Nuclear techniques in the detection and management of cancer

Interest is growing among developing countries in improved diagnostic methods

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Cancer is a major health problem of our times. Expectations that a single causative agent — and thus a single cure — would be identified have now been dispelled and current medical opinion favours the view that each cancer is unique and needs an individual approach. Many factors such as smoking, betel chewing, viral agents, and ultraviolet light have been identified as causing cancer. Recently, the possibly vital role of "cancer risk genes" (oncogenes), which are aberrant forms of normal genes, has been noted.

Significant, and sometimes spectacular, advances have also been made in cancer treatment, by conventional methods of surgery, radiation, and chemotherapy in addition to the more recent approaches of immunotherapy and genetic manipulation. There is no question, however, that, in most cancers, detection at the earliest possible stage still remains a key factor that determines the final outcome. In this regard, matters are not as satisfactory as would be desired. In lung cancer, for instance, only about 20% of cases are diagnosed before the disease has spread. Improved diagnostic methods are therefore required.

Improved diagnostic methods

There are two main areas in which recent developments have had a marked impact on the sensitivity (the proportion of patients correctly diagnosed by a positive test) and the specificity (the proportion of patients without cancer in which the test is negative) of cancer diagnosis.

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The first is the identification and quantification of substances known as "tumour markers", an *in vitro* method performed as a laboratory test on a specimen of biological fluid, usually blood.

The second group comprises *in vivo* methods, requiring the presence of the patient. In these methods, modern, often computer-assisted, organ-imaging procedures enable the detection of tumours. They also sometimes enable the characterization of tumours as malignant when they are still small and confined to the primary site of disease. Each group of tests, when combined with clinical data, contributes to early diagnosis as well as to follow-up or evaluation of the progress of the disease following treatment.

The role of nuclear techniques

Nuclear techniques have made very significant contributions in these diagnostic areas. Specifically, they provide convenient means to detect tumour markers and to visualize tumours by *in vitro* and *in vivo* tests using radionuclides.

Tumour marker detection. Tumour markers comprise a number of substances expressed by many, but not all, tumours. They are released into the circulatory system where their presence or concentration may indicate the existence of the tumour itself.

A few of these are chemical substances normally produced by the body ("eutopic markers"). Their elevated levels may indicate the presence of malignancy. Elevation of calcitonin levels, especially after stimulation with Pentagastrin, is a good example of such a biochemical marker for early diagnosis of medullary cancer of the thyroid. Another would be adrenocortical hormones.

Other biochemical markers may be "ectopic". They are produced at an abnormal site when cancer is present. For example, adrenocorticotrophic hormone (ACTH) is a normal product of the pituitary gland but elevated levels occur in cancer of the bronchus and some other organs.

Other markers are "oncofoetal". They are normally present during varying periods of embryonic and foetal life. They may exist at low concentrations in adults, but reappear in large quantities in certain malignancies — for example, alpha feto-protein (AFP) and carcinoembryonic antigen (CEA).

A final group may be classed as "mutational". Examples of these include paraprotein in myeloma and a mutation of P53 protein in cancer of the bronchus and colon.

Nearly two dozen tumour markers detectable by radioimmunoassay are commonly available. They include AFP; beta human chorionic gonadotrophin (HCG); beta-2 microglobulin; CA 15-3; CA 19-9; CA-50; CA-125; carcinoembryonic antigen (CEA); cortisol; gastrin; HGH; insulin; MCA; neopterin; NSE; PAP; prolactin; PSA; PTH; SCC; TCT; TG; and TPA.

Unfortunately, the vast majority of tumour markers are neither organ- nor disease-specific. They are not uniquely produced by any single organ. Elevated levels of CEA, for example, are found in malignancies of the gastrointestinal tract, breast, lung, ovary, and thyroid. CA-125 levels are elevated in more than 80% of the cases of ovarion cancer but also in pelvic inflamatory disease, endometriosis, cirrhosis, and pancreatitis.

Neither are tumour markers specific for malignancy. This is because they are produced by normal tissues and those that have undergone non-malignant change. Prostate specific antigen, for example, is expressed by the normal prostate, in benign hyperplasia, as well as in prostatic cancer.

