REVIEW ARTICLE

The Diagnostic Role of Radiolabeled Antibodies

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Introduction

AntiCEA-immunoscintigraphy whole-body scan (anterior view) in patient with history of medullary ca. of thyroid: detection in the neck area of two occult tumors (T), which had escaped conventional morphological imaging. Basal and stimulated calcitonin as well as CEA were restored to normal after surgery (30 months of follow-up) (Figure 1).

Antigens

In order to image tumors with antibodies, one must target those antigens on the tumor cell that are different, either qualitatively or quantitatively, from antigens on surrounding normal cells. Ideally, the targeted antigens would be unique to tumor cells (ie, not found in any normal tissue in any amount). However, in the real world, most “tumor-associated antigens” are also found in some normal tissues, although sometimes at lower density or in less accessible sites (eg, intracellular) than in tumors. The sensitivity and specificity of radiolabeled antibody imaging depends, in part, on the degree of expression of accessible antigen in the tumor site vs normal tissue [1].

Among the first antigens targeted for immunoscintigraphy were the oncofetal antigens (eg, carcinoembryonic antigen [CEA] and alpha-fetoprotein [AFP]). Later, monoclonal antibody development led to the discovery of other, previously unidentified tumor-associated antigens, (eg, tumor-associated glycoprotein 72 [TAG-72], novel CEA epitopes, and epithelial membrane antigen [EMA]), which has expanded the repertoire of available agents [2].

Isotopes

In selecting radioisotopes for imaging, one must consider a variety of factors, many of which are mutually exclusive (the “no free lunch” phenomenon) [3]. For example, one would like to have high count rates while limiting the radiation dose to the patient. Therefore, isotopes that have a high efficiency of interaction with the gamma camera crystal (eg, technetium-99m) are preferred over those with a low efficiency (eg, iodine-131). Technetium-99m also has a relatively short half-life (approximately 6 hours), which, coupled with its relatively low-energy gamma photon, means that a larger administered activity (which translates to more counts per second) can be given for the same patient radiation dose. Iodine-131, on the other hand, emits not only a higher-energy gamma radiation but also beta particulate radiation, which increases patient radiation dose without contributing to imaging. Both technetium-99m and iodine-131 are relatively inexpensive—an advantage in this era of cost consciousness [4].

For optimal lesion detection, however, a high target-background ratio is needed, which means that blood and normal tissue levels have decreased while tumor levels remain high [4]. With whole antibodies, this frequently requires waiting 24 hours or more for clearance from normal tissues to occur. The “ideal” isotope from the standpoints of efficiency and radiation dose, technetium-99m, has a short, 6-hour half-life. This means that by 24 hours, the count rate will have dropped to a point that would require prolonged imaging time to acquire suitable images, which, in turn, increases the probability that patient...

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movement will degrade those images. Iodine-131, with its 8-day half-life, presents no such problem. The chemistry of technetium-99m is also less favorable for antibody labeling than that of iodine [5].

Iodine-123 is chemically identical to iodine-131 but has no beta emission. Its efficiency of interaction with the gamma camera crystal is almost as good as that of technetium-99m. The half-life of iodine-123 is 13 hours, which is acceptable for 24-hour imaging but marginal past that point. Unfortunately, iodine-123 is relatively expensive and is less readily available than iodine-131 or 99mTc [5].

Indium-111 has a favorable half-life (approximately 3 days) for delayed imaging. However, it tends to accumulate in normal liver tissue, which decreases its usefulness for the detection of liver metastases. It is also expensive, but is currently the only isotope used with an FDA-approved monoclonal antibody imaging agent [6].

Antibodies

Polyclonal Antibodies

The first antibodies used for imaging were produced by immunizing an animal (usually a mouse) with human tumor cells or cell extracts and then harvesting and purifying antibody from the animal’s serum or ascitic fluid. This method yielded a variety of antibodies against a wide spectrum of antigens, some of which were “tumor specific” and others of which were more ubiquitous in tissues. Since these antibodies were derived from many B-lymphocyte clones, the term “polyclonal” is used to describe them. As might be expected, substantial lot-to-lot variation occurred when this production method was used [7].

Monoclonal Antibodies

In 1975, Kohler and Milstein [8] developed a method for selecting specific clones of cells that produced pure antibody against a single antigen; hence these antibodies were termed “monoclonal.” As initially described, animals were immunized with a target substance (eg, whole cells, membrane extract, or a purified antigen source), as is the case for the production of polyclonal antibodies. Subsequently, however, the splenic B lymphocytes were harvested and fused with mouse myeloma cells to form immortal “hybridomas,” which could be grown in cell culture. Each hybridoma clone produced a single antibody, which could be screened for immunoreactivity with the desired antigen. Cells from desirable clones could then be grown in animals (ascitic fluid) or in artificial cell culture systems to produce large amounts of pure antibody. This technology permitted the mass production of antibodies for clinical use.

