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Comments and Critique

Proliferation Markers in Breast Cancer

THE ARTICLE by Gaglia and colleagues [1] provides an opportunity to comment on an area of current interest, the clinical use of biological indicators. The criteria proposed by McGuire [2] provide the basis for using biological staging as a potential alternative or as a complement to conventional pathological stage for prognostic and therapeutic purposes.

The prognostic relevance of biological markers should be assessed in patients receiving local-regional therapy alone in order to avoid the confounding effect of successive systemic treatments on subclinical metastases. The results also need to be verified by the reproducibility of findings reported by different centres.

These two requirements have been met only partially for Ki67 since the prognostic relevance of this proliferation index has been investigated in breast cancer patients heterogeneous for stage [3–8]. An overview of available reports has shown controversial results even from studies dealing with patients homogeneous for stage, i.e. node-negative [3, 6–8] or node-positive tumours [3, 6]. The paper by Gaglia and colleagues [1] fails to clarify the prognostic value of the Ki67 index, since not only node-positive but also about 80% of node-negative patients were treated with tamoxifen—and we know that slowly proliferating tumours, which are probably oestrogen receptor positive (ER+), can benefit from endocrine treatment [9–11]. Node-positive patients were treated with endocrine and cytotoxic therapies. The similar distribution of slowly and rapidly proliferating tumours in patients receiving these two systemic regimens, (which is unexpected considering the retrospective nature of the study), fails to resolve this crucial point, since available data show that slowly and rapidly proliferating tumours can be differently influenced by the two forms of systemic treatment [9–14].

An extensive analysis of node-negative tumours submitted to local-regional therapy is needed to evaluate biological variables and cell kinetic indices. Such a verification has been performed for flow-cytometric S-phase cell fraction (FCM-S) [15–18] and [^{3}H] thymidine labelling index (^{3}H-dT LI) [19–23]. In the paper by Gaglia and colleagues [1] the median bromodeoxyuridine labelling index (BrdU LI) was double the median ^{3}H-dT LI reported in several previous studies [21–24]. Similarly, Ki67 needs to be more carefully evaluated bearing in mind the nature of its relationship with proliferating cells or the persistence of the antigen in G_0 cells [25]. Methodological aspects need to be more thoroughly investigated to permit comparison of the results from different studies. The lack of quality control is reflected by the wide variability of the median values of Ki67 index reported in the different studies. They range from 4 to 22% [4–7, 26–30], and the median value of the present study would be ranked as medium–low. It is generally believed that the median value of each centre can be used as a standard cutoff, regardless of intercentre variability. However, there are doubts relating to the variability not only among median values from different centres but also to the relationship between Ki67 and other reference biological and clinical factors. For example, it is difficult to accept that in the same series of patients the two cell kinetic variables, which should indicate the S-phase cell fraction (BrdU LI) or the cell growth fraction (Ki67), have similar values which are closer than would be expected.

Moreover, the results reported in Dr. Gaglia's paper indicate a weak relationship between Ki67 and BrdU LI and no congruency of results from the clinical analysis of the two cell kinetic variables. These results suggest that the two cell kinetic variables do not have the same biological significance and that Ki67 is a more important predictor of clinical outcome than BrdU LI. However, these statements are relevant only if derived from studies on node-negative tumours treated with local-regional therapy alone, since the clinical outcome in patients treated with systemic therapy results from the potential biological aggressiveness and the impact of treatment on tumour progression. The two cell kinetic variables could provide different information on prognosis and response to treatment.

In conclusion, the search for biological indicators of prognosis and response to clinical treatment needs to be pursued. However, there is a requirement for quality control programmes [31] and series of patients of adequate size and duration of follow-up (depending on the known clinical history of the tumour), which, for resectable breast cancer, should be at least 5 years.

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In vitro Assays for Antitumour Activity: More Pitfalls to Come?

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CHOOSING A GOOD screening system to search for antitumour activity of chemicals is not a trivial problem. It is even more complicated if, in addition to screening for antitumour activity the objective is also to predict tumour response in the clinic. Until a few years ago, the search for new agents relied on tests that made use of mouse leukaemia cells and a limited number of mouse and human xenotransplants. These were mostly fast growing cells and led to the discovery of some active agents. However, these models might not adequately resemble the biology of slow growing human tumours and thus might produce false negative results. Indeed, the results were unsatisfactory for the development of clinically active drugs against common forms of human cancer, particularly lung, breast and colon cancer [1]. The screening of the vast number of available chemicals could only be expanded if there was a fast, simple, sensitive, reproducible and in expensive assay that could be automated. The MTT assay was most promising and was adapted for large scale screening [2–5]. With all the publicity associated with the assay it is important that we remind ourselves of its limitations, in particular that it does not provide a direct measure of cell growth.

In this issue of the *European Journal of Cancer*, a study by Pagliacci and coworkers addresses the limitations of the MTT

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