Attempts have been made to establish "cut off points", or levels strongly suggestive of malignancy. This approach has proved fairly satisfactory in some cases (AFP, HCG, CA-125); however, it is not completely satisfactory because benign tissue necrosis, inflamation, and haemorrhage can also result in elevated levels of the markers concerned.

For these reasons, the greatest value of tumour marker measurements lies not in initial or early diagnosis. Rather, it lies mainly in the assessment of prognosis, post-therapy follow-up, detection of early recurrences; and, when used in combination with organ-imaging methods, in monitoring tumour burdens or size. Even in the case of thyroglobulin, a rare example of a marker that is 100% organ-

specific, its diagnostic value in thyroid cancer is almost nil. However, it is very useful in monitoring the response to treatment. If this has been effective, values will normalize, but if the condition is resistant to treatment they will remain high.

Tumour markers are immunogenic or can be made so by well-established means. Therefore, they may be used to produce antibodies which may be polyclonal (derived from a multiplicity of cell lines in an experimental animal) or monoclonal (from a single cell line by a special method known as the hybridoma technique). The latter method, which is now generally used, has greatly extended the sensitivity, specificity, and the range of analytical methods for the detection of tumour markers.

This simplified representation shows some of the more common tumour markers and their primary sites of production: 1) NSE in small cell lung cancer; 2) TPA and CEA in lung cancer; 3) AFP in primary liver cancer (hepatoma); 4) CA-50 and CA 19-9 in pancreatic cancer; 5) Beta-2 microglobulin in leukemia; 6) AFP and CA-125 in ovarian cancer; 7) AFP and HCG in testicular cancer; 8) Beta-2 microglobulin in malignant lymphomas; 9) TPA, ER^{+-}, and CEA in breast cancer; 10) CA 19-9 and CEA in colon cancer; 11) CEA, TPA, CA 19-9, and CA-50 in colorectal cancer; 12) PSA and PAP in prostatic cancer.
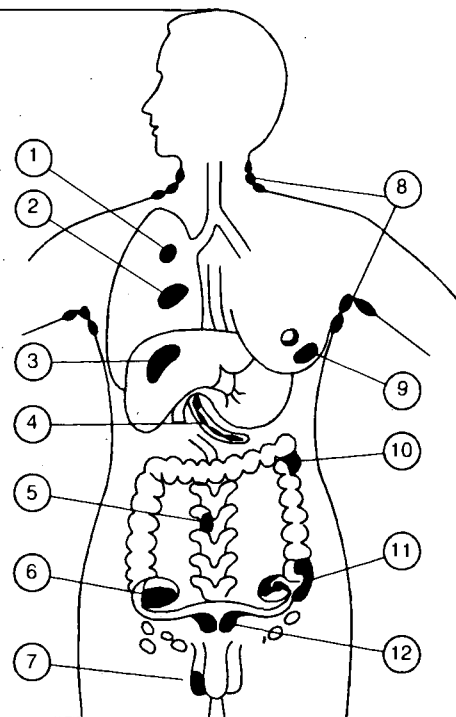
The more popular techniques for *in vitro* detection and measurement of tumour markers in biological fluids are based on reactions between the marker itself, serving as an antigen, and its corresponding antibody. A typical example would be CA-125, which is a cancer antigen. Its antigenic determinant is recognized by the monoclonal antibody OC-125 raised by using an ovarian cancer cell line (OVCA 433) as immunogen. Antigen-antibody reactions may be monitored by non-isotopic methods, such as enzyme-linked immunoassays (ELISA) where an enzyme is used to assess the end point or degree of binding.