Modifications of Monoclonal and Polyclonal Antibodies

Radioimmunodetection studies have been carried out not only with intact immunoglobulin but with MoAb fragments as well [9]. These have included F(ab’)2 and, more frequently, Fab’ fragments. The smaller the molecule, the faster its clearance from serum [10]. The Fab unit can “penetrate” the target by quickly leaving the vascular space and entering the tumor. This fragment is not as immunogenic as the intact antibody because it lacks the Fc portion of the parent molecule. The residence time of this radioimmunocomplex in tissue is short and therefore may not be ideal for the detection and treatment of certain solid avascular tumors. However, Fab’ fragments tend to be retained in the kidneys for extended periods, limiting their usefulness in the detection of disease around the kidneys. Moreover, fragments show less affinity for target antigens than intact immunoglobulin, and they are more difficult to produce and purify to clinical standard [11]. Genetic engineering techniques are able to replace the Fc portion of the murine MAb with the human equivalent, which is called a chimeric antibody, or both the Fc portion and the frame leaving only the CDRs of murine origin. This is called a “human reshaped” antibody or a CDR grafted antibody. While the antigenicity of murine antibodies poses a problem for multiple-use applications, the longer half-life of mouse/human chimeric antibodies or genetically humanized antibodies may increase nonspecific radiation from prolonged retention in the blood [12].

Potential Problems

Human Antimouse Antibodies

The tendency of mammalian immune systems to generate antibodies against foreign proteins, the very basis of monoclonal antibody production, also creates difficulties in the use of these substances in humans. The incidence of human antimouse antibodies after injection of whole murine antibody depends, in part, on the impact of the primary malignancy on the patient’s immune response, as well as on the amount of protein administered [13]. Therefore, patients with malignancies such as the lymphomas have a lower incidence of human antimouse antibodies than do patients with solid tumors (32% to 100%) [14].

The production of human antimouse antibodies in patients is worrisome for several reasons. The obvious concern when dealing with the injection of a foreign protein into patients is that of allergic reactions. However, serious allergic responses, such as anaphylaxis, have fortunately been extremely rare. Minor reactions in patients who have developed human antimouse antibodies, although more common, are not as frequent as one might expect [15,16].

Another problem is that once a patient has developed his or her own antibodies against the murine antibodies, any subsequently injected murine antibody will form complexes with the patient’s antimouse antibody. These antibody complexes are quickly cleared by the reticuloendothelial system, which prevents the murine antibody from reaching its intended target. Therefore, imaging these patients would produce the equivalent of a liver/spleen scan rather than a “tumor scan.” [17].

The presence of human antimouse antigens in a patient’s serum can also result in erroneous results in a variety of laboratory assays that utilize murine antibodies (eg, radio-immunoassay and enzyme-linked immunosorbent assay). Perhaps most
important for the oncology patient is the fact that many assays for tumor-associated serum markers used to detect early recurrence may become difficult to interpret in the presence of significant levels of human antimouse antibodies [18].

Spatial Resolution

Nuclear imaging has inherent limitations on the spatial resolution that can be achieved. One means of improving the lower limit of detection for lesions is to employ single-photon emission computed tomography (SPECT). This technique results in the equivalent of a three-dimensional map of isotope distribution in the body and enhances contrast resolution, making possible the detection of smaller and/or less intense concentrations of isotope. Lesions as small as 0.5 cm have been detected using technetium-99m SPECT. Single-photon emission computed tomography is also helpful in anatomic localization of lesions, compared to planar imaging alone [19].

Despite the improvements provided by SPECT imaging, radio-labeled antibody imaging alone is rarely sufficient to pinpoint the location of malignant lesions. This is due partly to the spatial resolution problem mentioned above and partly to the fact that normal structures (ideally) are not imaged to any great extent, therefore removing the usual reference points upon which one depends for exact anatomic localization. A potential solution to this problem is the fusion, by means of computer applications, of SPECT images of the distribution of labeled antibodies with CT or MR images. This fusion process is being studied at several centers, and eventually commercial software packages should become available for routine clinical use [3, 20].

Clinical Utility of Radioimmunodetection

During the past 25 years, there has been no shortage of clinical papers that have attempted to answer critical clinical questions concerning RID. Many of these publications present results for relatively small numbers of patients, without full clinical and/or pathologic correlation. Usually, not all known lesions (those abnormalities that are seen on CT, MRI, ultrasound, or physical examination) are detected with MoAb imaging. These studies have a sensitivity range of 67% to 89%, with larger lesions seen more easily than smaller lesions. The lack of visualization of a known lesion may be due to factors such as tumor necrosis, poor blood flow to the lesion, or lack of antigen expression, although these possibilities are not (i.e., cannot be) verified. Few of these papers discuss or present follow-up data on normal (negative) studies. In addition, different isotopes, antibodies, antibody forms, and imaging protocols have yielded different imaging results, causing some controversy and disagreement regarding the usefulness of RID studies [12].

One investigator states that there appears to be ample data to justify an optimistic outlook for the long-term role of RID studies in the clinical management of patients with a wide variety of human cancers [21]. Most RID studies have shown sensitivities (for surgically proven lesions) in the 70% to 80% range, with higher specificity in many instances. Thus, we are dealing with a test that does not detect all lesions. Although RID may miss up to 30% of lesions (as in the case of colorectal cancer), it can be used effectively with CT and other imaging procedures in selected circumstances to increase the accuracy of tumor detection [16].

