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Perspectives and Commentaries

Counting and Sampling Errors: (Mis)interpretation of Data from Tritiated Thymidine Labelled Human Tumors

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(A COMMENT ON: Lambert M. Tritiated thymidine labelling *in vitro* of human cancer of the breast: counting error and sampling error. *Eur J Cancer Clin Oncol* 1986, **22**, 781–785.).

IN A recent paper [1], M. Lambert attempted to demonstrate that there is substantial counting and sampling error in tritiated thymidine labelling indices (TLI's) derived from samples of cancers of the human breast. We take issue with 3 different aspects of his study: (a) the material used in his analyses, (b) the presentation of raw data and statistical method employed, and (c) the main conclusions of the paper.

As regards the material available, first, it comes as no surprise that there was indeed sampling error (or sampling differences) between specimens taken at random from the same tumor. This fact reflects the well-known heterogeneity in cell populations of malignant tumors. Heterogeneity may be due to the disorganized vascularization of the tumor [2], to aging of tumor cells (the malignant potential being reduced in older cells), or to the possible presence of more than one cell line within the same tumor [3]. The variability of TLI may also be due to the labelling techniques themselves. With *in vitro* labelling techniques, only a few cell layers in the vicinity of the surfaces in contact with the medium containing the labelled material adequately incorporate it. Hence, the TLI rapidly decreases beyond these cell layers. The intensity of the labelling may also be increased spuriously by the presence of polyploid cells which probably play a minor role in tumor growth and in its proliferating capacity. Thus it is difficult to interpret the alleged 'sampling error' if these structural causes of heterogeneity are not taken into account. However, in our experience, it is possible to identify areas of rather homogeneous TLI in spite of the global heterogeneity within the same tumor. These areas might correspond to different cell populations, each with its own proliferating capacity. We agree with Lambert that it is important to measure and possibly to report the TLI in several different areas, but it is equally important to define in advance which of these measurements will eventually be used in further analyses. In our studies of colorectal cancers [4], we decided somewhat arbitrarily to use only the highest TLI that could be measured reproducibly in each tumor. The rationale for so doing is that the highest TLI is likely to correspond to the area in which proliferation is fastest, and which may therefore be most responsible for the overall tumor growth.

As regards the presentation of raw data and the statistical method used to analyze them, it is misleading to perform 22 statistical tests with the 22 repeated counts obtained from the same slide, and conclude that there is substantial counting error if a few of them turn out to be significant at the 0.05 level (Table 1 of Lambert's paper). As a matter of fact, only 2 out of these 22 tests were 'significant' at the 0.05 level, which is no more than can reasonably be attributed to chance alone. If one insisted on making an overall inference from these 22 tests, one would have to lower the level

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of each test to approximately 0.0025 in order to preserve the overall level of significance of 0.05 chosen by the author [5]. None of the individual tests comes even close to the 0.0025 significance level, so that Table 1 shows excellent agreement between the TLI's determined from 2 independent counts upon the same slide, not substantial counting error as said by Lambert.

The same remarks hold true for the data and analysis of Table 2, though in this case there is indeed substantial evidence of heterogeneity between different specimens of the same tumor (subject to the reservations made above as to how these specimens were selected). What is more striking in Table 2 than this 'sampling error', however, is the fact that the TLI's vary much more between different tumors than between 2 specimens of the same tumor. The largest difference between 2 specimens of the same tumor is less than 4-fold, while the largest difference between the TLI's of different tumors is almost 40-fold, an order of magnitude larger. We suggest that the recourse to inadequate *P*-values has seriously distorted what the data themselves showed so blatantly, and may have misled the reader into excessive pessimism about the value of TLI measurements. To make our point clear, we wish to draw an analogy with a simple situation: to state that there is heterogeneity of TLI within a tumor is analogous to stating that students in a classroom do not all get the same grades at their exams. That observation is of no interest by itself. What may be of interest, though, is to study the differences in grades between classrooms (granted that there are also differences within each classroom), a question that falls within the realm of analysis of variance [5]. Similarly, what is of interest in the situation at hand is to examine and test differences in TLI's between 'groups' of tumor appropriately defined. We have used this approach to compare the TLI's of colorectal mucosas taken from 46 patients affected by various digestive pathologies (inflammatory bowel diseases, adenomas, adenocarcinomas). Our results showed that there were highly significant statistical differences in TLI's between these various pathologies in spite of substantial variability in TLI within each pathology [4]. In conclusion, the heterogeneities discussed by Lambert do not preclude carefully taken TLI measurements to show strong correlations with different clinical states. The truly interesting question is whether TLI can act, at least in some diseases, as an indicator of the disease process and therefore, hopefully, as a prognostic factor.

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