Radioimmunoassay's Role in Patient Management

Brigitte Petitte, Diane Bialczak, Linda Fink, and Deborah Golightly

Loyola University Medical Center, Maywood, Illinois

This is the third article in a four-part series on nuclear medicine updates. Upon completion of this article, the nuclear medicine technologist should be able to (1) identify new and standard RIA procedures, (2) utilize RIA values with imaging exams, and (3) recognize future applications of tumor marker assays with imaging techniques.

While health care professionals are aware of the contributions to quality care made by nuclear medicine, most are more familiar with the role of imaging than with radioimmunoassay (RIA).

In 1960, Berson and Yalow introduced an immunologic method of measuring plasma insulin in humans using RIA. This led to a Nobel Prize for both investigators. Many RIA procedures have been developed since then which allow clinicians to quantitatively measure peptide hormones in serum and plasma samples. Today, RIA techniques have greatly advanced and assays are available for drugs, tumor associated antigens, and many other compounds.

With the introduction of monoclonal antibodies, the assay of a number of specific tumor markers was possible. The clinical value of any assay, including tumor markers, depends on sensitivity, specificity, and whether progression of disease is reflected. Until recently, tumor markers played a minor role in monitoring the progression of a variety of malignancies. Of tumor markers commonly used, carcinoembryonic antigen (CEA) was used most widely. CEA is only useful in patients whose tumors produce high levels of antigen (*1*). These tumors are most commonly of endodermal origin. Elevated serum levels of CEA are often found even after the completion of radiotherapy. In addition to CEA, new tumor markers PSA (prostatic specific antigen), CA-125, CA 19-9, and CA 15-3 have proven quite helpful if disease persists.

One of five deaths in this country is attributable to some form of cancer (*2*). Common cancers are stomach, lung, breast, colorectal, and cervical (*3*). Tumor markers specific for these cancers have been developed. Success in treatment depends largely on early detection and treatment. The highly specific and sensitive tumor markers for a particular malignant disease can be an important tool in the early detection and management of cancer, to direct treatment, and to detect recurrence. Historical patient data is crucial as a reference in evaluating a patient (*4*). Increases in tumor markers despite treatment can indicate that a change in treatment should be considered. Many routine chemistry tests can be indicative of metabolic imbalance but are unable to differentiate tumor effects from other causes (*5*).

For reprints contact: Brigitte R. Petitte, CNMT, Department of Nuclear Medicine, Loyola University Medical Center, 2160 S. First Ave, Maywood, IL 60153.

SPECIFIC TUMOR MARKER ASSAYS

Prostatic Specific Antigen

In vitro tests are noninvasive, cost less, and are more sensitive than in vivo tests for diagnosing prostate cancer. A staff urologist is quoted as saying, "Almost every month there is at least one article in the journal *Urology* about the use of prostatic specific antigen (PSA) assay." This urologist relies on the PSA result in the management of his patients.

The measurement of PSA concentrations is an important tool for monitoring patients with prostatic cancer (*6*). PSA assists in determining the potential and actual effectiveness of surgery or other therapies. When PSA values are reported, they are graphed to show the trend of data from early assays onward. This gives the physician a clear picture of the patient's status.

PSA is specific for normal or malignant prostatic tissue. It is present in normal, benign, hyperplastic, and malignant prostatic tissue (*7*). It is present in metastatic prostatic carcinoma and found in prostatic fluid, as well as seminal plasma. PSA has not been found in other normal tissue. PSA is becoming the most widely used test to aid in the prognosis and management of patients with prostate cancer. The antigen used in this procedure has been identified and purified to be specific for prostatic tissue (*8*). The following case studies illustrate the value of the PSA assay.