Application in Oncology

In Colorectal Cancer

Currently, the most researched form of human cancer in terms of RID studies is colorectal cancer [22]. In most of the studies, patients with suspected recurrence or newly discovered cases with metastatic disease have been evaluated. It has been concluded until now, that radioimmunodetection allows a survey of the entire body to be made for evidence of recurrent or metastatic disease with far lower radiation burdens and costs than CT. Thus, radioimmunodetection appears to be of greatest utility in the follow-up of patients at high risk for recurrent or metastatic colon cancer [23].

Three types of antigen are used for making MAbs for RID:

First, the dedifferentiation antigens such as carcinoma embryonic antigen (CEA) which is present on most colon cancers. Detection rates near 70% with specificities near 90% for extrahepatic lesions have commonly been reported. Tumors as small as 0.5 cm have been detected with SPECT technology, but usually reported lesions are >2 cm in size [24]. In most studies, a combination of RID and CT scanning increased the accuracy of the diagnosis [25].

Anti-CEA MAb versus CT:

Most studies agree that radioimmunodetection is not as sensitive as CT scanning for the detection of hepatic metastatic disease but is more specific (41% versus 84%) [23, 26]. Especially when indium-111-labeled antibodies have been used, considerable “nonspecific” hepatic uptake has precluded visualization of hepatic metastases as areas of increased (“hot”) tracer uptake. Rather, these lesions, especially when >3 cm, appear to concentrate less tracer than surrounding parenchyma, thus providing nonspecific information comparable to a liver/spleen colloid scan. Fuster et al., [23] reported that CT is more sensitive also in the detection of lung metastasis (82% vs 27% for AntiCEA-MAb).

For the detection of extrahepatic intra-abdominal metastases, radio-immunodetection is probably the procedure of choice. Radioimmunodetection appears to be more sensitive than CT for recurrent disease in the extrahepatic abdomen (66% versus 34%) and pelvis (74% versus 57%) (as detected by 99Tc-antiCEA-Mab (arcitumomab). The combined sensitivity of both CT and RID was 88%. The current limitation of one-time use (due to the invariable occurrence of HAMAs) in the patient, peaking about a month after murine antibody infusion) precludes its use as a screening procedure. Yet, the sensitivity of MRI (93%) was found to be higher than anti-CEA [27].

Although few large clinical trials have been carried out to adequately assess the specificity of radioimmunodetection, all studies carried out to date suggest that the technique is more
specific in the detection of abdominal disease in all situations other than large hepatic metastases [28].

A patient with a history of colon cancer, a rising CEA, and a negative radiologic workup is one in whom the RID examination may have a major impact on management and outcome. Knowing the precise location of a recurrent tumor can play an important role in planning additional surgery. However, the role of RID in the diagnosis of primary colorectal cancer is more limited. Detection of synchronous lesions in patients with a limited abdominal workup may be helpful in planning patient management [29].

Fuster and his colleagues [26] stressed on that fact that low target to non-target ratios in antiCEA-MAb visualized lesions make SPECT mandatory (Figure 2). They found that SPECT was able to identify 35% of lesions which were not readily apparent on the planar images without significantly increased the number of false positive results. In addition, SPECT displayed a superior ability to delineate the extent and distribution of a patient’s disease. Technically, they added that after injection of radiotracer, 3 hours are necessary to allow antiCEA-MAb uptake in tumour sites. A delayed SPECT after 24 hours post-injection is also recommended, but only when suspected bowel uptake has been seen on early images and results should be read with caution [24].

Goldenberg and Nabi [30] added that lesion detection is in part related to lesion size, with a sensitivity of 80% for lesions over 2 cm. Detection of lesions smaller than 1 cm is 60%. CEA-Scan has been shown to have potential clinical benefit in one-third of colorectal cancer patients [24].

**FDG-PET versus 99mTc-AntiCEA-MAb:**

Schiepers et al. and Willkomm et al. [31, 32] compared the results of FDG PET are with those of CEA-Scan, and they found that both imaging modalities were more or less similarly sensitive in detecting local recurrence with better image quality for FDG PET. However, if correct tumor staging is required, FDG PET should be preferred because in CEA-scan there were missed lymph node and lung metastases which were probably caused by small tumor size (<2 cm).

**Second type of antigens** may be extracted from malignant cells such as the tumor associated glycoprotein, (TAG72) expressed by more than 80% of colorectal adenocarcinomas, more than 95% of common epithelial ovarian carcinomas, and the majority of breast, non-small-cell lung, pancreatic, gastric, and esophageal cancers evaluated [33]. MoAb B72.3 detects TAG-72 and has a high degree of specificity for tumor tissues and is generally not reactive with normal adult tissues. The antibody is commercially available as a murine IgG1 MoAb, conjugated to indium-111 and known as Oncoscint CV/OV (Satumomoab pendetide) and may be available as a chimeric antibody as is anti-CEA with similar accuracy as anti-CEA.

It was approved by the FDA in colorectal and ovarian cancer for the detection of extraperitoneal abdominal disease. The most important indications for its use in colorectal cancer as being reported are:

1. **As an alternative to second-look laparotomy to detect occult colorectal carcinoma in persons with suspected recurrence suggested by an elevated carcinoembryonic antigen (CEA) level, but who have no evidence of disease on conventional imaging modalities (including CT scan); or**

2. Detection of occult colorectal carcinoma in persons about to undergo a potentially curative resection of an apparently isolated recurrence located at a single site (e.g., lung or liver) which has been identified on conventional imaging modalities (including CT scan) and for whom the detection of occult lesions elsewhere would alter the surgical management

Gamma camera imaging is performed after administration of the Oncoscint, with optimal diagnostic images acquired at ~72 to 120 hours, after the blood pool background has cleared [34].

