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Commentary

Monitoring of Patients with Nonseminomatous Testicular Cancer by Nuclear Magnetic Resonance Spectroscopy of Plasma

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(A COMMENT ON: Berger S, Pflüger KH, Etzel WA, Fischer J. Detection of tumours with nuclear magnetic resonance spectroscopy of plasma. *Eur J Cancer Clin Oncol* 1989, **25**, 535–543.)

RECENTLY, it has been suggested that water-suppressed proton nuclear magnetic resonance spectroscopy (NMR) of plasma is a potentially valuable approach to the detection of cancer and the monitoring of therapy [1]. Since then there has been a controversy in the literature about the NMR test and its ability to detect cancer [2–8]. In the March issue of this journal, Berger *et al.* [9] report the results of the NMR plasma test from 284 patients and conclude that even at very high magnetic fields there is a severe overlap between the groups of patients with untreated cancers and normal controls.

In a footnote to this paper Berger *et al.* quote the results of a study that was done in collaboration with us on 203 plasma samples of patients with nonseminomatous testicular cancer and healthy male blood donors. As these results had not been published before and as we had performed a similar study in collaboration with Dr. Fossel [10], we appreciate that we are given the opportunity here to report about the two studies, to compare the results and to let the reader draw the conclusion.

For both studies blood was collected in Vacutainer tubes containing EDTA. Plasma was immediately separated by centrifugation and stored at 6°C for a maximum of 21 days. Samples were randomly assigned an identification number and then were sent within 24 h from the West German Tumor Center either to the Beth Israel Hospital in Boston or in the more recent study to Dr. Berger's laboratory in Marburg. Up to this point there was no difference in drawing blood or plasma handling.

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In the study done in collaboration with Dr. Fossel, plasma of 142 patients with nonseminomatous testicular cancer and 57 healthy male blood donors (C) was examined by NMR. In 54 of the cancer patients (TU) active tumor was confirmed by imaging techniques or surgery at the time blood was drawn for this study. The remaining 88 patients showed no evidence of disease (NED) after successful treatment by surgery and/or chemotherapy. These patients came to the hospital outpatient clinic for regular physical examination. The corresponding specifications of the patients of the study done in collaboration with Dr. Berger were: 56 C; 47 TU; 100 NED. The mean age ($x \pm$ S.D.) of the 400 males was 31 ± 9 years and there were no differences of age between the groups of the two studies.

All proton spectra were obtained with use of Bruker AM Fourier transform spectrometers. In the first study; plasma was analyzed exactly in the manner described by Fossel and coworkers [1, 11] at a resonance frequency of 360 MHz at 20°C. In the second study the temperature was 25°C and magnetic field strength 400 MHz. Special care was taken to optimize magnetic homogeneity, i.e. water linewidth before suppression was <4 Hz. Plasma (0.4 ml) was placed in an NMR tube of 5 mm OD. The water proton NMR signal was presaturated for 6 s before the 90° observation pulse. After signal averaging and Fourier transforming 16 free induction decays, the aliphatic region was plotted and the full width at half the height of the methyl and methylene resonances was measured. The average of these two values in Hz was used as the indicative value. The respective clinical data of patients were

not known to anyone involved with the NMR measurements.

Mean values ± S.D. are given throughout the text. Kruskal–Wallis analysis was used to test the null hypothesis in comparing groups. In the first study, specificity and sensitivity calculations were done defining values ≤30.5 Hz as diagnostic for the presence of active tumor.

Table 1 shows the average methyl and methylene linewidth determined in the two different laboratories for our patients. Whereas the differences between C, NED and TU were statistically significant when determined in Boston, there was no difference between the three groups in the second study. In the first study 49 out of 54 TU patients had values ≤30.5 Hz. The remaining five patients were between 34.2 and 38.3 Hz. So we defined values below or equal to 30.5 Hz as tumor specific. Doing so, we calculated a specificity and sensitivity of the test of 92.4 and 90.7%, respectively. This compares to a specificity of 95.3% and a sensitivity of 24.1% estimated in the same patients for α-fetoprotein and 98.4 and 27.7% for human chorionic gonadotropin, respectively. There were no relapses in the 77 NED patients with normal NMR values, but in four out of 11 NED patients with false positive NMR values a relapse occurred 1–4 months after the test.

In order to avoid the possible influence of age, sex and non-malignant disease, a homogeneous population of patients with nonseminomatous testicular cancer and healthy age-matched males were used. Water suppressed NMR of plasma differentiated well between patients with and without clinical evidence of tumor and healthy controls in the first study. In contrast, the second study yielded no differences between the groups of a very similar patient population although plasma was prepared and handled identically. The only methodological differences were magnetic field strength and observation temperature. From data on magnetic field strength dependence of linewidth [11] one would expect the difference between cancer and non-cancer to be even higher at 400 MHz. On the other hand the different observation temperature would have been one reason for the conflicting results [11].

In summary, for a plasma test to have clinical implications, high reliability as well as reproducibility are prerequisites. In the hands of Dr. Fossel the NMR test yielded reliable results, i.e. patients with active testicular cancer could be separated from NED patients and healthy controls with high specificity and sensitivity. However, these findings could not be reproduced in a different laboratory in a subsequent study.

Table 1. NMR spectroscopy of plasma in patients with nonseminomatous testicular cancer

Patient status	First study		Second study	
	Number of patients	Linewidth (Hz) (Mean ± S.D.)	Number of patients	Linewidth (Hz) (Mean ± S.D.)
Active tumor (TU)	54	29.2 ± 2.8*†	47	31.5 ± 4.2
No evidence of disease (NED)	88	35.8 ± 4.5*‡	100	33.2 ± 4.4
Healthy controls (C)	57	39.1 ± 3.4†‡	56	33.3 ± 5.5

*$P < 0.001$.
†$P < 0.001$.
‡$P < 0.01$.

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