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Letters

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Letter to the Editor:

Comments on *Adaptation of the Cellscan Technique for the SCM Test in Breast Cancer* Rahmani *et al., Eur. J. Cancer*, 32A, No. 10, pp. 1758–1765 1996

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WE READ with interest the article by H. Rahmani and associates in the September issue of the *European Journal of Cancer* [1]. We would like to comment on both the methodological approach and the diagnostic criteria applied to define positive and negative patients, having ourselves used the Cellscan instrument (from April 1993 to May 1995) in order to validate this method for the early detection of breast cancer [2].

On the basis of this experience, we would like to debate the questionable decision of using non-specific stimulants for the detection of breast cancer, rather than a specific breast antigen or a tumour associated antigen (TAE).

Cellscan is an instrument which can detect a variation of membrane electropolarisation in stimulated lymphocytes. Since we exploit this peculiarity, in our opinion, the only appropriate candidate antigen is a TAE and no other non-specific stimulant which at the most might yield some information on the immunological status of patients. Moreover, the determination of the basal value of the lymphocyte polarisation of each patient is mandatory, and may be obtained by incubating a lymphocyte suspension for the *same* time and at the *same* temperature of specifically stimulated lymphocytes. For this purpose, we tested the reliability of the Cellscan for measurement of the basal value with and without the conditions specified. Also, because of the fluctuality of electropolarisation values on the same grid, it is advisable to perform an average of at least three Cellscan measurements for each stimulation. Therefore, it is hazardous to confirm or exclude the diagnosis of tumour by the simple ratio $P_{EF}/P_{PHA} >$ or < 1. We rather suggest the following criteria to distinguish positive and negative cases:

$$1 - \frac{\text{basal}}{\text{BrAg}} \times 100 > 5\% \text{ or no stimulation: } \textit{negative}$$

$$1 - \frac{\text{BrAg}}{\text{basal}} \times 100 > 5\%: \textit{positive.}$$

Table 1. Examples of the Cellscan results

Number	Basal value	PHA	EF	BrAg	Diagnosis
1	0.213	0.205	0.200	0.214	Healthy
2	0.241	0.219	0.228	0.216	Breast cancer
3	0.214	0.215	0.231	0.211	Healthy
4		0.194	0.178	0.186	Healthy
5		0.211	0.217	0.206	Breast cancer
6		0.222	0.256	0.246	Benign lesion

In Table 1, we report the various results achieved in 6 of our cases by applying Rahmani's and our criteria, respectively.

Case number 1 was a healthy control who would be positive according to the RR_{SCM} ratio. In contrast, according to our calculation, the person would be negative since there is no difference between basal value and BrAg. In case number 2 the difference between BrAg and the basal value is sufficient to confirm the positivity of the sample.

Case number 3 is problematic: she would be negative for the first step of the RR_{SCM} ratio, and positive according to the second step, i.e. BrAg stimulation. However, the BrAg and basal value are almost the same, and our interpretation is a negative diagnosis. In the last three examples, in which the basal value is not available, the decision criteria should be:

$$1 - \frac{\text{EF or PHA}}{\text{BrAg}} \times 100 > 5\% \text{ or no stimulation: } \textit{negative}$$

$$1 - \frac{\text{BrAg}}{\text{EF or PHA}} \times 100 > 5\%: \textit{positive.}$$

In fact, in all 3 cases, the diagnosis of positive or negative is determined by a strong difference (>5%) between the specific stimulant and the control value, rather than the RR_{SCM} ratio $>$ or < 1, according to which extremely low differences aquire a highly significant meaning. Otherwise, in the case of a balance between stimulants, the logical conclusion is that TAE has the same effect as any other antigen and the subject is therefore negative.

Finally, it is mandatory to verify both the vitality and the concentration of the lymphocytes before stimulations and scanning so as to avoid misinterpretations due to membrane alterations.

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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.

1. Rahmani H, Deutsch M, Ron I, *et al.* Adaptation of the Cellscan technique for the scan test in breast cancer. *Eur. J. Cancer* 1996, **32A**, 1758–1765.
2. Birindelli S, Colnaghi MI, Pilotti S. New SCM (structuredness of the cytoplasmatic matrix)-based approach in breast cancer detection. *Tumor* 1996, **83**, 550–553.

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Response from Rahmani and Associates

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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