The **third approach** is to use an epithelial surface antigen that is remote from the blood, but which the architectural disruption of the malignancy exposes in greater density to blood. PR1A3, also called 1A3, is such an antibody which reacts with a fixed antigen on the surface of colonic crypt cells resulting in a high specificity for colorectal cancer. Normal lymph nodes do not show abnormal uptake since the antigen is fixed to the colorectal cancer cell surface [35].

**Intraoperative Radiomunodetection**

In patients with colon cancer, intraoperative radiomunodetection using iodine-125-labeled MoAbs has been shown to be very useful in the detection of abdominal metastatic disease, especially in patients who have resectable hepatic metastases (or who are to undergo intra-arterial infusion chemotherapy for inoperable hepatic metastases), to ensure that there is no evidence of extraperitoneal disease, thus altering significantly the surgical management in approximately 30% [36].

**Ovarian Carcinoma**

Patients with ovarian cancer can benefit from RID. Second-look

Figure 2: A)Planar images obtained after bladder catheterization show an abnormal anti-CEA-MAb uptake in the presacral region (big arrow) which is better localized in SPECT slices (B). We can see radiotracer uptake in the site of colostomy (small arrow). The CT was non-conclusive between recurrence and postsurgical fibrosis. A local recurrence of a sigmoid tumour was confirmed after surgery by histologic analysis.
surgery may be avoided if a scan shows diffuse disease. RID can guide the surgical approach if a recurrence is accurately localized. RID has been reported to have a sensitivity of 60% to 80% in this disease. Anti-CEA MoAbs were the first to be used for imaging of epithelial ovarian tumors, but, in the majority of recent studies, MoAbs B72.3, OC-125, and OVTL3 have been used with greater success [37].

Current diagnostic modalities have limitations in patients with either primary or recurrent ovarian cancer. Small tumor deposits, tumors in normal-sized lymph nodes, and diffuse mililiary disease (carcinomatosis) are not always detected by CT and/or MRI scans. It is also difficult for these scans to differentiate between recurrent disease and post-irradiation and post-surgical changes. Furthermore, serum tumor markers such as CA-125 are frequently negative in patients who have recurrent disease [38].

In a recent multicenter trial, 103 patients with known ovarian cancer underwent a RID study with 111In-labeled MoAb B72.3 (Oncoscint). The sensitivity per patient was 68%, with a specificity of 55%. Patient management was positively influenced in 27% of the cases [39]. However, because Oncoscint CV/OV can cross react with benign tumors of the ovary, this RID system has not been recommended in the workup of primary ovarian carcinoma. The investigators of this multi-center trial concluded and consequently recommended by FDA [40] that RID was helpful in determining the location and extent of extrahepatic disease, mediastial spread, and carcinomatosis in women with suspected recurrent ovarian cancer. An advantage of RID was the disclosure of omental metastases by SPECT, whereas neither CT nor transabdominal ultrasound could conclusively reveal these tumor sites. RID was also effective in detecting occult disease in 35% of patients, which significantly contributed to better patient management. Satumomab pendetide can be the primary imaging study in patients with rising serum markers (CA-125) [34].

In an important prospective multicenter French trial of 111In OC-125, recurrent ovarian cancer in 47 patients could be excluded with RID alone, whereas RID combined with CT had an even higher sensitivity (>80%) for detecting recurrent tumor sites in early stage disease [38]. In another study on the use of 111In OVTL3 F(ab')2 fragment, ovarian tumors were detected in 16 of 17 patients (94%). Of the 45 tumor sites found at surgery, 67% were localized by RID, whereas CT and ultrasound visualized 53% and 23%, respectively [41].

SM3 (anti-stripped mucin 3) is a MAb against a core protein epitope of the polymorphic epithelial mucin antigen and was devised when it was shown that cancer cells have a reduced ability to glycosylate glycoprotein in their surface membrane. Typical specificity in ovarian cancer for 99mTc MAb such as SM3 is 75%, sensitivity 100% and overall accuracy 93% with a positive predictive value of 92%, a negative predictive value of 100% at a prevalence of 76% [35].

The majority of studies reported in the literature to date clearly show that MoAbs are safe and effective agents for evaluation of recurrent ovarian carcinoma. The two clinical settings where RID can have the most impact are (1) before and after chemotherapy, for assessment of response (to eliminate the need for second-look surgery) and (2) for assessment of the locale of recurrent/residual tumor after “curative” surgery when serum markers indicate recurrent disease [37].

**Lung Carcinoma**

Both small-cell (SCLC) and non-small-cell carcinoma (NSCLC) of the lung have been studied by means of RID techniques. A multicenter study using a 99mTc-labeled anti-lung antibody Fab unit (NR-LU-10) yielded promising results for staging of SCLC and NSCLC, with sensitivities of 77% and 88%, respectively [42,43]. RID did very well in staging of patients with SCLC. In fact, the single total-body survey with MoAbs was as accurate as the combination of the “standard” battery of tests that are used for staging of these patients (that is, CT of the head, chest, and abdomen, bone scan, and bone marrow aspiration). Patients with extensive disease at the time of diagnosis were accurately staged by RID with a >95% positive predictive value [4].