However, radioisotopic methods — radioimmunoassay (RIA) or more often immunoradiometric assay (IRMA) — offer many advantages in sensitivity, precision, convenience, and cost. These are microanalytical methods that essentially depend on reaction between the substance whose detection or measurement is desired (analyte), which in this case is a tumour-associated antigen, and a suitable binding agent (reagent), which in this case is a monoclonal antibody. Attachment of a suitable radiolabel (generally iodine-125) to the antigen (RIA) or the antibody (IRMA) enables the determination of the fractional occupancy of the available binding sites at the end of the reaction. It may be said that they are in fact the methods of

Specificity characteristics of some common tumour markers

Marker	Tumours	Specificity
AFP	hepatoma gonad	>80%
CA 15-3	breast cancer ovarian cancer	>80%
CA 19-9	GI-tract (stomach, liver, bileduct, pancreas, colorectal cancer)	>65%
CA 125	ovarian cancer pancreas	>70%
CEA	most solid tumours (in non smokers!)	~60%
NSE	oat cell lung cancer apudomas seminoma	>70%
PAP	prostatic cancer	80%
PSA	prostatic cancer	50%
SCC	ENT cancer lung uterus	>80%
TCT	med. thyroid cancer	>80%
TG	thyroid cancer (post therapy)	>80%
TPA	breast, lung, colorectal cancer, bladder, uterus	~40%

choice, universally applicable to tumour markers of whatever nature.

Known information about the sensitivity and specificity characteristics for some common tumour markers serves to underscore the point that tumour marker measurements may not be generally applicable in making early or initial diagnoses of cancer. *(See table.)* Rather, they

are more useful in the monitoring of treatment and in providing lead time for detecting recurrences. A few exceptions exist. These include the eutopic markers already referred to; they are commonly used for the screening of high-risk groups — for example, human chorionic gonadotrophin (HCG) measurements to detect the development of chorionic cancer in patients who have had hydatiform moles.

Tumour marker measurements can sometimes be useful in assessing the stage of development or severity of malignant disease. Normal levels of the markers AFP and CA-153 are found in the early stages of breast cancer (stages 1 and 2). However, they are both elevated in stages 3 and 4 where local or distant metastases have occurred. Here again, however, both can be elevated in non-mammary malignancies.

The problems of inadequate sensitivity and specificity may at times be overcome by measuring several tumour markers together, combined with sophisticated statistical methods of data analysis. Such "tumour marker panels" may provide the best discrimination between benign and malignant lesions and also between different cancer cell types. This approach, recently adapted to lung cancer, has improved diagnostic accurancy. Combined measurement of beta HCG and AFP have also been found useful in the clinical staging of testicular neoplasms.

In general, however, in spite of the recent advances and the advantages of sensitive and precise radioisotopic techniques, tumour marker measurements remain complementary to other diagnostic procedures, at least for the detection of cancer during its early stages.

Organ visualization: In this field as well, methods using radionuclides exist alongside non-isotopic alternatives, such as diagnostic radiology, computerized tomography (CT), and magnetic reasonance imaging (MRI).

Examples of radionuclides that are themselves efficiently delivered to tumours on intravenous administration are very few. They probably are confined to iodine-131 to differentiated thyroid cancer after removal of the gland itself, and to iodine-131 MIBG to tumours of chromaffin tissue, (phaeochromocytoma, neuroblastoma, paraganglioma). Technetium-99m can now be universally used as the radionuclide of choice, even for labelling of monoclonals. Nonetheless, it may demonstrate space-occupying lesions but cannot distinguish malignant lesions from others, such as cysts or even inflamatory processes, either when delivered by itself or in combination with a variety of chemical agents that show affinity for particular organs, such as sulphur colloid for the liver. Among other radionuclides used by them-

selves, gallium-67 has a sensitivity of about 90% and a specificity of about 75% for detection of lung tumours. However, it does not provide good discrimination of malignancy.

More efficient systems for delivery of radionuclides to tumours have been developed as monoclonal antibodies to tumour-associated antigens became available. For example, malignant melanomas express an antigen called P97. A monoclonal antibody, appropriately labelled, can be targeted against this. Given intravenously, the antibody binds to the antigen at the surface membrane of the tumour cells. The sites of antibody deposition may then be visualized by external imaging. This procedure, known as radioimmunoscintigraphy (RIS), is becoming increasingly popular as better antibodies and labelling techniques with more convenient isotopes are being developed.

localizing the antibody after administration to the patient. The technique itself is quite non-invasive. It consists of administration of the labelled antibody to a patient (who does not generally need special preparation) and imaging after a suitable period (which may be from a few hours to a few days).

