DNA flow cytometry of breast cancer fine needle aspirates

Sir – There are a number of points which I believe should be raised with regard to the article by Mullen and Miller (1989) on DNA flow cytometry of breast cancer fine needle aspirates. First, I would point out that the authors have omitted to reference previous contributions on the same topic (Spyratos et al., 1987; Remvikos et al., 1988a; Briffod et al., 1989). Second, as a cytometrist, I believe that data file histograms should be presented (rather than redrawn ones) as these usually contain extensive information on the quality of the preparations (baseline, noise, skewed peaks, aggregates). Third, the method of calculation of S-phase values should always be stated.

The most important weakness of the article is the definition of the variations on the histograms. For instance, a modification of more than 5% in DNA index is considered significant, where the standard deviation inherent in the method of calculation (internal standard) is 4%. From a statistical point of view, a threshold of at least twice the standard deviation should be used, i.e. 8%. Incidentally, the committee on nomenclature of flow cytometry published its recommendations in 1984 (Hiddeman et al., 1984), stipulating that animal cells should definitely not be used as calibration standards. To my knowledge, these recommendations have been recognised by the Society of Analytical Cytology and are followed by most cytometrists. As for the modifications of cell populations, this is a very delicate point that should be treated with much caution. A variation in the proportion of the diploid cells could first of all be due to the presence of a variable contingent of normal cells (lymphoid infiltrate for example). Therefore, a precise score of the normal cells must be established on control smears before a threshold of 10% for a substantial difference between duplicate analyses can be validated. Breast cancers are heterogeneous. This has been shown by Auer et al. (1980), but also by our comparison of FCM and cytogenetics for the same tumour (Remvikos et al., 1988b), with sometimes a paraploid and an aneuploid clone being simultaneously present. Variations in the proportions of the different cell populations in repeated samplings are therefore to be expected.

A final point I wish to make concerns the usefulness of iterative FNA in the assessment of response to treatment. By analogy with in vitro experiments, modifications in the cell cycle distribution are primarily to be expected (for instance accumulation of cells in S or G2M). This was shown in the pioneering work of Vindelov et al. (1982) and has been confirmed for breast cancers (Spyratos et al., 1988; Remvikos et al., 1988c).

DNA flow cytometry (FCM) is a promising method in clinical oncology, in particular because of the possibility of measuring DNA index and S-phase fractions (Kalionemi et al., 1988). It was recently shown that DNA-ploidy and S-phase fraction are significantly related to response to cytotoxic chemotherapy (Briffod et al., 1989; Remvikos et al., 1989). I believe that the article by Mullen and Miller can only bring unjustified confusion to the minds of clinicians who may be interested in FCM.

Yours etc.,

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Response to the letter from Dr Remvikos

Sir – The letter from Dr Remvikos makes a number of criticisms of our paper (Mullen & Miller, 1989) which we feel are worth defending. With regard to the originality of the article and the possible omission of other work (Spyratos et al., 1987; Remvikos et al., 1988; Briffod et al., 1989), we feel our work is indeed original. Both Spyroats and Remvikos, while commenting on the clinical importance of sequential FNAs for flow cytometric DNA analysis, do not provide any data pertaining to reproducibility and variability of such serial FNAs, although both studies, like our own, do provide data comparing FNAs with surgically excised biopsies. The third study (Briffod et al., 1989) was not in print when our manuscript was accepted for publication, hence its exclusion (along with Remvikos et al., 1989).
In response to the comments relating to the way in which we presented the DNA histogram data, we felt that diagrammatic representations were the clearest way of illustrating the similarities/differences exhibited. Had the article been published in a more cytometrically orientated journal, we may well have used the raw histograms. Debris was not a problem since most samples yielded good quality histograms. S-phase values were calculated using the para-1 software supplied by Coulter for use with the EPICS-C.

Defining the degree of abnormality for the S-phase fraction is, as Dr Remvikos states, a controversial issue – indeed we spent considerable time choosing such an arbitrary value. However, providing this value is kept constant for all analysis, results should remain valid. Chicken red blood cells (CRBC) were used as an internal standard in this study since, using CRBC, a batch preparation may give a reproducible estimation of the DI of the diploid peak (0.997 ± 0.039). The DI of aneuploid populations was calculated from the diploid peak channel and not the CRBC channel. Despite recent recommendations (Hiddeman et al., 1984), other studies continue to use only CRBCs (Abandowitz et al., 1987; Askens ten et al., 1988; Dressler et al., 1988; Stål et al., 1989).

Concerning the modifications of cell populations which are seen to occur, we have clearly stated in the text that changes may be due to (a) the proportion of tumour to non-tumour cells in the aspirate, and (b) heterogeneity. To ‘score’ FNAs for the number of ‘normal’ cells before DNA analysis may be a valid criticism although this clearly applies to all FCM analysis, regardless of source material. We fully agree with Dr Remvikos in that variations in the proportions of different cell populations in repeated samples are to be expected due to heterogeneity (Auer et al., 1980; Kallioniemi et al., 1988); indeed that is why serial FNAs may be so susceptible to inter-sample variation.

While Dr Remvikos regards variations observed in serial breast tumour FNAs as being analogous to in vitro experiments, it is important to remember that studies involving cell lines are concerned with single homogenous populations and are therefore intrinsically less likely to show significant variations. The heterogeneous nature of breast tumours can only increase the inter-sample variations.

We do not underestimate the potential of FNAs in yielding important prognostic information about breast cancer. However, we would wish to emphasise the potential pitfalls of interpreting such sequential aspirates. While it is true that sequential treatments may be associated with marked differences in the DNA profile from breast cancer cells, our data clearly show that similar changes may be observed following either no treatment or clinical failure to treatment. It would therefore be irresponsible to suggest that changes in the DNA content of FNAs represent an accurate method of assessing clinical response. However, with the advent of better markers of cellular proliferation and regression, this technology may well become an invaluable tool.

Yours etc.,

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