Commentary

The challenge of measuring circulating estradiol at low concentrations

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See related Commentary by Dowsett and Folkerd: http://breast-cancer-research.com/content/7/1/1

Abstract

Demand for measuring estradiol at low concentrations is increasing, and the widely used 'direct' radioimmunoassays that do not require a preliminary organic purification step may be inadequate in patient care because of their limited accuracy. In observational epidemiology, however, the main concern is to obtain a correct ranking of individuals' hormone concentration relative to the true level (as determined through a 'gold standard'). Despite differences in the absolute scale of measured and true concentrations, correct ranking will permit calculation of unbiased estimates of hormone–disease associations. In prospective studies, the major concerns are the limited volume of often irreplaceable specimens and the need to perform a large number of assays within a reasonable period of time. Organic purification is often not feasible because of sample volume requirements and logistic difficulties, and so the development of accurate, rapid and inexpensive methods to measure sex steroids at low concentrations would represent a valuable new research tool for both clinicians and epidemiologists.

A timely commentary by Dowsett and Folkerd [1] in the January issue of this journal directs attention to the shortcomings of commonly used direct immunoassays for estradiol, which are especially problematic when the analyte's concentrations are very low [2]. Until recently, analyses of estradiol at low concentrations would have been highly unusual, given that the assays were intended mostly for assessment of reproductive function in premenopausal women. The situation has greatly changed, however. In clinical oncology the demand for low-level determinations has been fueled by the introduction and ever growing use of aromatase inhibitors. In research, this need has been stimulated by the publication of a series of prospective cohort studies [3,4] offering consistent evidence that breast and endometrial cancer are associated with measurable, differential elevations in the extremely low circulating estrogen concentrations that are typical of older, postmenopausal women. Bearing in mind the prospect of increasing demand for measurements of estradiol and other sex steroids at low concentrations, the nature and extent of the technical difficulties connected with performing such determinations should be carefully considered and addressed.

There is no question that in clinical practice the use of rapid and inexpensive immunoassays that skip the costly and time-consuming purification step is hard to justify, given the likelihood of inaccuracies in measurements, which may in turn lead to erroneous and possibly harmful clinical decisions. If one considers the technical issues that are involved in measuring estradiol at low concentrations, including competing binding, cross-reactivity, and matrix effects, it is difficult to dispute the validity of Dowsett and Folkerd's warnings. In short, it seems unjustified and perhaps bordering on the irresponsible to base clinical decisions in patients receiving treatment with aromatase inhibitors, or tamoxifen, on estradiol assays that do not include an organic extraction step.

Although it is unquestionable that clinical practice demands high degrees of accuracy that could not be met with direct immunoassays, a distinction must be made between the requirements of patient care and those of observational epidemiologic research. Indeed, epidemiologists working in the area of endogenous hormones and cancer are keenly aware of the limitations of direct assays and have long attempted to address and clarify the problems connected with their use [5–7]. Key prerequisites for the selection of laboratory assays for epidemiologic studies are adequate reproducibility and accurate ranking of individuals according to long-term concentrations of the circulating compound of interest [6]. As for sex steroids, direct assays have been found to be sufficiently reproducible for use in epidemiologic research, despite the existence of considerable inter-laboratory variations [2,6,7]. The correlations between measurements obtained by direct and indirect radioimmunoassays

and between indirect radioimmunoassays and mass spectrometry, taken as the 'gold standard', have also been reasonably high, at least when direct assays have been carefully chosen [6,7]. In a validation study in post-menopausal women from the New York University Women's Health Study [6], only two out of the five tested direct kits for measurement of estradiol exhibited good correlations with the indirect assays.

However, the standardization of the absolute scale of assays remains problematic, and it is evident that direct assays consistently overestimate estradiol or other sex steroid concentrations. Nevertheless, what matters the most in epidemiologic research is the measurement's relative ranking, which allows the computation of reliable estimates of relative risk. The results of the Endogenous Hormones and Breast Cancer Collaborative Group study [3], which pooled together the data from nine prospective studies, offer no indication that the magnitude of the associations between concentrations of several sex steroid hormones (including estradiol) and breast cancer risk in postmenopausal women differed markedly according to the direct or extraction laboratory methods used. Although these results are reassuring, further confirmation is necessary.

Reliability and validation assessments of the performance of direct immunoassays before initiating large-scale studies of hormone concentrations in precious biologic specimen should be available in all epidemiologic studies. Correct scaling of the measurements enhances the comparability of results between studies and facilitates pooled analyses of findings, which is a natural step forward in our efforts to understand hormonal effects on disease risk and is the most efficient way to combine data resources and scientific expertise. Furthermore, accurate estradiol concentrations, in combination with sex hormone binding globulin measurements, may be used to calculate the bioavailable fraction of estradiol [6,8,9].

In observational epidemiology, two major issues come into play, which are almost completely irrelevant in clinical practice. The first concerns the limited quantity of available analyte. For example, because hormonal determinations must be based on samples drawn well in advance of cancer diagnosis, all of the studies included in the Endogenous Hormones and Breast Cancer Collaborative Group [3] – extensively cited by Dowsett and Folkerd [1] – were case–control studies nested in prospective cohorts. The common characteristic of this type of study is that they rely on serum or plasma samples stored for lengthy periods that are usually available in small, finite volumes. Although some of the studies were able to use organic extraction, in normal circumstances the need to preserve highly valuable, irreplaceable biologic material dictates that the smallest amount of analyte possible be used. We believe that, at least until new methods become available, such a task is best achieved through carefully chosen direct immunoassays. However, it could be argued that when multiple steroid assays are to be performed, the same test material could be used for the organic extraction and chromatographic separation steps, thus reducing differences in volume requirements with direct assays.

A second, equally important issue is that extraction assays tend to be substantially labour intensive and create nontrivial logistical complications in research using large numbers of individuals. An advantage of direct immunoassays is their relative simplicity, which makes them amenable to automation and further reductions in time and costs [6]. A fast automated analysis allows samples to be thawed and analyzed for several hormones within a single day, which, in the case of studies involving quantification of a battery of analytes from various hormone groups, will reduce the necessity for repeated thawing of samples and/or preparation of multiple small volume aliquots, which might increase losses and affect sample quality.

In conclusion, epidemiologists and laboratory scientists should continue working together to improve techniques and strategies that will limit the effect of measurement and laboratory errors. High priority should be given to improvement in the accuracy of direct assays and the sample volume and labour requirements of extraction assays. Of course, it goes without saying that assays that are grossly unreliable would lead to excessive misclassification and misleading results. The effect of other sources of measurement error, such as those related to short-, medium- and long-term physiologic variations in hormone concentrations, sample handling and storage conditions, should not be forgotten and adequately accounted for.

In the case of estradiol and postmenopausal breast cancer, because of the limits imposed by the small volume of analyte usually available in prospective cohort studies and other logistical constraints, epidemiologists have tended to favour the use of direct assays with the understanding that such methods, although not perfect, will provide a reasonably good estimate of the underlying true concentrations. In spite of the possibility that the true risk associated with estrogen exposure might have been somewhat underestimated as a result of measurement error, the substantial impact that the Endogenous Hormones and Breast Cancer Collaborative Group's observations had on our understanding of the role of estradiol in breast cancer speaks for itself.

Competing interests

The author(s) declare that they have no competing interests.

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