February 10, 1992

THE FUTURE OF TUMOR MARKERS

Noel R. Rose, MD, PhD

DR. SCHWARTZ: We've spoken about the present, and now we'd like to try to delve into the future a bit. It's a great pleasure for me to introduce Dr. Noel Rose. Dr. Rose is Professor and Chairman of the Department of Immunology and Infectious Disease of the Johns Hopkins University School of Hygiene and Public Health. Dr. Rose will discuss the Future of Tumor Markers. Noel.

DR. ROSE: Thank you, ladies and gentlemen. I want to thank my friend Mort Schwartz for giving me such an "easy" topic: The Future of Tumor Markers. I was enthusiastic until I heard that they were going to tape this session, and somebody might be able to play it back five years from now.

The best way of predicting the future is to look critically and searchingly at the past. This morning I plan to go through a case history of the tumor marker that I personally have been most personally concerned with, prostatic acid phosphatase or PAP. I'd like to describe the rise and the fall of the use of PAP as a tumor marker and then see what kinds of lessons for the future we can derive from the case history. I shall first list the possible applications of tumor markers and then conclude with some discussion of the problems, real and potential, or applying them in the clinical setting.

Possible Applications of Tumor Markers

- Early diagnosis
- More precise diagnosis
- Monitoring of treatment or recurrence
- Evaluation of host immune response

Applications of tumor markers have been well discussed by Drs. Herberman and Schwartz already. I shall just highlight four major areas: Detection, a more precise diagnosis, monitoring of treatment and recurrence, and, perhaps the most exciting, speculative evaluation of host immune response. The latter topic is a bit beyond the conventional applications of tumor marker themselves, but I think may come to fruition in future years.

Let us return to each of these possible applications at the end of our discussion.

My involvement with tumor markers started about the time that the "war on cancer" was declared by President Nixon in the early 1970's. Like many other basic scientists, I was asked to serve on one of the site-oriented cancer panels. I ended up on a "working cadre" that was devoted to prostate cancer and was asked, with a group of colleagues, to review the existing knowledge of prostatic cancer. We were then to project ways of improving the diagnosis, treatment, and perhaps even prevention of this group of cancers. We agreed that the first step was to improve diagnosis, since this was one tumor that was accessible to surgical treatment if detected early, before spread occurred.

It was well known that in patients with prostatic cancer there was an elevated level of a particular enzyme in the blood, acid phosphatase. Acid phosphatase is produced by the prostatic epithelial cells, and is liberated in large amounts when malignant change occurs. Presumably, this elevation occurs because of a loss in the orientation of the prostatic cells. Rather than being secreted into seminal fluid, the enzyme enters the blood stream.

Although an elevation of acid phosphatase is characteristic of prostatic cancer, it also occurs in other diseases, because acid phosphatase is not specific for the prostatic cell. In fact most tissues have acid phosphatases. Numerous efforts have been made to see if the prostatic enzyme differs in some way from acid phosphatase present in bone, white blood cells, liver, kidney, etc. In fact, previous bio-chemical studies did suggest that there is a prostate-specific isoform of the enzyme. The form of acid phosphatase that was inhibited by tartrate was more characteristic of prostate, but still not specific enough so the test was of maximal value.

I thought that there might be an immunochemical difference between the prostatic form of acid phosphatase and acid phosphatases produced elsewhere. With a research associate, Vita Milisaukas, we were able to show that we could produce antibodies in rabbits that reacted specifically to the prostatic form of acid phosphatase and did not react with the acid phosphatases derived from other tissue sources. This reagent seemed
to meet the requirements for a useful biochemical test for a tumor marker. We then developed a very simple immunochemical test. It was called rocket immunoelectrophoresis. We took samples of patient's serum or urine, and electrophoresed them into an agar gel containing the antibody. The distance that the material migrated depended upon the concentration of acid phosphatase. We could then detect the acid phosphatase by doing an enzymatic stain in the agar gel, since by good luck the antibody did not interfere with the enzymatic activity of the acid phosphatase. By measuring how far migration had occurred, we could quantify the amount of acid phosphatase in the samples.