Figure 1 shows steadily increasing levels of PSA in a 71-yr-old male. The increasing levels show progressive disease despite therapy. All other diagnostic tests were negative until December 1990 at which time a bone scan showed metastases. The urologist voiced his frustration at seeing continuous high results on his patient with all other procedures negative. He knew that the disease was progressing but was unable to locate further disease. The rising levels, however, indicated that a

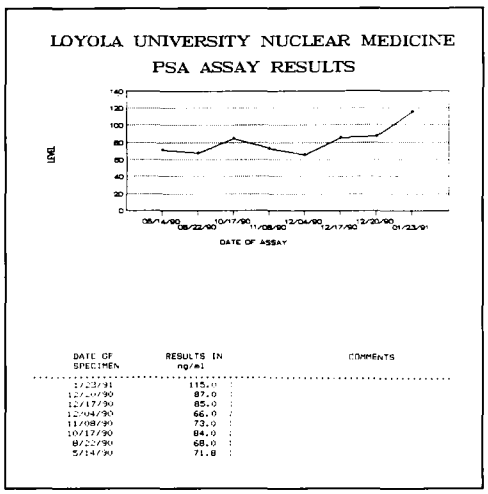

FIG. 1. 71-yr-old patient having PSA levels monitored while simultaneously undergoing radiation therapy.

change in treatment was necessary. PSA testing is also valuable in observing patient trends following successful treatment of prostate cancer.

The PSA assay results in Figure 2 are for a 77-yr-old male. A computed tomography (CT) scan indicated no abdominal or pelvic lymphadenopathy. X-rays and a bone scan were negative. A needle biopsy was consistent with Gleason Grades 2 and 3 adenocarcinoma of the prostate. A metastatic workup was negative. The PSA level was consistent with the biopsy. The urologist decided to perform a radical prostatectomy on this patient in August 1990. As seen on the graph, the patient's postoperative PSA result dropped to a negative level. PSA levels as well as other diagnostic tests will continue to be ordered on this patient to observe evidence of recurrent disease. If the patient's PSA level begins to rise, it will alert the physician that more aggressive treatment is necessary.

Figure 3 shows increasing PSA levels in the next patient. A bone scan of the same patient (Fig. 4) demonstrates evidence of metastatic bone disease consistent with the increasing PSA levels.

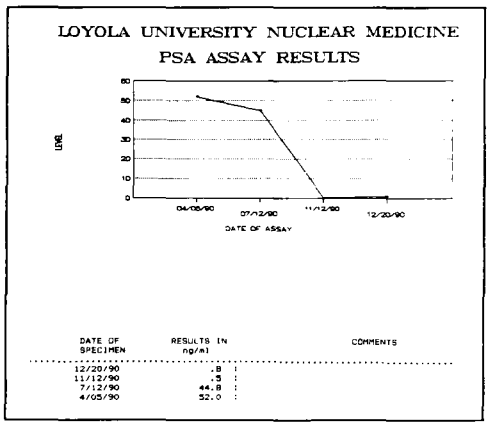

FIG. 2. Results of PSA monitoring of a 77-yr-old male diagnosed with Stage C-3 prostate cancer in April 1990. Results show marked decrease after surgical resection performed June 1990.

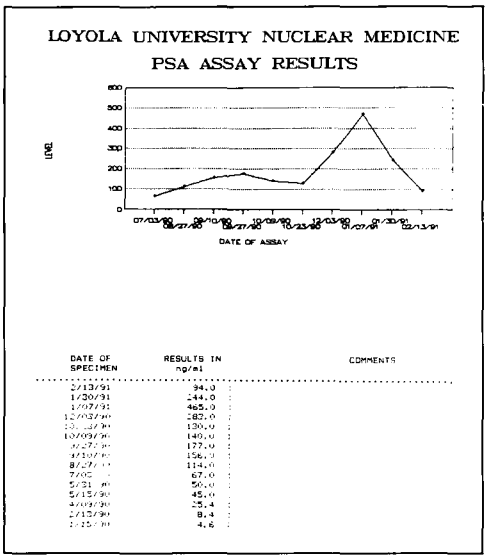

FIG. 3. Typical PSA profile for a 72-yr-old male followed for recurrent disease after a total prostatectomy. The level increases steadily following chemo- and radiation therapy.

CA-125

CA-125 is a cancer antigen found in serum of women with primary epithelial invasive ovarian cancer. The measurement of CA-125 is used to supplement second-look surgery for residual ovarian carcinoma in patients. A level of 35.0 U/ml or greater is indicative of residual tumor (*9*). The CA-125 assay has proved valuable in predicting the presence of subclinical disease in those patients whose antigen levels are elevated and who are in clinical remission.

