

## Scintigraphic detection of colon carcinomas with iodinated monoclonal antibodies

V. Artiko<sup>1</sup>, M. Petrovic<sup>1</sup>, Z. Jankovic<sup>2</sup>, Lj. Jaukovic<sup>2</sup>, D. Sobic-Saranovic<sup>1</sup>, I. Grozdic<sup>1</sup>, S. Odalovic<sup>1</sup>, S. Pavlovic<sup>1</sup>, E. Jaksic<sup>1</sup>, M. Zuvela<sup>1</sup>, B. Ajdinovic<sup>2</sup>, S. Matic<sup>1</sup>, V. Obradovic<sup>1</sup>

<sup>1</sup>Center of Nuclear Medicine, Clinical Center of Serbia, Faculty of Medicine, University of Belgrade, Belgrade; <sup>2</sup>Military Academy Belgrade, Belgrade, Serbia

### Summary

**Purpose:** The aim of this study was to evaluate the clinical reliability of the immunoscintigraphy with iodinated monoclonal antibodies for the detection of metastases and recurrences of colon carcinomas.

**Methods:** A total of 45 patients with colon carcinoma was investigated with gamma camera, after intravenous application of iodinated monoclonal antibodies.

**Results:** The sensitivity of the method was 90%, specificity 86%, positive predictive value 93%, negative predictive value 80% and accuracy 87%. There was statistically significant relationship between immunoscintigraphic and ultrasonographic (US) findings ( $p=0.005$ ). Also, there was significant relationship between immunoscintigraphy and Dukes

stage ( $p=0.019$ ). Tumor marker levels were not significantly correlated with immunoscintigraphic findings ( $p>0.05$ ). Significant difference was noted in patients with positive findings for malignancy on US and immunoscintigraphic findings ( $p=0.006$ ), i.e. patients with positive findings for malignancy had more frequently immunoscintigraphic findings of malignancy. Correlation with other diagnostic procedures (rectoscopy, colonoscopy, CT) did not show significant correlations.

**Conclusion:** We conclude that immunoscintigraphy can be helpful in the detection of metastases and recurrences of colon carcinomas.

**Key words:** colon carcinoma, I-131, immunoscintigraphy, monoclonal antibodies

### Introduction

More than 50% of patients with surgical resection of primary colorectal carcinoma will develop disease recurrence within the next 2 years [1]. Prompt diagnosis of the recurrent disease will influence the final patient outcome. The diagnostic modalities most frequently used are barium enema and colonoscopy, which is considered as the gold standard. However, there are other imaging noninvasive modalities currently used for disease staging, restaging, choice of the biopsy site, as well as for follow up after completion of therapy and for radiotherapy planning. Anatomical imaging methods, such as US, computed tomography (CT), and magnetic resonance imaging (MRI), are mostly used. Thus, for local staging, superficial tumors are best evaluated using endorectal US, which can estimate the tumor ingrowth into the rectal wall layers. More advanced local tumors are best

imaged using MRI. However, CT is not considered accurate in the early detection of a recurrence of colorectal carcinoma due to the difficulties to provide the accurate data owing to the distorted local anatomy after operation and to differentiate the fibrotic areas from the viable tissue after radiotherapy. This modality has, however, certain advantages, such as the performance of virtual colonoscopy. For the assessment of nodal metastases, neither US, nor CT can be reliably used for clinical decision making, because of the usually small size of many affected lymph nodes (<1 cm). MRI, using a lymph node specific contrast, can be useful in the detection of nodal disease. For the detection of distant metastases, abdominal US is used as a primary diagnostic tool for the detection of liver metastases, especially with contrast agents. Chest X-ray is used also as the first-choice technique, but multidetector CT is nowadays the best imaging method for staging and follow-up of these patients, because it

Correspondence to: Vera Artiko, MD, PhD. Center of Nuclear Medicine, Clinical Center of Serbia, Visegradska 26, 11 000 Belgrade, Serbia.

Tel/fax: +381 11 3615641, E-mail: veraart@beotel.rs

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allows visualization of the liver, abdomen in its entirety and the chest in one session. MRI is commonly used for the detection and characterization of liver lesions [2,3].