We published this method in 1972 in Clinical Chemistry, "Immunochemical Quantification of Prostatic Phosphatase." It turned out that normal individuals did not have detectable levels of acid phosphatase in their serum or urine using this simple immunochemical test, whereas about 50 percent of patients with various stages of prostatic cancer did have positive reactions. We then had a simple test that very nicely distinguished normal individuals from about half of the patients with prostatic cancer. It obviously was not sufficiently sensitive for general use. The same rabbit antiserum to PAP was labelled with fluorescein and used to stain biopsy specimens. If we tested a number of different cells for the presence of acid phosphatase using a histochemical method, acid phosphatase was present in many tissues and cells, but using immunofluorescence, which depends on the specific antibody, we found that acid phosphatase is present only in prostatic tissue. Positive reactions were seen with tissue from patients with benign prostatic hypertrophy or prostatic cancer. So, antibodies could distinguish the prostatic form of acid phosphatase from the acid phosphatase derived from other sources at the level of single cells. We will return to this point later.

Ouchterlony tests with this antiserum showed that this acid phosphatase was present in prostatic tissue, seminal fluid, and ejaculate, and not present in other cells or fluids. So it appeared that this rabbit antigen might serve as a guide for isolating PAP. With Dr. B.K. Choe, we attempted to purify the prostatic form of acid phosphatase and to develop a more sensitive immunochemical test. Using purified prostatic acid phosphatase characterized biochemically we determined the molecular weight and the substrate specificity of the enzyme. We had gotten down to a single component with a molecular weight of about 102,000. Because the very simple rocket immunoelectrophoresis was not sensitive enough for clinical application, we developed a double antibody immunoenzyme assay. With it, we could detect low levels of prostatic acid phosphatase in serum of normal individuals and measure slightly more in individuals with benign prostatic hypertrophy, and progressively more with various stages of prostatic cancer. Since about 15 percent of men with benign prostatic hypertrophy (BPH) had positive reactions, we arbitrarily adjusted the cutoff point so that BPH patients were all negative. At that degree of sensitivity, about 33 percent of men with Stage one prostatic cancer were positive, whereas in Stages two and three about 60 and 70 percent were positive. Patients with Stage four metastatic disease had over 90 percent positive reactions.

A number of other investigators took up this test. Tests for PAP were advocated as screening tests, which is something we had never envisioned. We had thought of the tests for early diagnosis and monitoring. Commercial companies started putting out something they called a "male PAP test." For a short time, there was fantastic enthusiasm for it. Of course, the "crash" occurred when it was realized that this assay was not suitable as a screening test. The presence of acid phosphatase in the serum in low amounts is normal, and only increased amounts may be used diagnostically. Like most of these tests, it does not discriminate well enough to screen populations. It may have some value in evaluating individuals with clinical evidence of prostatic cancer or in following patients after surgery.

There are cases where a PAP test of bone marrow may be useful in making a clinical decision based on the presence of metastasis. An interesting extension was the use of immuno-fluorescent methodology itself. That allowed us to do histological and cytological studies and find where PAP is actually being produced.

That information put us on another track. With Dr. E. Pontes, we hit upon the idea that this technique, using a fluorescent-labeled antiserum to PAP, could be useful in staging the disease, and knowing where metastasis has occurred. By looking at bone marrow aspirates, one could see nests of metastatic cells in smears of bone marrow. The antiserum did not react with the form of acid phosphatase that is normally found in bone marrow. The antiserum did not react with the form of acid phosphatase that is normally found in bone marrow. The antiserum did not react with the form of acid phosphatase that is normally found in bone marrow. The antiserum did not react with the form of acid phosphatase that is normally found in bone marrow. The antiserum did not react with the form of acid phosphatase that is normally found in bone marrow. This immuno-fluorescent method became a useful test for metastasis to bone marrow.

Then an unexpected finding occurred. We encountered a patient who had an extremely high level of "prostatic acid phosphatase." The urologists explored him very thoroughly and found that he had no prostatic cancer at all! What he did have was pancreatic cancer. Yet,
immunochemically the acid phosphatase in this patient's serum was identical to the prostatic form of acid phosphatase. We then remembered that inappropriate expression of cell-specific antigens can occur in malignancy. Inappropriate expression of antigens is a characteristic of many types of malignant cells. When the patient died, we were able to get tumor tissue and actually isolate acid phosphatase from the liver metastases. It proved to be identical in every respect to the prostatic form of acid phosphatase.

So-called tumor-specific, or tumor-associated, antigens may be inappropriately expressed by many types of cancer cells. We looked at a number of different kinds of tumors, and we found examples of two breast cancer patients who had elevations of prostatic acid phosphatase. In the strict sense of the term, these are not "false positives," but are immunochemically correct reactions. However, they may not correspond with the particular tumor that we had in mind.