The fact that most antigens expressed by tumour cells are neither organ- nor tissue-specific exerts the same deleterious effect as in the case of the *in vitro* methods discussed earlier. Most monoclonal antibodies used in RIS are tumour-associated rather than tumour-specific and only a small fraction (0.01% to 0.001%) of the injected radioactivity finally localizes in the tumour. The rest is found in the blood, and in normal organs such as the liver, spleen, and kidneys. As a result, only large tumours are usually detectable in areas where

After a suitable radio-pharmaceutical is injected into a patient, its distribution in a selected organ can be visualized.
(Credit: ORNL)
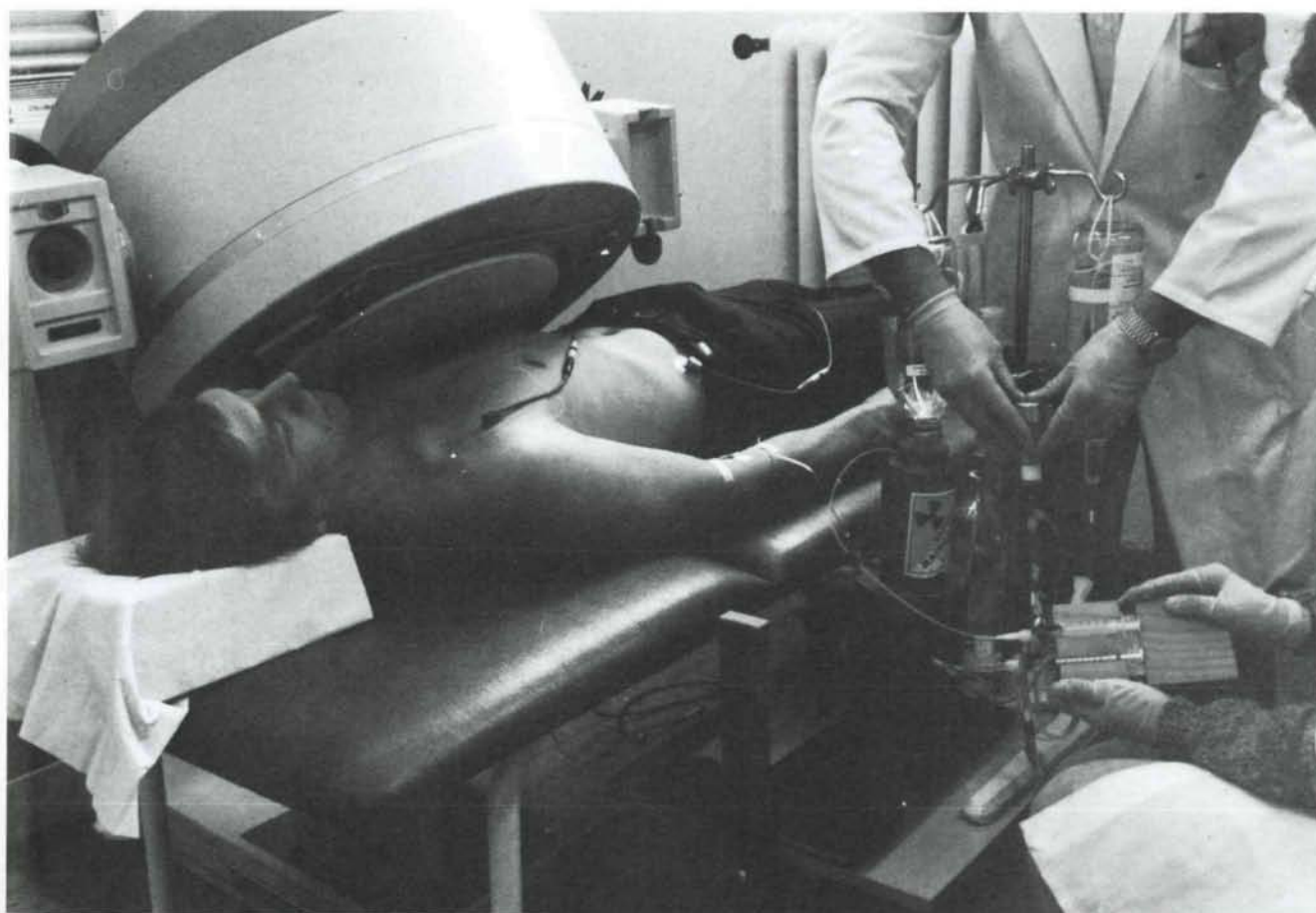
From the above example, the general requirements for a RIS system would be clear. These would be an antigen specifically expressed by cancer cells; a good quality (high affinity and specificity) monoclonal antibody against this; a suitable label with a labelling system that leaves immunoreactivity unimpaired; and an external imaging system for