In another multicenter trial, patients with NSCLC were evaluated with the same MoAb in an effort to determine whether RID could increase the accuracy of clinical staging, thus potentially eliminating unnecessary surgery [4]. Of 89 patients with NSCLC, 86% of those with extensive disease were accurately identified by RID, with a positive predictive value >99%. RID was particularly helpful in identifying mediastinal disease; the mediastinum is a region that is difficult to identify on CT. Currently, CT and MRI scans are only 60% to 70% sensitive for detection of mediastinal involvement with cancer in patients with NSCLC.

The role of RID studies in patients with: (1) NSCLC would be to provide more reliable information on regional (mediastinal) nodal involvement that would affect surgical management, (2)SCLC to detect the extent of systemic disease. This information may help guide the medical management of the patient and are currently being studied extensively in Europe and USA; at least one of them has been recommended for FDA approval [35].

**Malignant Melanoma**

Several clinical trials have shown that melanoma can be imaged with MoAbs. A recent multicenter trial in which RID was used in patients with known metastatic melanoma yielded encouraging results. Detection rates were dependent on lesion size, location, and vascularity. Liver and bone lesions combined showed a detection rate of 87% for lesions of all sizes and 90% for lesions > 1 cm. For subcutaneous and lymph node lesions combined, these rates were 68% and 77%, respectively. Lung lesions were the least sensitive, with a detection rate of 55% [44]. Accordingly, several investigators, concluded that the detection of previously unknown sites was particularly helpful in directing patient management [45, 46]. However, these MoAbs have been limited in their application by their low sensitivity in the detection of parenchymal disease.
and by their high false-positive rates in the detection of regional lymph node involvement in primary melanoma [46].

B-Cell Lymphoma

Technetium-99m-labeled LL2 Fab’ (anti-CD22) is being currently studied in phase II trials and will probably play an important role in the evaluation of B-cell lymphoma [47]. As yet, however, there is no firm evidence suggesting that adioimmunodetection is more sensitive than radigallium scintigraphy, although it will probably be more specific [48].

Breast Cancer

A number of clinical trials with different antibodies and labels have been used for breast cancer imaging, including CEA, B6.2, B72.3, M8, 791T/36, 3E1.2, 3C6F9, HMFG1, and HMFG2 [49]. The study results are quite variable, as can be expected when different MoAbs, routes of administration, radiolabels, and patient groups are used. Much effort has been directed toward developing a RID system that would primarily demonstrate breast cancer metastases in regional lymph nodes that are difficult to detect with standard radiologic procedures or on physical examination. Multiple studies with different antibodies have demonstrated sensitivities in the high eighties and specificities in the low nineties for lymph node metastases [2, 50]. Some investigators used SPECT technology, and others used planar imaging techniques only. Although many of the studies show promising results, additional prospective trials are needed for assessment of the diagnostic accuracy of RID in preoperative patients [47].

Prostate Cancer

For patients thought to have localized disease amenable to cure, either by radical retropubic prostatectomy or radiation therapy, accurate assessment of the local extent of the tumor is critical. Unfortunately, there is no accurate or reliable noninvasive imaging modality that can adequately assess pelvic lymph node involvement, and this makes surgical staging necessary. Routine 99mTc bone scans are not specific for prostatic cancer. Similarly, abdominal/pelvic CT studies fail to detect lesions in localized prostatic cancer (sensitivity = 44% to 69%). Transrectal ultrasound frequently fails to differentiate stage B (local disease) from stage C (extra capsular extension) prostate cancer, which is necessary to identify tumors that are amenable to surgery. The use of RID in men with suspected localized disease may enhance the clinician’s ability to evaluate patients accurately in a noninvasive fashion. Active investigations with MoAb (Cyt-356) in men with limited or local disease continue with promising for the detection of soft-tissue lesions [51]. Prostascint is a murine IgG1 monoclonal antibody that targets prostate-specific membrane antigen (PSMA), which is more highly expressed in malignant cells (especially metastatic lesions) [52]. The ProstaScint® scan was approved by the U.S. Food and Drug Administration in 1997 for the staging evaluation of men with a high risk of disease spread beyond the prostate [53]. Generally, the main suggestions for its use in cancer prostate till now is in:

1. Preoperative staging of newly diagnosed persons with biopsy-proven prostate cancer that is thought to be clinically localized after standard diagnostic evaluation, but who have a moderate to high probability of occult extraprostatic metastasis;

2. Staging of post-prostatectomy persons or persons treated with radiation therapy in whom there is a high suspicion of undetected residual prostate cancer or cancer recurrence Manyak et al. [54] recently demonstrated, in men who underwent pelvic lymphadenectomy for high-risk nodal disease based on serum PSA level, Gleason score, and advanced clinical stage, that the ProstaScint® scan was associated with a sensitivity and specificity of 62% and 72% for nodal disease, respectively. In comparison, the sensitivities of CT and MRI were 4% and 15%, respectively, and the specificities for both were 100%. Therefore, use of ProstaScint®, together with serum PSA, histologic grade, and clinical stage, appears to provide additional predictive information.