That finding led us into our final study, and leads us to the futuristic part of the talk. We proceeded to fragment the PAP molecule. There are two ways to break down the molecule. One is to break it chemically through enzymatic treatment, or with oxidation and reduction. The other is to clone the gene encoding PAP and express parts of the gene. With Dr. Choe, we tried both strategies, and were able to determine that there are a number of antigenic sites on the molecule. We were able to localize the three major antigenic sites in different parts of the molecule. At the same time we produced monoclonal antibodies to PAP in mice. These antibodies were highly targeted in their action and reacted with different parts of the molecule. Had we gone on with this work, then we would have tried to sort out the individual determinants on PAP, in order to identify the parts of the molecule that are most characteristic for the prostatic form. That would give us, we think, a much more specific reaction. Moreover, if we eliminate the shared parts of the molecule, we can push the sensitivity of the test to a much greater level.

That's the story of rise and fall of the prostatic acid phosphatase. I think it exemplifies what happens in the study of tumor markers. In a broader context investigations of tumor markers go through several maturational steps. The four ages in the development of tumor markers is shown in the following slide.

(Insert slide)

Four Ages in the Development of Tumor Markers

1. Age of discovery
2. Age of enthusiasm
3. Age of disillusion
4. Age of reality testing

First there is an age of discovery, which may extend over a period of many years. It often begins with someone finding an antigen that seems to be specific for a particular type of tumor. Most of these antigens turn out to be re-expressed fetal antigens or embryonic antigens. Other antigens turn out to be those that are characteristic of all rapidly proliferating cells, and still other antigens are characteristic of cells that have a metabolic shift to the anaerobic metabolism. Another pathway to discovery leads to antigens that are unique for certain types of cells. When tumors arise, cell-specific antigens are sometimes overexpressed. Prostatic acid phosphatase is such an example. Most of the efforts to look for tumor-specific markers have ended with some kind of tumor-associated antigen.

This first step of discovery may take some years of basic research and results in the demonstration that a certain antigen seems to be present in tumors and not in the equivalent normal tissue. That apparent difference gives rise to the next stage, the age of enthusiasm, when the discovery looks like a new tumor marker. At this stage we'll find article upon article comparing patients with tumors of various types. The test may be commercialized. In practice, other ill patients with other types of tumors or with non-malignant disease will be tested. When this happens, the age of disillusion sets in, because the difference between the patient group and the control groups gets smaller and smaller. The discriminating power of the test progressively becomes less.

Since my job is to talk about the future, I want to emphasize the fourth age, the age of reality testing. Is it possible to develop immunologic tests that mark the presence of tumors? I think its going to take the kind of strategy that gets to the molecular level and determines exactly what configurations are "specific" for cancer. (I use quotation marks around the word, because I'm not convinced there is ever going to be any determinant that's perfectly specific for cancer.) Certainly there are
some antigenic determinants that are relatively overexpressed by cancer cells, and by a judicious selection of monoclonal antibodies, I think we can develop tests that will be clinically adequate indicators of cancer. Probably much of that is going to arise from the sort of molecular work described above.

1. "Tumor" antigens are not tumor-specific
   - re-expressed fetal/embryonic antigen
   - amplification of normal tissue antigen
   - proliferation antigen
   - stress antigen
   - acute phase antigen
   - microbial containment
   - Oncogene

2. Inappropriate expression of tumor antigen
   - non-malignant diseases
   - unrelated malignancies

3. Cross-reactivity
   - shared antigens
   - "polyreactive" antibodies

4. Technical difficulties
   - antibody specificity and affinity
   - separation of free/bound antigen
   - sensitivity/specificity of detection system
   - need for multiple antigens
   - formation of immune complexes
   - timing of samples
   - establishing normal range

What are the problems, real or potential, in developing tumor markers? The first is that tumor antigens, to the extent that we know them now in humans, are not truly tumor-specific. We have talked about tumor-associated antigens, which result from re-expression of fetal antigens, overexpression of cell-specific antigens, antigens of inflammation, antigens of proliferation, or stress antigens. Heat shock proteins, for example, keep getting rediscovered as "tumor antigens" because the tumor cells are, in a sense, under stress. C-reactive protein, an example of an acute phase reactant, is present in malignant serum and not in normal serum, but it's nothing more than an acute phase reactant. The literature is replete with studies of microbial products from microorganisms harbored by tumors and are not present in normal cells. Those represent secondary contamination of certain types of tumors. For example, acid fast organisms have been implicated from time to time. Most important, we must be aware of oncogenes, these normal constituents which get overexpressed during malignant change, at least in experimental animals. We have no reason to doubt that oncogenes occur in humans and act very much the same way. There are many reasons why we are not going to enjoy the luxury of having an entirely tumor-specific antigen.