The oncologist sees the CA-125 level as a predictor of recurring disease. An example would be a woman with known ovarian cancer who, subsequent to treatment, has been in remission for several years. Suddenly the CA-125 level is elevated. This could be an indicator that disease has recurred. The physician is now alerted that there is a need to undertake

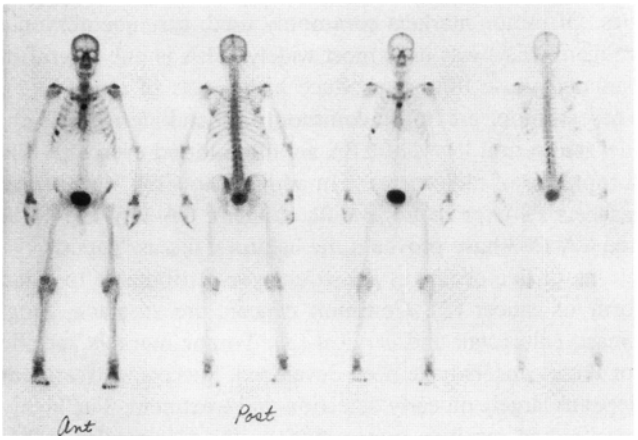

FIG. 4. Bone scan with metastatic indices for a 72-yr-old male diagnosed with prostate carcinoma. The patient's PSA result on Figure 3 (114 ng/ml, 30 times normal) correlates with the extent of metastatic disease on his bone scan.

a second-look operation. The CA-125 level is also useful in monitoring patients undergoing chemotherapy. The physician uses the test to monitor the rate of decrease in CA-125 after two to three cycles of chemotherapy.

The assay results shown in the next three figures are typical. Figure 5 shows results from a patient who had her first CA-125 level determined in August 1989. The results were negative at that time but her levels gradually increased during the year. The patient had a mastectomy in 1975. The breast cancer had metastasized to the ovaries, resulting in an elevated CA-125 level. In November, surgery was performed and she is currently on chemotherapy. Along with a monthly CA-125 level, other diagnostic studies were being performed. The other studies were negative while the CA-125 increased. This result alerted the physician to the need for additional tests.

CA-125 results in Figure 6 follow a patient who had a total hysterectomy in 1988 following the diagnosis of ovarian cancer. Two years post-chemotherapy the patient became symptomatic. A CT scan showed a pelvic mass. The patient's CEA level was negative and CA-125 was markedly elevated. The level dropped consistently as treatment was administered. This drop in the level is indicative of successful treatment.

Figure 7 demonstrates how an increasing CA-125 level can alert the physician to recurrent disease warranting an aggressive second-look operation. This 38-yr-old female was diagnosed with left ovarian cancer in December 1989. After surgery and treatment, the patient remained asymptomatic until September 1990. Note the slight increase in the CA-125 level of August 1990. Unfortunately, no level was ordered in September. However, in that month pathology identified well differentiated adenocarcinoma compatible with endometrial carcinoma. The October CA-125 result correlates directly with the patient's clinical status and clearly shows marked decreases of CA-125 as treatment progressed.

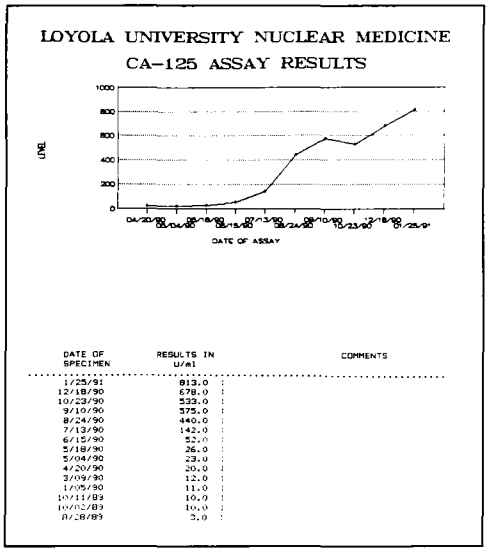

FIG. 5. Abnormal CA-125 levels on a 64-yr-old female originally presenting with breast cancer in 1975. The patient did well until June 1990. All other diagnostic tests were negative.

