In our validation of Cellscan, we found only 50% of true positive results. In fact, we screened 88 women with benign breast lesion, 207 women with breast carcinoma, and 325 healthy donors, and positivity was 50% among breast cancer patients, 34% among women affected by benign disease, and 27% and 22%, respectively, among healthy female and male controls. In conclusion, it is mandatory to revaluate critically the diagnostic criteria applied so as to avoid the risk of misclassifying a subject.

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Response from Rahmani and Associates

H. Rahmani, M. Deutsch, I. Ron, S. Gerbat, R. Tirosh, A. Weinreb, S. Chaitchik and S. Lalchuk

The Jerome Schottenstein Cellscan Centre for Early Detection of Cancer, Department of Physics, Bar Ilan University, Ramat Gan, 59200, Israel

WE HAVE carefully read the letter from Birindelli and associates and feel that this indicates a lack of understanding of the SCM phenomenon and its measurement.

Firstly, intracellular fluorescein fluorescence polarisation (IFFP) was the measured parameter, and not membrane electropolarisation, nor membrane properties, as indicated by Birindelli and associates.

The authors state that basal (control) value determination is mandatory and that the use of phytohaemagglutinin (PHA) is superfluous. Being consistent with this approach leaves only 3 out of 6 cases in Table 1.

Variability in IFFP between different cells on the same cell carrier is natural and expected (see Figure 3b of Ref. [1]). This is irrelevant to performing repetitive measurement on a number of cell carriers in order to evaluate the IFFP mean of means [1].

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Secondly, the coefficient of variation (CV) of basal measurements over a number of cell carriers was approximately 2% in our study [1]. Thus, a 5% ($\backsim 2\sigma$) deviation from the mean is significant. This figure applies to incubations with and without PHA, encephalitogenic factor (EF) and tumour antigen extract (TAE) and was firmly based upon a total error analysis including machine performance, blood drawing, separation, stimulation, staining, etc. Hence, we do not see how and why natural IFFP variations lead Birindelli and associates to conclude that "...it is hazardous to confirm or to exclude the diagnosis of tumour by the simple ratio of $P_{EF}/P_{PHA} >$ or < 1".

Changes in IFFP following incubation with PHA are determined to a large extent by the different cell populations. The original Cercek separation procedure [2], defined as mode (b) in [1], yields two bands, I and II. In healthy controls, band-I cells respond by decreasing IFFP upon incubation with PHA, but not with EF or TAE. The opposite occurs in cancer patients. This has been confirmed by others [3].

Pritchard and associates [4] showed that when the 'band-II cells' are challenged with PHA, IFFP decreases in patients, but not in healthy controls, in contrast to the band-I cells' behaviour. Incubation with EF or TAE does not induce a decrease of IFFP in band-II cells.

Birindelli and associates have used mode (a) [1] of cell separation. With this procedure, one cell layer is obtained which contains both bands. This yields a positive PHA reaction in healthy controls as well as cancer patients. Hence, the decrease in IFFP following PHA or TAE will be influenced by the relative representation of each band in the measured sample.

The population ratio band-I:band-II is approximately 3:1—both having the same $IFFP_{basal}$ and intensity. Therefore, healthy controls and cancer patients having an RR_{SCM} of 1.11 and 0.90 (obtained from band-I alone), would have yielded approximate RR_{SCM} values of 1.08 and 0.925, respectively, had mode (a) separation been employed. Thus, a correct mode (a) separation would still enable the use of the yes/no RR_{SCM} general parameter—an advantage especially for one lacking a good proven TAE. Yet, incorrect separation might result in a change in the band-I:band-II ratio, thus yielding biased RR_{SCM} values. We offered our criteria [1] in order to deal with these possibilities.

Birindelli and associates suggest giving up the use of PHA, which "at most, yields some information on the immunological status of the patient". This statement ignores findings indicating that lymphocyte stimulation by PHA and other lectins is reduced in patients with cancer, and has even been proposed as a prognostic parameter for breast cancer [5].