Since these antigens are tumor-associated, we can predict that they may be inappropriately expressed in non-malignant diseases upon occasion, and that unrelated malignancies will overexpress them. We say one specific example of prostatic acid phosphatase being overexpressed in a pancreatic tumor. If we deal with whole antigenic molecules, we can predict that many of the tumor antigenic determinants are shared with normal tissues. They are shared because almost every molecule in the body contains determinants that are widely distributed on normal tissues as well as malignant tissues. When we use monoclonal antibodies that recognize very short sequences of amino acids, we can expect that some monoclonal antibodies are going to react with these widely distributed sequences. Immunologists now speak of "polyreactive" monoclonal antibodies; that is, monoclonal antibodies that react unexpectedly with an antigenic determinant on many different molecules. Such reagents may react strongly with tumor cells but can give misleading results. Only when we study reaction at a molecular level is there any chance of finding a really discriminating antibody.

There are many technical difficulties in the test itself. Most tests for tumor markers are going to be looking for circulating antigens in the blood stream. The specificity and affinity of an antibody are reciprocal functions. We usually want to have the most sensitive test, but the higher the sensitivity the greater the possibility of the loss of specificity. In most immunoassays we need to find better ways of separating bound from free antigen, or ways of distinguishing antigen that is bound to antibody. We probably would do well to look at multiple tests for the same tumor. I mentioned PAP as a test of the past because prostate specific antigen (PSA) has rather replaced it as the best marker of for prostatic cancer, but there may be a virtue in a multi-antigen test. Maybe doing both tests, PAP and PSA, in the case of
prostatic cancer, for example, has more predictive value than a single antigen test.

One of the interesting possibilities that offers some chance for a new approach is that some of the re-expressed antigens are themselves antigenic in the same individual and elicit production of antibodies. So, immune complexes of antigens and antibody can occur. While on one hand those can interfere with the test, they also provide an opportunity to demonstrate an antibody response. Even more interesting than antibody response is the T-lymphocyte response. Looking for cytotoxic T-cells in cancer patients is not yet a standard procedure and might be of great interest.

Other problems related to testing for tumor markers include the timing of samples. Then, most tumor markers are going to be constituents that are normally present, and we need to establish a normal range in order to look for a significant elevation.

Let us now return to the subject with which we opened this talk: namely the clinical application of tumor markers. Too frequently, they are thought of as screening tests useful for early diagnosis of cancers. The important issue is not the time of diagnosis but what that diagnosis leads to. What will be the follow-up? In some instances, earlier treatment is beneficial. Sometimes, however, early treatment merely leads to the artifact of longer survival because of the lengthened time between diagnosis and death. In the case of prostatic tumors, we may be more interested in their behavior — their aggressiveness — than in the mere presence. Tumor markers that can predict tumor growth or spread, or evaluate host response, are the goals that we seek.

Let me just sum up. We can predict the future of tumor markers best by looking at the past. I’ve picked one case history, the prostatic acid phosphatase. That test went through a typical life-cycle of these tests, with a long latent period of many years while discovery is going on, followed by a wave of enthusiasm and overenthusiasm, where some thought we could replace good clinical judgement with some simple test. Invariably, this overenthusiasm has been replaced by a period of disillusion, and many have taken an overly pessimistic view of the whole approach of immunochemical tests for tumor markers. I hope the future will be an age of reality, when we’ll balance what we now know to be the weaknesses and the defects in this approach against the potential value. Using some reality testing, I think the future will give us tests that will be valuable in the early diagnosis of some cancers and are useful in monitoring progression and looking for recurrence.

Perhaps the most exciting opportunities in the future will be looking for people at inordinate risk for developing cancer, by detecting genetic abnormalities favoring the origin of malignant cells. Genes that enhance or suppress the tendency to develop a particular tumor may well be tumor markers of the future. Thank you.