A recent multicenter study documented the incidence of prostate fossa (PF), pelvic node, and extrapelvic (EP) uptake of prostascint among different clinical settings [55]. From this investigation and others [45], the approximate values for diagnostic parameters in the post-surgery setting are sensitivity = 75% (extraprostatic) and 92% (PF), specificity = 86%, positive predictive value = 81%, and negative predictive value = 67%. According to these results, Jani and his colleagues [56] recently used RIS to identify the patient population most likely to benefit (or not to benefit) from RT and, in theory, this would lead to improved locoregional control of persistent or recurrent prostate cancer and improve the ultimate outcome of prostatectomy patients. They demonstrated that in the select group of patients who have a rising PSA postradical retropubic prostatectomy (RPP) or are at high risk of failure post-RRP, and in many cases having negative bone scan or abdomen or pelvis CT scan, the RIS scan provides additional information that is useful in guiding the radiotherapy (RT) decision making. This was demonstrated in 2 ways: (a) altering the decision to offer RT and (b) altering the decision to offer RT to a different target volume than initially intended (Figure 3).

Non-Oncological Applications of Radiolabeled Antibodies

Inflammation with and without Infections:

Scintigraphic detection of inflammation makes possible the determination of both the localization and the number of inflammatory foci throughout the body. Since scintigraphic images are based on functional (physiological and/or biochemical) changes of tissues, inflammatory processes can be visualized in their early phases, when anatomical changes are not yet apparent. Moreover, the effectiveness of anti-inflammatory therapies can be monitored since scintigraphic imaging enables accurate staging of inflammatory diseases.
Over the last 30 years multiple approaches to visualize inflammatory foci using radionuclides and gamma cameras have been developed. All inflammatory processes develop along a known sequence: locally increased blood supply, leakage of fluid, small molecules and proteins, and infiltration of cells. Scintigraphic imaging of inflammation can be achieved in various ways, depending on which aspect of the inflammatory process is to be addressed (Table 1) [57].

Nonspecific Uptake in Inflammatory Foci (Polyclonal Immunoglobulin):

Polyclonal immunoglobulin (HIG) has been introduced more recently for imaging inflammation (Figure 4), and in spite of early claims of several active mechanisms of localization, is now known to accumulate on the basis of increased permeability with consequent nonspecific extravasation of HIG [58]. $^{111}$In or $^{99m}$Tc-labeled HIG has been extensively tested in a large number of clinical studies. It has shown excellent performance in the localization of musculoskeletal infection (Figure 5) and inflammation [59]. In addition, good results have been reported in pulmonary infection—particularly in immunocompromised patients [60, 61], and abdominal inflammation [62]. Poor sensitivity of radiolabeled HIG is found in the diagnosis of endocarditis and vascular lesions in general, due to long lasting high levels of circulating activity. A general limitation is the long time span between injection and final diagnosis (24-48 h) [57].

Although, $^{99m}$Tc-HIG is commercially available the evidence suggests that $^{111}$In-HIG is better, especially in more chronic inflammation/infection [63]. This is because the $^{111}$In transchelates from HIG to extravascular local proteins, leaving the HIG to diffuse back into plasma [64]. $^{99m}$Tc-, on the other

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<td>Enhanced blood flow</td>
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Table 1: Overview of Nonspecific and Specific Radiotracers for Imaging Inflammatory Processes. (Rennen et al., 2003)
these methods aimed to label white blood cells in vivo. Such labeling procedures can be easier and do not require handling of potentially contaminated blood. The use of radiolabeled monoclonal antibodies against surface antigens as present on granulocytes was one of the first attempts to accomplish in vivo labeling of granulocytes. At least three $^{99m}$Tc-labeled anti-granulocyte antibodies have been tested for imaging inflammation: anti-NCA-95 IgG (BW250/183), a Fab’-fragment of IgG directed against NCA-90 (Immuno-MN3, leukoscan’: anti-CD66), and anti-SSA-1 IgM (LeuTech’: anti-CD15). Each of these anti-granulocyte antibodies made possible an accurate delineation of inflammatory foci. It was soon realized that the in-vivo behavior of these labeled anti-granulocyte antibody preparation did not mimic the behavior of radiolabeled granulocytes.

In general, blood clearance of the IgG preparations was much slower, giving a high background radioactivity that decreases slowly with time. For that reason the time interval between injection of the labeled antibodies and the acquisition of images is relatively long in order to get good target-background ratios. Furthermore, no initial lung entrapment was seen and splenic uptake was much lower, while the preparations based on antibody fragments (Fab, Fab’) had a much higher renal excretion. Becker et al. [10] showed that less than 10% of the radiolabeled BW250/183 antibody as present in the blood was actually associated with granulocytes. These observations indicated that the anti-granulocyte antibody approach for imaging inflammation, although feasible did not represent a method to label white blood cells in vivo. It is now generally accepted that radiolabeled anti-granulocyte antibodies localize in inflammatory foci mainly by nonspecific extravasation as a result of locally enhanced vascular permeability, and that binding of the antibody to infiltrated granulocytes in the inflamed tissue may contribute to the retention of the radiolabel in the focus [57].