surrounding or background activity is low. One recent promising advance in this field is the use of artificial antigens, such as the so-called panadenocarcinoma antigen Ag15OH82. Also, certain tumour-associated antigens (CEA, AFP, HCG) are available in purified form and animals can be immunized with these rather than with tumour extracts.

Advances in radiolabelling techniques also have resulted in major improvements. Technetium-99m is the isotope most commonly available in nuclear medicine laboratories worldwide. It is very suitable, in view of its short half life, for use in RIS because most antibody uptake by tumours occurs within the first 24 hours. However, other isotopes, such as iodine-131 and indium-131, have been used when there was no good method for labelling of antibodies with technetium-99m at hand, or the imaging was done after a longer interval. Recently, however, convenient methods of technitium-99m labelling of antibodies have been described that can be used in nuclear medicine laboratories based in hospitals. The methods are not drastic and leave the immunoreactivity of the antibodies unimpaired.

In regard to instrumentation, the basic requirement is for a planar gamma camera with attached computer. Single photon emission computer tomography (SPECT) may offer the advantage of better resolution, especially with double or triple headed cameras. This question, however, still seems undecided and authorities disagree as to whether SPECT in RIS causes more problems than it solves.

As in the case of *in vitro* techniques, RIS is more useful for detection of recurrent cancer and for follow-up after treatment than for primary detection, and is generally more sensitive than CT in this respect. With RIS, the sensitivity of detection of recurrent cancer of the gastrointestinal, genito urinary, and gynaecological systems in nearly 100% and the specificity is 80%. These are better than the corresponding figures for the *in vitro* techniques. *(See the cover photo of this edition for a typical example of a colonic cancer recurrence detected by RIS, using a monoclonal antibody directed against CEA.)*

The most recent development in the use of radionuclides for detection of malignant lesions is positron emission tomography (PET). This is a sophisticated method using shortlived radioisotopes, such as fluorine-18, oxygen-15, and carbon-11, that are produced in a cyclotron. PET does not merely localize a tumour but provides information on its functional or metabolic status, which can be the key to its characteriza-

tion as being malignant. It can even go further, in brain tumours, for example, and establish the degree of malignancy or grade of the tumour. Neither CT nor MRI can accurately do this. It seems certain to be the technique for the future, provided it can be made more convenient and less expensive for general use.

IAEA interests and activities

Over the years, the IAEA has contributed to the creation of a network of well-equipped and competent RIA and nuclear medicine laboratories at major hospitals, universities, and other institutions in the developing world. The high interest of these laboratories in working on RIA for tumour markers and on RIS comes as no surprise, given the greater availability of the necessary reagents (including monoclonal antibodies and technetium-99m), instruments (RIA equipment and gamma cameras), and trained personnel.

In response to the interest, the IAEA's Nuclear Medicine Section of the Division of Life Sciences has intensified its work in these fields. Last year, in May 1990, a seminar was organized on the application of nuclear techniques to the early diagnosis of cancer in developing countries. In 1991, individual research contracts awarded over the years will come to a head when co-ordinated research programmes, at a global level, start in the fields of tumour marker assays (using AFP in liver cancer as a model) and in RIS (using CEA in colorectal cancer as the model). Consultant meetings, attended by international experts, have already been held to advise on the technical as well as logistical aspects of project implementation.

Over the next few years, an intensive programme of work under research contracts and agreements, workshops, training fellowships, and research co-ordination meetings is foreseen. These programmes will make no small contribution to the firm establishment of such methods in developing countries and to the enhancement of the role that nuclear techniques play in the promotion of human health.