fitting the data with a best-fit curve may suggest a trend toward line narrowing for untreated cancer samples compared to healthy controls, but a mean difference in line width of 1.7 Hz, in the triglyceride range of 75–300 mg/dl, is small compared to the scatter of points (Fig. 1).

Comparison of the present results of randomly collected preoperative bloods (Fig. 1) with the col-

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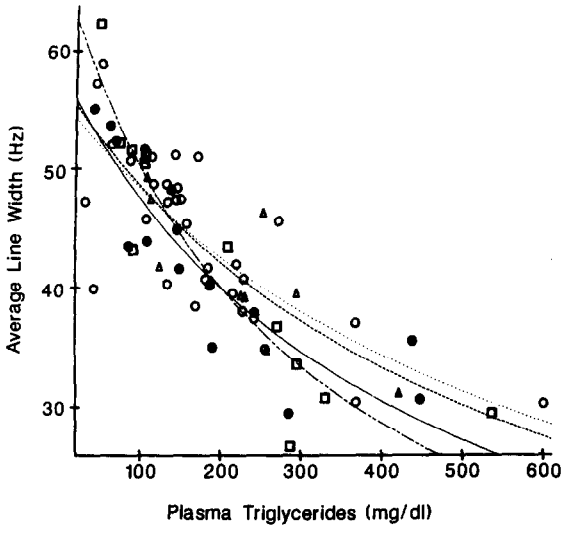

Fig. 1. Average plasma lipoprotein line widths versus triglycerides for the following groups: healthy (●)··· (n = 34); untreated malignancy (○) — (n = 14); treated malignancy (△) --- (n = 10); and assorted other diseases (□) -- (n = 15). The curves are a best-fit of the simple equation $y = 1/(ax + b)$. *Blood samples from untreated cancer patients were obtained prior to biopsy on biopsy-proven cancer samples. Plasma samples contained EDTA and did not show evidence of hemolysis. They were stored at 4°C and never frozen. Samples were shimmed using the* H_2O *free induction decay to a* H_2O *line width of 3.7 Hz or less.* ^1H *NMR measurements at 500 MHz and 20°C include line broadening of 2 HZ.*

lection of bloods selected for examination by Dr Fossel [4] shows that most cancer samples do not exhibit line widths of 43 Hz or less unless the triglyceride levels are higher than 100–200 mg/dl. We have tested some possible variables, such as use of trace levels of several detergents, that might possibly cause selective narrowing of cancer samples, but the narrowing that we found was nonselective (not shown). Most of the samples with line widths that are well below the curves (Fig. 1) have diminished levels of high-density lipoproteins or low-density lipoproteins.

In view of the dependence of line width on triglycerides, reports of a clear separation in means between groups, as described by Fossel [1], do not provide support for the proposed blood test, since they can be explained by differences in triglyceride levels (Fig. 1) [6]. A difference in age between cancer patients and healthy workers who frequently serve as control populations for these studies is one factor that would be expected to effect test results. Other investigators have reported major overlap between groups [6–20]. For example, we estimate, from the results reported by Berger *et al.* at 300 MHz [7], that the false positive and false negative rates are each about 38%. We therefore do not agree with Dr Fossel who stated that Berger *et al.* 'separate a control group from an untreated cancer group' [1]. Even though we believe our procedures have more precision, as evidenced by less scatter in our measurements (Fig. 1) than others, and even though we have used more favorable conditions [1] than earlier studies, we are still unable to detect malignancy by this method.

Several attempts have been made to understand lipoprotein–lipid line widths in terms of additivity of lipid components and by isolation of lipid fractions [5, 9–13, 21]. Unusual spin–spin relaxation (T_2) properties of a glycolipid associated with cancer have also been observed [22]. If ^1H NMR spectra of plasma contain unique information about human health, more research will be needed on experimental conditions and methods of interpretation of data. Although we have serious doubt that it is possible to detect cancer using the procedures described by Fossel *et al.* [1, 2], certain aspects of the methodology could conceivably be valuable for characterizations of ^1H NMR spectra of blood plasma and for the study of human diseases.

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