The $^{99m}$Tc-labeled antigranulocyte Fab’-fragment (Leukoscan”) has been registered in Europe as an infection imaging agent [65]. It is murine monoclonal antibody fragment specific for surface glycoprotein designated as Nonspecific Cross-reactive antigen (NCA-90) (surface glycoprotein) on neutrophils not present on monocytes or lymphocytes (Figures 6 and 7). It has been applied to visualize infectious foci in patients with a sensitivity between 80 and 90% (Figure 8) [66]. It was useful in the evaluation of vascular graft infection and prosthetic heart valve infection. Good results were also obtained in the evaluation of patients with inflammatory bowel disease, although the agent appeared to be less accurate compared to labeled granulocytes [67]. Pulmonary infections with the exception of lung abscesses were not visualized. Peripheral bone infections were adequately visualized, but the sensitivity decreased in case the focus was located closer to the spine [57].

E-Selectin and Endothelial Role in Inflammation:

An interesting approach to imaging inflammation is to target endothelial adhesion molecules that are expressed during inflammation [66]. Endothelium is metabolically

Figure 5: Anterior image of the lower part of a 41-year old female patient with focal infection in the left ankle and around the left hip prothesis, 24 h after injection of 740 MBq $^{99m}$Tc-HIG.
very active and orchestrates leucocyte migration through the activation of a cascade of adhesion molecules, which govern leucocyte margination, adherence, spreading and eventually transendothelial migration. Adhesion molecules attractive for imaging inflammation are those that are: (1) only expressed during inflammation (i.e. are not constitutively expressed), (2) present only on endothelium, (3) expressed on the luminal side of the endothelial cell, (4) not shed into the circulation, and (5) internalized along with the monoclonal antibody following binding. E-selectin, a member of the selectin family, fulfills these specifications rather well. It is synthesized de novo over a period of about 45 min by the endothelial cell in response to several cytokines. After antibody binding to E-selectin, the immune complex is internalized, with only minimal shedding into the circulation, in contrast to vascular cell adhesion molecule-1 (VCAM-1), for instance, which is largely shed when it binds specific antibody [68]. Bhatti et al. [69] have characterized E-selectin expression which is closely linked to lymphocyte and neutrophil migration indicating its effectiveness in imaging chronic inflammation (Figure 9).

Jamar et al. [70], compared the efficacy of $^{111}$In-anti-E-Selectin mAb and $^{99m}$Tc-labelled human non-specific immunoglobulin in imaging of rheumatoid arthritis and they found that E-Selectin was better in sensitivity and specificity with higher joint-to-soft tissue ratio (Figure 10).

**Cardiac Diseases:**

**Indium-111 Antimyosin Antibody and Myocardial Damage:**

Loss of cell membrane integrity allows cell swelling and heralds cell necrosis. In cardiomyocytes, loss of sarcolemma allows soluble intracellular macromolecules (such as troponin, creatine kinase, and myosin light chains) to wash out in the bloodstream and be measured as an indicator of severity of necrosis. On the other hand, the insoluble macromolecules (such as heavy chains of myosin) remain immobilized until removed by the scavenger cells. An antibody specifically directed against the heavy chain of myosin (antimyosin antibody) allows differentiation of necrotic cells (with disintegrated sarcolemma) from viable cells (with intact sarcolemma) [71]. The necrotic myocardial regions, which allow binding of antimyosin antibody to the exposed myosin heavy chain, can be noninvasively localized by radionuclide imaging if the antibody is appropriately labeled with a γ-emitter and is administered intravenously. Antimyosin antibody has
been successfully used for the detection of myocardial necrosis associated with acute myocardial infarction [72]. Antibody uptake in infarction occurs discretely in the myocardial territory supplied by the occluded coronary artery [73]. Nakata and Shimamoto [74] added that monoclonal antibody uptake is only dependent on the extent of infarcted myocardium and the intensity of uptake cannot predict the patency of an infarcted coronary artery. Yamada et al. [75], reported a good inverse relationship between antimyosin uptake and thallium-201 uptake with the classification of these patients into match, mismatch and overlap patterns (Figures 11 and 12).

Cardiovascular disorders characterized by multifocal myocardial necrosis, such as myocarditis and cardiac allograft rejection, show diffuse antibody uptake in the cardiac region [73, 76]. All patients with histologically verified myocarditis have positive antimyosin scan findings representing high sensitivity [71]. In contrast, no antimyosin-negative patients have biopsy evidence of myocarditis (Figure 13). Therefore, antimyosin antibody has been recommended as a useful screening tool in adults with suspected myocarditis [75]. The feasibility of using antimyosin scintigraphy in children with clinically suspected myocarditis has not been investigated.

On the other hand, Kremer et al. [77] reported their results of a pilot study using 111In-AM scintigraphy to detect early myocardial damage before the ability of the conventional methods to detect cardiac dysfunction in patients treated with anthracyclines as a critical number of cells may be damaged before the functional myocardial impairment is detected.