Morphological and functional imaging modalities, such as immunoscintigraphy with single photon emission computerized tomography (SPECT), as well as positron emission tomography (PET), provide valuable clinical information in the diagnosis of metastatic and recurrent disease, owing to their ability to detect viable tumor tissue, especially since fusion and hybrid techniques (PET/CT and SPECT/CT) have been introduced. Although PET and particularly PET/CT, mostly using  $^{18}\text{F}$ -fluorodeoxyglucose present great diagnostic potentials in all aspects of staging patients with metastatic and recurrent colorectal carcinomas, they are very expensive and not widely available. Recently, radioimmunoguided surgery (RIGS) was introduced, using conventional gamma and positron emitting radionuclides [4]. Immunoscintigraphy can also be used as a basis of application of immuno- or radioimmunotherapy [5].

The aim of this study was to evaluate the reliability of immunoscintigraphy with iodinated monoclonal antibodies for the detection of metastases and recurrences of colon carcinomas.

## Methods

A total of 45 consecutive patients (34 males, 11 females, mean age  $61.33 \pm 9.23$  years, range 38-78) with diagnosed colon carcinoma (primary, with recurrence and/or metastases) were included in the present study. Most of them ( $n=38$ ) were operated on with curative intent and investigated during 6 months - 3 years after surgery. In the remaining 7 patients with metastatic disease clinical diagnosis was carried out through several diagnostic methods (colonoscopy, CT, MRI, US, tumor markers levels and biopsy). Seventy-five percent of the patients ( $n=30$ ) had initially Dukes stage C disease and 12.5% ( $n=15$ ) stage A.

### Immunoscintigraphy

Immunoscintigraphy was performed using 30-min infusion of IMACIS 1, a cocktail of 111 MBq  $^{131}\text{I}$ MAb 19-9 F(ab')<sub>2</sub> and MAb anti CEA F(ab')<sub>2</sub>. Potassium iodide (600 mg/day) was administered orally for 10 days (starting 24 h before the injection) to block the uptake of free  $^{131}\text{I}$  into the thyroid gland. Imaging was carried out after 96-120 h. Planar images (~6 min per image, or at least 200,000 kcounts over the whole field of view), including anterior and posterior projections of the thorax, abdomen, and pelvis, were obtained using large field of view gamma camera, fitted with a parallel hole high energy collimator. In order to achieve more precise estimation of the localization of the pathologic lesions, as well as to increase the target-to-background ratio, dual isotope acquisition ( $^{99\text{m}}\text{Tc}$ -sulphur colloid) and subsequent subtraction of the obtained images were carried out.

### Patient selection

Selection of patients was based on anamnestic data, physical

examination, blood analyses, US, barium enema, rectoscopy/colonoscopy, CT, MRI, tumor markers estimations, and clinical follow-up of at least 6 months. The investigation was performed whenever there was a rise in serum levels of CEA and CA 19.9 and metastases or recurrences could be not located based on clinical, radiological (chest X-rays), imaging or endoscopic findings.

### Statistical considerations

Statistical analyses were performed using the SPSS programme (v.18 for windows). We calculated sensitivity, specificity, positive and negative predictive values, as well as accuracy. Correlations were estimated with Spearman's bivariate correlation test and  $\chi^2$  test was used for estimation of the differences between the methods used.

## Results

There were 27 true positive (TP), 12 true negative (TN), 2 false positive (FP) and 3 false negative (FN) findings. Of the 27 TP patients 16 had recurrences, 6 metastases, while 5 had both recurrences and metastases.

The sensitivity of the method was 90%, specificity 86%, positive predictive value 93%, negative predictive value 80% and accuracy 87%.

Bivariate correlation analyses showed that there was positive statistically significant relationship between immunoscintigraphy and US findings ( $r_s=0.504$ ,  $p=0.005$ ) and also between immunoscintigraphic findings and Dukes stage ( $r_s=0.420$ ,  $p=0.019$ ).