Thirdly, the two diagnostic criteria suggested by Birindelli and associates are seemingly simple mathematical expressions. Nevertheless, in spite of all our efforts, we failed to see the logic behind them. In fact, they are two pairs of complementary inequalities, and apart from the missing brackets around

$$1 - \frac{\text{basal}}{\text{BrAg}}$$

they are erroneous. Mathematically speaking, if one of the expressions is greater than a given number (5%), then its

complementary must be less. If data are applied to these formulae, the result is that a patient can be sick and healthy concurrently.

In response to Birindelli and associates' analysis of Table 1: Case 1 is not positive since

$$RR_{SCM} = \frac{0.200}{0.205} = 0.98$$

which represents a change in IFFP of less than 5%. We would define this as "no decision" (and run it again); case 2 is positive since $RR_{SCM} > 1$ and IFFP decreased by at least 5% in response to TAE; in case 3,

$$RR_{SCM} = \frac{0.231}{0.215} = 1.074$$

and the change in IFFP due to TAE is less than 5%, suggesting a negative result; case 4 is false positive by our criteria; case 5 is positive since $RR_{SCM} = 1.028$ is in the no decision range, but the decrease in IFFP in response to TAE is greater than 5%; case 6 is negative since $RR_{SCM} = 1.15$.

Finally Birindelli and associates' results show a ratio of less than 2 between positive findings, among patients and those among healthy controls (50%/27%). Since these results are based mainly on $IFFP_{basal}$ and $IFFP_{BrAg}$ measurements, a serious doubt is cast as to the validity of the antigens they used, and their ability to differentiate between healthy controls and cancer patients. Birindelli and associates did not use our own TAE preparations. Based on our experience, purification and calibration procedures should be carried out with great care since these influence the performance of TAE which is greatly affected by its molecular characteristics and concentration. Therefore, when a new TAE is introduced, calculating the sensitivity and specificity per antigen or even per time period, defined according to the overall system performance, is recommended.

In conclusion, considering problems stemming from cell heterogeneity, we are now exploiting the Cellscan to analyse individual and subpopulations of cells in order to optimise measurement of the SCM phenomenon. Special software and hardware tools have been developed in our laboratory for this purpose. These tools are now in use.

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Non-steroidal Anti-inflammatory Drugs in the Treatment of Colorectal Cancer

G. Morgan

Iechyd Morgannwg Health, 41 High Street, Swansea SA1 1LT, U.K.

GOOD EVIDENCE indicates that the regular consumption of non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin reduces the risk of fatal colorectal cancer [1]. The potential of NSAIDs in the treatment of colorectal cancer has received little attention. The purpose of this letter is to highlight this important possibility.

Pharmacologically, NSAIDs inhibit the cyclo-oxygenase (COX) enzyme leading to the reduced synthesis of prostaglandins (PGs). PGs such as PGE_2 are produced in excessive amounts by colorectal cancers and appear to contribute to a number of deleterious pathological effects [2]. These include maintenance of cancer blood flow, immunosuppression, cachexia and metastatic potential. Given these properties, the use of NSAIDs in the treatment of colorectal cancer is logical. Two clinical studies have addressed this possibility.

In 1982, Lipton and associates [3] reported a randomised trial with 66 colorectal cancer patients. Aspirin was given to reduce platelet aggregation in an attempt to prevent haematogenous cancer spread. For patients randomised to receive aspirin, 600 mg twice daily for 2 years did not prevent metastatic spread. Despite this rationale, however, this study is seriously flawed since the dose of aspirin given was too high for platelet inhibition. Furthermore, the degree of PG inhibition that can be achieved with this dose of aspirin is, at best, modest. More positively, in 1995 Preston and associates [4] noted that ibuprofen (400 mg three times daily for 3 days) attenuated accelerated whole-body protein kinetics in 7 colorectal cancer patients. Although this study implicates PGs as mediators of cachexia, it remains to be seen whether the long-term administration of ibuprofen alters the survival rate in patients with malignant disease.

More recently, an inducible isoform of COX, COX-2, has been implicated as producing excessive PGE_2 in colorectal cancers [5]. Additionally, NSAIDs have been shown to induce apoptosis (programmed cell death) in colorectal cancer cell lines [6]. Further clinical studies with NSAIDs in

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