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I Detected My Cancer with My Smart Phone

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If Alexander Graham Bell were to come back to life and see what the telephone looks like today, compared with his own invention, he would definitely be astonished. It may not even be appropriate to call today's devices "telephones." They are multitasking gadgets capable of a myriad of other operations. It is astonishing that the Apple iPhone claims over 350 000 specialized applications (better known as "apps").

On the basis of their multitasking capabilities, many have claimed that "smart phones" could have important applications in medicine, including the automatic transmission and sharing of laboratory data, images, and so forth in real time, for more effective patient care *(1)*. In a recent issue of the journal *Science Translational Medicine*, Haun et al. described a micro–nuclear magnetic resonance (micro-NMR)[5] device for the rapid molecular analysis of human tumor samples *(2)*. In the editor's summary, the title was modified to read "A Micro-NMR Smart Phone for Detecting Cancer." Unfortunately, the editor, in his effort to draw more attention, portrayed the smart phone as an integral part of this futuristic diagnostic device. In this case, however, the smart phone was only a minor player that merely controlled the NMR device, an operation that could probably be performed more conveniently with a remote control or a button on the NMR unit. Nevertheless, we describe this pioneering technology in an effort to realistically evaluate its usefulness and performance as the technology stands today. It is common in the diagnostic and biomarker field for advances like this one to be oversold and for overly optimistic views to be expressed regarding their clinical utility. The phenomenon of declining interest in published reports over time has become known as the "decline effect" *(3)*. Some examples of oversold and subsequently failed cancer biomarkers have recently been discussed *(4)*. Therefore, let's see how this technology works and how it performs in real-world applications.

For patients with suspected intra-abdominal malignancies, fine-needle aspirates were collected by conventional techniques, and the samples were placed in tubes containing saline. After centrifugation and resuspension, the cells were treated to undergo measurements of either extracellular or intracellular antigens according to their respective protocols. Monoclonal antibodies against the targets of interest (the authors of this report quantified 9 different candidate proteins) were labeled with TCO [(*E*)-cyclooct-4-enyl 2,5-dioxopyrrolidin-1-yl carbonate] and reacted with the cells. After washing away excess antibody, Haun et al. reacted the cells with magnetic nanoparticles conjugated to Tz [2,5-dioxopyrrolidin-1-yl 5-(4-(1,2,4,5-tetrazin-3-yl) benzylamino)-5-oxopentanoate]. The cells were washed again and introduced into the micro-NMR device for signal generation. The antigen concentration is directly related to the magnitude of the NMR signal.

The NMR system used in this study is a third-generation instrument and highly portable, with a footprint of only 10 × 10 cm. Microfluidic devices are incorporated into the NMR system for multichannel, multiparametric analysis of various proteins.

The premise of these investigators was that the 9 proteins analyzed (selected from literature reports describing their overexpression in cancer compared with healthy tissues) can be used to separate cancer from noncancer tissues. The authors reported their best clinical results for a panel of 4 protein biomarkers: MUC-1 (mucin 1, cell surface associated), HER2 [also known as ERBB2: v-erb-b2 erythroblastic leukemia viral oncogene homolog 2, neuro/glioblastoma derived oncogene homolog (avian)], EGFR (epidermal growth factor receptor), and EpCAM (epithelial cell adhesion molecule). We focus on these markers in our further discussion of this invention.

The initial analysis was performed with 50 patients, 44 with malignant lesions and 6 with benign lesions. The 4-marker combination correctly classified all 44 malignant lesions (sensitivity, 100%) and 4 of 6 benign lesions (specificity, 67%), for an overall accuracy of 48 (96%) of 50 samples. To verify these findings with an independent sample set, they analyzed 20 ad-

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[5] Nonstandard abbreviations: NMR, nuclear magnetic resonance; TCO, (*E*)-cyclooct-4-enyl 2,5-dioxopyrrolidin-1-yl carbonate; Tz, 2,5-dioxopyrrolidin-1-yl 5-(4-(1,2,4,5-tetrazin-3-yl)benzylamino)-5-oxopentanoate; MUC-1, mucin 1, cell surface associated; HER2, also known as ERBB2: v-erb-b2 erythroblastic leukemia viral oncogene homolog 2, neuro/glioblastoma derived oncogene homolog (avian); EGFR, epidermal growth factor receptor; EpCAM, epithelial cell adhesion molecule.

ditional samples, of which 14 were malignant and 6 were benign. They correctly classified all 20 samples (100% accuracy). The authors concluded that their method exceeded the standard of care that used conventional cytology and histology analyses, which had overall accuracies of 74% and 84%, respectively. Notably, the authors described 1 patient sample that cytology and core biopsy analyses had deemed to contain only inflammatory cells but that the micro-NMR analysis unequivocally classified as malignant. The patient was found to have metastasis 2 months later.

Taken at face value, these data are very impressive and demonstrate that this micro-NMR technology provides faster, better, and likely cheaper point-of-care data for differentiating malignant from nonmalignant lesions, compared with the cytologic and histochemical techniques, which are slower, require more sample, and are likely more expensive.