Tumor markers levels were not significantly correlated with immunoscintigraphic findings ( $p>0.05$ ). Chi square test showed significant difference in patients with positive findings for malignancy on US and immunoscintigraphic findings ( $p=0.006$ ). Correlation with other diagnostic procedures did not show significant differences ( $p>0.05$ ; Table 1). Figures 1 and 2 demonstrate two characteristic cases with disease recurrence in the colon, liver and the lymph nodes.

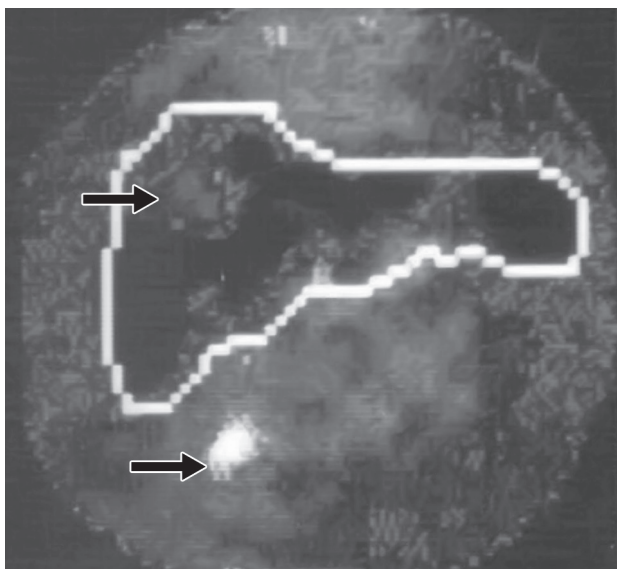
## Discussion

Noninvasive imaging methods include nuclear

**Table 1.** Correlation of immunoscintigraphy with other diagnostic methods

	Immunoscintigraphy	
	$r_s$	$p$ -value*
Tumor marker level	0.577	0.134
Colonoscopy	-0.310	0.226
Rectoscopy	0.138	0.539
US	0.504	0.005
CT	0.500	0.170
Dukes stage	0.420	0.019

\* $p$ -value with Spearman's correlation test



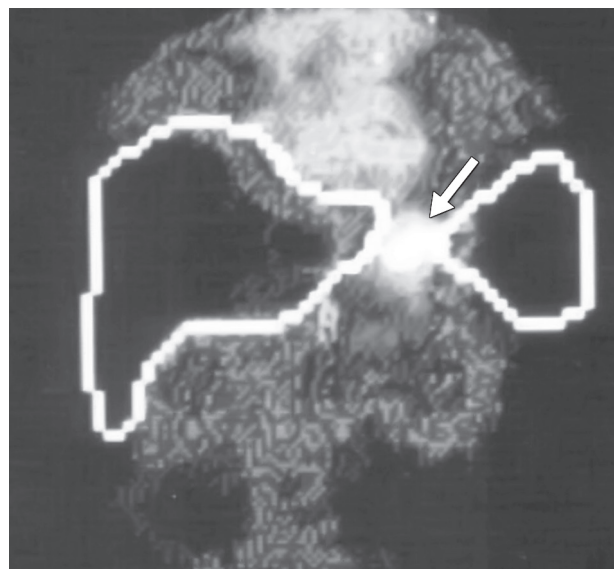
**Figure 1.** A patient with colon adenocarcinoma, two years after surgery. Immunoscintigraphy, anterior view, after subtraction of radiocolloid image, showed two hot spots in the abdomen: one in the ascending colon pointing out recurrence (arrow) and the second in the upper part of the right liver lobe, pointing out liver metastases (arrow).

medicine tests, MRI, CT and US. Monoclonal antibodies are high-affinity molecules that can be used for specific, high-signal delivery from the cell surface. However, studies indicate that there are several factors that influence successful targeting and imaging. These include stability of the monoclonal antibody fragment, the labeling chemistry (direct or indirect), whether critical residues are modified, the number of antigens expressed on the cell surface, and whether the target has a rapid recycling rate or internalizes upon binding [6].

In this study, 45 patients were investigated. There were 27 TP, 12 TN, 2 FP and 3 FN cases. From 27 TP patients 16 had recurrences, 6 metastases, while 5 had both recurrences and metastases. TN results were registered in 12 patients. In two patients with local inflammation, FP findings were obtained. In 3 patients FN results were obtained because of the small size of the lesion (1.5 cm).