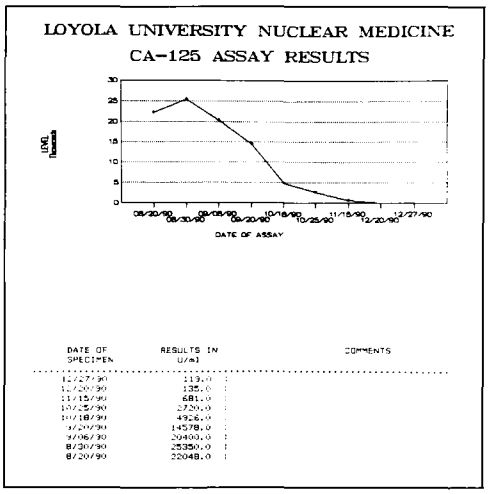

FIG. 6. In August 1990, this 49-yr-old female had a recurrent pelvic mass after a total hysterectomy 2 yr earlier. The CA-125 levels shown on the graph follow an aggressive course of chemo- and radiation therapy.

CA-15-3

The CA-15-3 assay is used for monitoring breast cancer patients (*10*). Often this tumor marker is positive long before there is any clinical evidence of disease. The final study discussed will focus on a breast cancer patient. We started monitoring CEA levels on this patient in January 1987. The patient was diagnosed with cancer of the breast in 1979. All of her CEA levels were negative even though her bone scans, along with other diagnostic procedures, clearly indicated metastases. The first CA-15-3 ordered on this patient was in January 1989. This level clearly indicated recurrent disease.

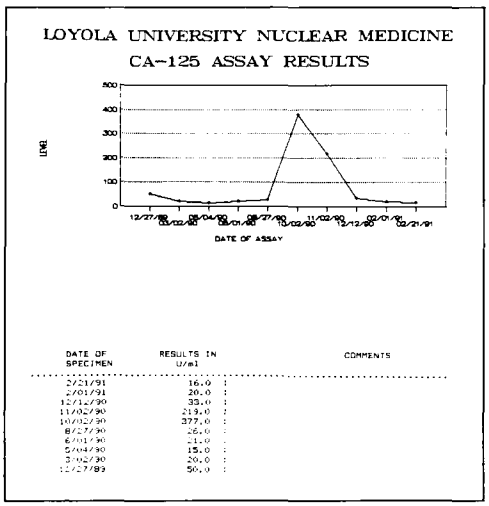

FIG. 7. CA-125 levels for a 38-yr-old patient treated for ovarian cancer in December 1989. The patient did well until an elevation in CA-125 level was observed on 8/27/90. Surgical biopsy results in early September confirmed recurrence of disease. Elevated results obtained in October and November are indicative of spread of the disease. CA-125 levels dropped markedly in December after successful therapy.

Her CA-15-3 levels have continued to rise and fall throughout the past two years and correlate directly to the periodic success and failure of treatment.

As physicians gain experience and confidence in using monoclonal tumor markers, we see more orders for them. In the future, these monoclonal antibodies can be developed for use with monoclonal antibody imaging techniques. The visualization of a tumor will allow the physician to locate a tumor site and be able to visualize its size as well. The nuclear medicine physician can utilize the RIA tumor marker results to distinguish which patients would benefit most from monoclonal antibody imaging or therapy.

For example, if Anti CA-125 antibody was developed for imaging, the patient who would benefit most from a monoclonal antibody scan would be a patient who had an elevated serum CA-125 result because high uptake would be anticipated. The integration of RIA procedures with monoclonal antibody imaging techniques will provide vital information to the nuclear medicine physician and ultimately impact on quality health care of the patient. In the future, if imaging with monoclonal antibodies is possible, then treatment with therapeutic doses of radioactivity may be possible, targeting only the tumor cells. RIA is still the most accurate, sensitive, and specific assay for many substances.

OTHER USEFUL RADIOIMMUNOASSAYS

Some of the more common procedures done in our laboratory are:

Ferritin: Serum ferritin levels are indicative of iron stores present in a patient. Levels are useful in differentiating true iron deficiency from the body's failure to utilize these stores, often eliminating the need for an invasive bone marrow biopsy.

Digoxin: This allows direct measurement of serum digoxin levels quickly and accurately. It is important to rule out digitoxicity quickly and accurately. We are also able to filter out digibind to let the physician know how much the level has dropped after digibind has been administered.

Thyroid testing: This is used to determine the patient's thyroid status and to follow patients after iodine-131 therapy to see if the dose was indeed effective.

Angiotensin I: This measurement allows the analysis of plasma renin activity in peripheral and renal vein samples to aid in the differential diagnosis of renal arterial hypertension.

