COMMENTARY

SERUM TUMOUR MARKERS: IMPLICATIONS OF DISCORDANT RESULTS

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Since the topic of tumour marker assays was last reviewed in Disease Markers (Roulston, 1991; Ward, 1991) their use has become even more widespread and the need for close attention to the principles of quality control has become even more apparent.

Tumour marker immunoassay kits from different manufacturers can yield discordant results for both patient and control specimens. Such discrepancies have been reported for a number of tumour markers including carcinoembryonic antigen (CEA) (Bormer, 1991), alphafetoprotein (AFP) (Kelsten et al., 1988), human chorionic gonadotropin (hCG) (Cole et al., 1992), and prostate specific antigen (PSA) (Graves, 1993). Differences among assays have major implications for the use of tumour marker assays in assessing patients with malignancy. To provide results which can be interpreted accurately, same-method testing is necessary for serial tests in an individual patient. Assay discordance makes it impossible to compare patient results from laboratories using different methods. As a given laboratory changes immunoassay methods, new baseline results must be established for individual patients.

REFERENCE ANTIGEN PREPARATIONS AND THEIR LIMITATIONS

Tumour markers usually are measured by immunoassays, often using one or more monoclonal antibodies in the reaction. Although differences in standardization account for some differences in immunoassay results, they cannot explain all of the discrepancies seen. Even when assays are standardized using the same material, large differences in results still are seen in patient and control results. For example, use of the World Health Organization's International Reference Preparation 1st IRP 73/601 as a common standard for CEA would actually increase differences between some assays (Bormer, 1991). Discordant results have a number of causes, but nonequimolar antibody reactivity is probably the major cause. Nonequimolar reactivity refers to the same concentration of analyte showing different degrees of reactivity with the antibody used in a particular immunoassay kit. Large assay-dependent differences in results occur because of variation in antibody reactivity with various forms of analyte present in the specimen to be tested. Typically, the tumour markers are glycoproteins which can exist in serum in a variety of forms and it is this physico-chemical heterogeneity that accounts for some of the differences in reactivity with assay antibodies. Thus tumour marker kits from different manufacturers should not be used interchangeably, because the antibody source is the important factor in determining final assay results.

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Additionally, the kit used for testing should be identified on the laboratory report form in order to avoid confusion in interpreting test results (Aziz et al., 1993).

REACTION IN KINETICS, ANTIBODY SPECIFICITIES AND COMPLEX INTERACTIONS

Discordant results caused by nonequimolar reactivity of various analyte forms can occur by a variety of mechanisms. Nonequimolar reactivity in different immunoassays can be caused by assay configuration. That is, results can differ for various assays even though the antibodies used are the same: the kinetics of the immunoreaction, such as occur with variations in incubation time, can cause the differences in results. For example, prostate specific antigen (PSA) assays with short incubation times may capture the free form of PSA in higher proportion than the form complexed to \( \alpha_1 \)-antichymotrypsin and thus different results occur depending on the percentage of free and bound PSA present in the specimen (Graves, 1993). In contrast, the assay antibody itself can be the cause of nonequimolar reactivity. One or both the antibodies used in an immunoassay can have different affinities for one or more epitopes on the analyte in question. When dealing with tumour markers, this may lead to complicated interactions, because the markers often have multiple epitopes and may share epitopes with other molecules. For example, monoclonal CEA antibodies recognise different CEA epitopes and over a dozen CEA-related macromolecules have been identified. These related antigens show variable degrees of crossreactivity with CEA, presumably because CEA shares epitopes with other members of the family group (Bormer, 1993).

VARIATIONS IN THE ANALYTE

Various forms of the analyte including proforms and degradation products may or may not react with the antibodies used in a given assay thus leading to non-equimolar reactions. For example, some total hCG (hCG plus free beta subject) assays seriously underestimate or overestimate free hCG beta-subunits (Thomas et al., 1989). Additionally, differences in amino acid composition between proforms, degradation products and the ‘native state’ presumably can lead to secondary conformational changes in the peptide chain and thus to further differences in antibody reactivity. Some hCG assays, for example, fail to recognise nicked hCG (Cole et al., 1992). For those tumour markers that bind to protein, further complications arise because an epitope may be partially or wholly masked by the binding to protein. An example is PSA, which when added to serum, forms stable complexes with several proteins including \( \alpha_2 \)-macroglobulin. The \( \alpha_2 \)-macroglobulin appears to encapsulate PSA causing loss of immunoreactivity (Lilja et al., 1991).

CONCLUSIONS

Nonequimolar reaction in itself is not necessarily good or bad: an assay must be judged on its clinical performance. The phenomenon of nonequimolar reactivity, however, must be taken into account interpreting results from tumour marker assays. In using tumour marker immunoassays, care must be taken to use the same assay in following a given patient. Thus as changes in assays must be made, new baseline results must be established
for each patient being followed. Additionally, because differences in tumour marker form or crossreacting material may occur only in a minority of patients, particular care must be exercised when judging comparability of immunoassays. Although control results may reflect patient results only poorly, control data can be helpful in assessing the presence or degree of nonequimolar reactivity. For example, significant differences among PSA assays are demonstrated using control material that contain only free PSA (Howanitz, 1993), and large difference in CEA control results have been reported depending on the source of the CEA (Klee et al., 1987).

Laboratory staff have a number of responsibilities in helping their clinical colleagues when using tumour marker assay. Good practice should include labeling assay results with the kit or reagents used and providing assistance in obtaining new baseline results when assay kits are changed. Moreover, laboratory staff should work to ensure interassay precision and consistent results over time. Ideally, a stable standard material could be used to assess assay drift over the period that a particular assay is used. Unfortunately, when more than one form of analyte occurs and in proportions which vary among patient specimens, it becomes impossible to have a meaningful standard.

Assay limitations plus clinical problems such as large intraindividual variation can make interpretation of tumour marker results different. In spite of these problems, tumour marker assays continue to be one of the most cost-effective and reliable ways of obtaining objective data for following patients with a variety of malignancies.

REFERENCES