Radiolabeled Annexin A5 (V) and Imaging of Programmed Cell Death:

Apoptosis or PCD is known to occur during physiologic as well as pathologic processes in the human body, such as growth and inflammatory events on the one hand and Alzheimer’s disease or myocardial infarction (MI) on the other hand. With the latter, both cardiomyocyte dysfunction and irreversible cell loss might occur, which can lead to the decline in left ventricular contraction, one of the factors related to earlier death [78]. Better understanding of the cellular apoptotic pathway may
lead to the development of new therapeutic drugs that might limit this cardiomyocyte loss [79].

Radiolabeled annexin V, a human protein can be used to image apoptosis in vivo. The ability of radiolabeled annexin V has a reversible, calcium-dependent, nanomolar affinity for phosphatidylserine (PS). PS comprises 10-15% of the total phospholipids content of plasma cell membrane and is normally restricted to the inner leaflet of the plasma membrane lipid bilayer by an ATP-dependent enzyme “translocase”. Translocase in concert with a second ATP-dependent enzyme, “floppase”, that pumps cationic phospholipids such as phosphatidylcholine (PC) and sphingomyelin to the cell surface, maintains an asymmetric distribution of different phospholipids between the inner and outer leaflets of the plasma membrane. With the onset of apoptosis, PS is rapidly redistributed onto the cell surface within 30 to 120 minutes of signaling in culture (Figure 14). The number of anexine V binding sites per cell with the onset of apoptosis increases 100 to 1000-fold during apoptosis, reaching values of 3-4 millions in some cell lines. The redistribution of PS and PC across the cell membrane at the beginning of the execution phase of apoptosis is also facilitated by a calcium ion dependent deactivation of translocase and floppase and activation of third enzyme called, “scramblase”. The externalization of PS is a general feature of apoptosis occurring prior to membrane bleb formation and deoxyribonucleic acid (DNA) degradation.

Thimister et al. [80] reported a protocol of imaging including combined 99mTc-MIBI and 99mTc-annexin A5 images for a similar group of patients, the area at risk could be well defined on the MIBI pictures. Because of the storage of 99mTc-MIBI in the mitochondrion of cardiomyocytes (and lack of redistribution), imaging could be postponed until after revascularization was achieved. Furthermore, a MIBI image of the heart could still be seen vaguely (“99mTc-MIBI ghost-image”) in the unaffected myocardial area on the 99mTc-annexin A5 scintigraphy. This technique allowed better SPECT reconstruction and much better localization of the 99mTc-annexin A5 activity in the heart of all patients at the myocardial region corresponding to the 99mTc-MIBI defect. So the question remains whether enhanced 99mTc-annexin A5 uptake in the heart is caused by an ongoing cell-death program or whether it is more the expression of the presence of necrotic cells as can be seen in the final stage of cell death [81]. The decreased 99mTc-MIBI defect in the subacute phase, when compared with the acute phase of the MI, strongly suggests that at least part of the myocardial 99mTc-annexin A5 activity as present in the acute phase represents potentially reversible myocardial cell damage rather than necrosis (Figure 15). In addition, Thimister et al. [80] observed the decreased 99mTc-annexin A5 activity in a patient who was repeatedly studied 3 and 8 d after the MI onset. Because no activity remained after 8th d, regeneration of myocardial cells might have taken place, in which case PS expression is no longer present. This

**Figure 13:** (A) Absence of myocardial antimyosin uptake (HLR = 1.4) in a scan with normal findings. (B) Moderate myocardial antimyosin uptake (HLR = 1.8) in a patient with myocarditis. (C) Intense myocardial antimyosin uptake (HLR = 2.5) in a patient with biopsy-verified myocarditis. (D) Right ventricular endomyocardial biopsy demonstrating a central focus of necrotic myocytes surrounded by lymphomononuclear cell infiltrate (arrows) diagnostic of myocarditis (hematoxylin and eosin, x200).

**Figure 14:** Molecular basis of imaging myocyte apoptotis. As a result of enzymatic alteration, during apoptotic cell death, normal phospholipid asymmetry within the sarcolemmal lipid bilayer is lost, resulting in exteriorization of PS. Because macrophages have PS receptors, exposure of PS acts as an “eat-me signal” and apoptotic bodies are removed. An endogenous protein, Annexin V possesses nanomolar affinity for externalized PS (epitaph!) and is radiolabeled for noninvasive detection of apoptotic cell death.

**Figure 15:** Combination of acute 99mTc-MIBI & 99mTc-annexin A5 uptake in area at risk on day 1 in patient.
would imply either that the ischemic part of the MI has been restored to viable tissue or that cells have been removed as necrotic material. They concluded that the main clinical utility of this in vivo detection of cell death with 99mTc-annexin A5 scintigraphy would be the evaluation of new therapeutic strategies that intervene in myocardial cell death, the so-called programmed cell death. Reversal of programmed cell death can be done by reperfusion in the adjustable time.

Figure 16: Hypothesizing an ischemia-apoptosis-necrosis spectrum. Coronary artery occlusion leads to ischemic changes. Annexin A5 imaging data support that ischemia is indeed equivalent to early phase of apoptosis, which is represented by death receptor upregulation, PS exteriorization, and caspase-3 activation. In cardiomyocytes, cell death by apoptosis is unlikely since reduction in blood supply depletes adenosine triphosphate and death may ensue by secondary necrosis. PS-flagged death mechanisms may be amenable to annexin A5 imaging. Reversal of programmed cell death can be done by reperfusion in the adjustable time.

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