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The design of the study seems to comply to modern standards, the authors used fresh material; even cytokeratin labeling, the study was restricted to invasive ductal carcinomas.

I was nevertheless amazed by the fact that in their original population, only ductal and lobular carcinomas occurred, no other specific histological type being described.

In their series no quality control concerning the samples processed, seems to have been performed (100% of results in a population of 240 patients). In our experience such a quality control (cytologic or histologic control of the remaining tissue after flow cytometric processing) excludes roughly 10% of the samples. There is neither any precision concerning quality control of the histograms or SPF data, how much, if any, histograms were discarded because of too few events, a too large CV, too much debris and so on . . . , such a control would also discard about 20% of the samples.

The authors declare p. 142 that their study, I quote “is one of the largest prospectively performed study”, this is definitely wrong, numerous studies have included more patients; a study by Wenger et al. included over 127 000 patients [1].

The mere fact that in their study, ploidy and S phase were significant prognostic factors in monovariate analysis, but not in multivariate analysis, does not allow them to state that flow cytometry is of no interest for clinicians. This can also be construed as the fact that their study did not include enough patients to evidence the prognostic impact after multivariate analysis.

In their discussion the authors acknowledge that “In most studies, S phase fraction was detected as significant prognostic factor” but without any further review of the pertaining literature. Several lines later they state that only “one large study of Camplejohn et al. [2] with a long follow-up showed the prognostic impact of SPF in a multivariate analysis”.

As we [3–6] and Wenger [7] have previously shown, the literature is rather clear, when focused on studies using fresh or frozen material including at least 100 patients. DNA ploidy showed a prognostic impact in 17 out of 23 studies, 8 of them after multivariate analysis. SPF was correlated with prognosis in 21 out of 24 studies, after multivariate analysis in 17 of them. These results are a strong argument against the use of paraffin embedded material with its well-known drawbacks. The numerous studies published with this technique have shown such discrepant conclusions concerning the clinical interest of FCM, that several oncology panels, performing an analysis of the whole literature, have refused to endorse the clinical use of FCM data. So we found it rather paradoxical that Wimberger et al. who used fresh material, cite only a study using paraffin-embedded material [2].

In a node negative population of 600 patients [4], as well as in a further study including 1850 patients [6], we could show that SPF alone or combined to mitotic activity is a salient prognostic factor. Lymph node status only had a more important prognostic impact. The low proliferative subgroup in node negative patients allowed us to evidence a population of about 50% experiencing a very low percentage of carcinologic events (3% with a median follow-up of 55 months and about 10% of patients submitted to chemotherapy). In addition, the proliferative activity remained a potent prognostic factor in the whole and node positive population.

In a study limited to 104 breast carcinomas drawn from this large population [5] ploidy showed no relationship with survival or any other clinicopathological prognostic factor. But in the whole population it was a strong univariate prognostic factor, this data being only shown as an example, that statistical data must be considered with care, when the population studied does not include enough patients.
We are rather sure that SPF is a very important prognostic indicator which should be used in daily oncology practice, we think that Wimberger et al. should have been more cautious concerning their conclusions which are based on a limited study with no relevant review of the literature.

References


J.J. Michels

Dear J.J. Michels,

We thank you very much for your comment addressing the problems in establishing reliable DNA data and drawing scientific and clinical conclusions, demonstrating high competence and experience in the field of flow-cytometric DNA analysis. First of all we have to apologize that we obviously missed to review your results from Caen published in 2000 (Refs [1] and [2]). As outlined we do agree with respect to the statement that DNA-ploidy and S-phase fraction do have prognostic significance in breast cancer; however, we disagree concerning its clinical significance for decision making. Based on nearly two decades of experience in diagnostics and therapy in breast cancer patients we are convinced that determination of DNA-ploidy and S-phase fraction by flow-cytometry even if methodologically optimized may only marginally contribute to all other known prognostic parameters and its not the key for a new dimension of prognostic characterization of this disease. Following actual therapeutic guidelines for adjuvant therapy in breast cancer no change of therapeutic strategy can be based due to assessment of DNA-ploidy or S-phase fraction. To our opinion no clinical impact – apart from scientific interest – can be provided by determination of these parameters in clinical routine.

Concerning our work, in fact, we concentrated on invasive ductal carcinomas; however your impression, that there has been no quality control with respect to the samples processed is definitely wrong, on the contrary! First of all, inclusion criterium for our prospective analysis was analysable DNA-ploidy and thus resulting in 100% analysable samples with respect to this parameter. Each sample was prepared by an experienced pathologist from the same material used for fresh frozen section and therefore confirming diagnosis of breast cancer. In addition a second specimen of the analysed sample was frozen and analysed in case of DNA-diploid tumor, to confirm a second time the presence of malignant tumor tissue in the sample. Following tissue dissociation each cell suspension was additionally analysed for tumor cell viability cytologically. Concerning determination of S-phase fraction we defined exclusion criteria for histogram analysis as described in Kimmig et al. 2001 [3], where methodological aspects of our work are described in detail as mentioned in material and methods of the presented paper. Due to our rigid exclusion criteria we had in fact 55% of the samples which had no reliably analyzable S-phase fractions in the total collective which could be reduced by cytokeratin labelling to 39%. For comparison, in ovarian, endometrial and cervical cancer the range of non analyzable samples was between 10 and 24%. That fits well with the 20% of the samples in described in your collective. As we described in the cited paper: quality control was one of the most important issues of our work.

Concerning the prognostic significance of DNA-ploidy and S-phase fraction we definitely did not state that there is no significance at all. On the contrary, we stated that “the prognostic significance of DNA-ploidy and S-phase fraction in univariate analysis is evident and plausible in view of molecular biology”. Due to different published results concerning multi-
variate analyses and our own data however we doubt that there is a relevant additional impact of DNA parameters compared to known prognostic parameters. However we stated in addition “even when prognostic significance could be demonstrated in larger trials the expected survival advantage of the prognostic better groups would be too small to justify changes of therapeutic strategy”. In the last sentence of our conclusions we stated that the parameters should be determined to investigate scientific aspects concerning predictive significance for estimation of therapy success in case of adjuvant chemo- or hormone therapy; therefore we share the opinion there may be some potential of clinical significance with this respect and we underline your statement that DNA analysis should be exclusively done in fresh tissue.

Furthermore, we are very interested in your papers being in press and hope on the basis of your papers that we can intensify our discussion and cooperation of our two institutions in the future.

References


With best regards
R. Kimmig, M.D.
and for the authors
Dr. P. Winberger