The authors also presented some other data that were somewhat peripheral to their major findings but were clearly important. These data included their finding that repeated biopsy sampling along an identical coaxial needle path produces considerable heterogeneity (30% or greater), whereas aspirating samples from different regions of the same tumor yields an even greater variability (on the order of 90%). They further report considerable decreases in marker expression over time, with mean losses of about 100% within 1 h and about 400% at 3 h, a finding that emphasizes the necessity of either immediate analysis or elaborate preservation procedures immediately after sample retrieval.

Several aspects of this technology could moderate enthusiasm for it until more data are generated and more validation studies are published. First, the authors rightly compared their micro-NMR quantitative measurement of the biomarkers with measurements by conventional ELISA, fluorescence-activated cell sorting, and immunohistochemistry (their Fig. 2). Given the highly different operational principles of micro-NMR and these other techniques, we would expect a modest correlation (r^2 values of approximately 0.7–0.8). We were surprised that much higher correlation coefficients were observed (r^2 values of 0.99 with ELISA, 0.98 with fluorescence-activated cell sorting, and 0.93 with immunohistochemistry. To contrast these correlations with others that we usually encounter in clinical chemistry, we mention a recent comparison of 2 ELISA methods for vitamin D measurement (one by Diasorin and one by Immunodiagnostic Systems), which produced an r^2 value of 0.72. A comparison of these 2 methods with a reference method based on liquid chromatography–tandem mass spectrometry produced r^2 values not exceeding 0.87 (our unpublished data). Another surprising aspect described in the report of Haun et al. was the reproducibility of micro-NMR measurements for the same sample. The reported CVs were $<0.6\%$ overall and $<0.3\%$ for intracellular markers. Practicing clinical chemists will recognize that such reproducibilities are attained rarely, if ever, even for analytes requiring extreme precision (such as calcium measurements).

A crucial piece of information was inadequately described in the report. The authors pointed out that there is great variability between cell types in their biopsies, which typically contain, on average, approximately 30% leukocytes; however, the variability of both leukocyte and nonleukocyte cell populations in such biopsies was exceedingly high (see their Fig. 6, right panel). Because the authors reportedly analyzed approximately 200 cells on average, it is not clear whether the measured biomarkers were expressed per cell (as indicated in their Fig. 6) or per nonleukocyte cell (the latter cells are presumably cancer cells). If the measurements were expressed per cell, irrespective of the type of cell, then an excessive amount of leukocytes in the biopsies likely would have underestimated the amount of biomarkers measured per cell (assuming that leukocytes do not produce significant amounts of these biomarkers), whereas the measurement of these biomarkers in nonleukocyte cells would necessitate the enumeration of leukocytes and nonleukocyte cells in every clinical sample.

Irrespective of the above concerns, we believe that the authors' clinical validation of this device has a major limitation. The authors' original set included 44 malignant lesions and 6 nonmalignant lesions (see their Table 1). The authors identified 4 of the 6 lesions as nonmalignant and 2 as malignant. The extremely small number of samples in the nonmalignant group ($n = 6$) makes the calculation of specificity and accuracy less reliable. One wonders why the authors did not include more nonmalignant lesions in their evaluation. The same comment applies to their independent test set, which included samples from 14 patients with malignant lesions and 6 with nonmalignant lesions. The number of samples tested, especially in the nonmalignant group, is so small that any conclusions regarding specificity or accuracy are highly speculative.

Some aspects of this technology are quite impressive, however, including the size, portability, speed, multianalyte capability, and control of the device with a smart phone. The authors are optimistic that this technology could be used for many other medical applications, such as biopsies of other tissues and analysis of peripheral blood for rare cancer cells or microvesicles such as exosomes. We have concerns, however, that some of the reported analytical data (such as correlation with conventional techniques and precision) seem unattainable under routine testing conditions and that the clinical validation of the assay has been quite lim-

ited because of the exceedingly small number of tested samples, especially in the nonmalignant groups. The ultimate judge of this and similar technologies will be time, as well as independent validations by other groups in real clinical settings. Given the recent disappointments with many cancer biomarkers that failed validation *(4, 5)*, we caution the readers of *Clinical Chemistry* to reserve judgment on such advances, even if published in top-rated journals, until further independent testing is performed and published.

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References

1. Lippi G, Plebani M. Laboratory applications for smartphones: risk or opportunity? Clin Biochem 2011;44:273–4.
2. Haun JB, Castro CM, Wang R, Peterson VM, Marinelli BS, Lee H, et al. Micro-NMR for rapid molecular analysis of human tumor samples. Sci Transl Med 2011;3:71ra16.
3. Schooler J. Unpublished results hide the decline effect. Nature 2011;470:437.
4. Diamandis EP. Cancer biomarkers: Can we turn recent failures into success? J Natl Cancer Inst 2010;102:1462–7.
5. Cramer DW, Bast RC Jr, Berg CD, Diamandis EP, Godwin AK, Hartge P, et al. Ovarian cancer biomarker performance in prostate, lung, colorectal, and ovarian cancer screening trial specimens. Cancer Prev Res (Phila) 2011;4:365–74.