Methotrexate: One of the unique services we are able to provide to our physicians is the ability to measure methotrexate levels in chemotherapy patients. Methotrexate is an antimetabolite and immunosuppressive agent useful in the treatment of lymphomas, sarcoma, and leukemia. It inhibits the formulation of folinic acid which is necessary for cell division. Methotrexate blocks the DNA synthesis pathway and is selectively lethal to certain rapidly growing cells. It is damaging to bone marrow, intestinal tract, and lungs (*11*). The body eliminates this drug through the kidneys. Since toxicity can arise from delayed elimination, patient hydration is critical.

Physicians use leucovorin as a rescue agent to avoid toxicity, and timely methotrexate levels are vital for proper dosage. The physician needs to know the exact level within 24 hr of administration of the drug in order to determine the dose of leucovorin required to rescue the patient. Levels drawn at 48 hr are critical in determining whether a safe methotrexate level has been attained.

CONCLUSION

Radioimmunoassay procedures have become very sophisticated in the past 30 years. In particular, the development of monoclonal antibody technology has resulted in the isolation of specific tumor markers, which in the future may play an important role in evaluation of patients for monoclonal antibody scanning and therapy. Vital patient information can be obtained quickly, accurately, economically, and with minimal discomfort to the patient utilizing RIA procedures.

The more aware physicians become of the availability of various RIA procedures, the better they will be able to utilize the information obtained from testing, thereby enhancing the quality of patient care. Early detection is the key to successful treatment, and with the combined sensitivity and specificity of RIA procedures, the nuclear medicine laboratory offers important information to aid in early detection of various diseases and plays a crucial role in diagnostic nuclear medicine.

ACKNOWLEDGMENTS

We thank the attending physicians at Loyola University Medical Center who took valuable time to tell us why our procedures are important to them: Ketty Badrinath MD, Scott McKenzie MD, Lode Swinnen, MD and John Wheeler, Jr., MD. Special thanks go to: Robert Henkin MD, Loyola University Medical Center, and Robert Peiss MD, St. Francis Hospital, Blue Island, Illinois, for their input on why RIA is important in the nuclear medicine department.

REFERENCES

1. Rutanen EM, Lindgren J, Sipponen PL, Stenman UH, Saksela E, Seppala M. Carcinoembryonic antigen in malignant and nonmalignant gynecologic tumors: circulating levels and tissue localization. *Cancer* 1978:42:581–590.
2. American Cancer Society. Cancer statistics. *CA: Cancer J Clin* 1987;37:2–19.
3. Braun P, Hildenbrand LG, Izbicki J, Leyendecker LG. Clinical significance of measurement of carcinoembryonic antigen in serum of patients with carcinoma of the uterine cervix. *Arch Gynecol* 1981;230:263–273.
4. Van Nagell JR, Hadson S, Gay EC, et al. Carcinoembryonic antigen in carcinoma of the uterine cervix. *Cancer* 1982;49:379–383.
5. Felder RA, Hunt R, MacMillan III RH, Bruns DE. Two monoclonal based assays for carcinoembryonic antigen compound. *Clin Chem* 1987;33:700–704.
6. Liedtke RJ, Batjer JD. Measurement of prostate-specific antigen by radioimmunoassay. *Clin Chem* 1984;30:649.
7. Nadji M et al. Prostatic specific antigen: an immunohistolic marker for prostatic neoplasms. *Cancer* 1981;48:1229.

8. Killian CS et al. Prognostic importance of prostate specific antigen for monitoring patients with stages B to D prostate cancer. *Cancer Res* 1985;45:886–891.
9. Dodd J, Tyler JPP, Crandon AJ, et al. The value of monoclonal antibody (Cancer Antigen 125) in serial monitoring of ovarian cancer: a comparison with circulating immune complexes. *Br J Obstet Gynaecol* 1985;92:1054–1060.
10. Siegel R, Rae J, Mertz S, Geelhoed G, Foemel R. Predictive value of biomarkers CA 15-3, Lasa-P and CEA in diagnosing carcinoma in suspicious breast lumps (Abstract). *Proc Annu Meet Am Soc of Clin Oncol* 1987;6:7.
11. Camitta BM, Holcenberg JS. Safety of delayed leucovorin "rescue" following high-dose methotrexate in children. *Med and Pediatr Oncol* 1978;5:55–59.