Bivariate correlation analyses showed that there was statistically significant positive relationship between immunoscintigraphic and US findings ( $r_s=0.504$ ,  $p=0.005$ ). Also, there was significant positive relationship between immunoscintigraphic findings and Dukes stage ( $r_s=0.420$ ,  $p=0.019$ ), meaning that in advanced disease stages the clinical value of immunoscintigraphy is better, as well as that immunoscintigraphic findings correlate with the clinical disease course.

We also noticed that tumor marker levels were not significantly correlated with the immunoscintigraphic findings ( $p>0.05$ ). Correlation with other diagnostic



**Figure 2.** A patient with colon adenocarcinoma, 18 months after surgery. Immunoscintigraphy, anterior view, after subtraction of radiocolloid image, showed a hot spot (arrow) between liver and spleen, pointing out metastases in the abdominal lymph nodes.

procedures (Table 1) did not show statistical differences ( $p>0.05$ ).

Our results are in accordance with the results of other authors using this radiopharmaceutical. Thus, Chatal et al. [7], using  $^{131}\text{I}$  labeled CA19-9 monoclonal antibody or its F(ab')<sub>2</sub> fragments, showed significant accumulation in 66% of colorectal cancer sites. Baum et al. [8] obtained high sensitivity (82%) and specificity (90%), especially in the diagnosis of pelvic recurrences and intra-abdominal metastases. Our preliminary results using the same radiopharmaceutical, proved its value in the detection of recurrences, and in liver and extrahepatic metastases [4]. According to Jang et al. [9], similar to our results, the serum tumor markers level was not correlated with positive tumor uptake in immunoscintigraphy. Similarly, the sensitivity and specificity of immunoscintigraphy based on surgery, CT, US and pathology, were 90.5 and 100%, respectively. Like in our study, they emphasized the second-tracer technique as essential for anatomical landmark by using a double isotope scan. In addition, according to Sohn et al. [10], the sensitivity and specificity of radioimmunoscintigraphy with IMACIS1 were 80 and 100% respectively, tumor detection rate was not proportional to the level of serum tumor markers, second-tracer technique was essential for tumor localization as an anatomic landmark, tumor/non-tumor radioactivity was most elevated at 7 days delayed imaging and using planar scintigraphic technique it was possible to image most of the tumors. Furthermore, Naruki et al. [11] with IMACIS1 obtained accumulation of radioactivity in the primary



tumor and precise visualization of the liver metastases. Thus, detection of liver metastasis was better than in primary or recurrent tumors, with the recommendation of the delayed imaging (5-7 days). Like in our investigation, there was no correlation between serum concentration of CEA or CA19-9 and the visualization of tumors. Contrary to ours and the results of other authors, Holting et al. [12] using immunococktail of  $^{131}\text{I}$  labeled  $\text{F}(\text{ab}')_2$  fragments of monoclonal antibodies against CEA, with CA 19-9, found disappointing immunoscintigraphic results in comparison to other diagnostic modalities, especially concerning extrahepatic tumor diagnosis. Similarly, Schlag et al. [13], using the same radiopharmaceutical concluded that immunoscintigraphy cannot give information beyond that of conventional diagnostic tools for indication or planning of operative strategy in the treatment of recurrent colorectal cancer.

Some authors performed immunoscintigraphy with  $^{123}\text{I}$  labeled antibodies. Goldenberg et al. [14] using immunoscintigraphy with  $^{123}\text{I}$  labeled fragments,  $\text{F}(\text{ab}')_2$  and  $\text{Fab}'$ , of IMMU-4, and anti-CEA monoclonal antibody (Immu-RAID-CEA) showed that this imaging method complemented CT findings by confirming suspected tumors and disclosing occult lesions. Also, Bischof-Delaloye et al. [15] proved that immunoscintigraphy with SPECT based on  $^{123}\text{I}$ -labeled anti-CEA monoclonal antibody allows early detection of recurrence or metastasis of colorectal cancer, thus reducing the delay between diagnosis and treatment. Wong et al. [16] evaluated an engineered intermediate-molecular-mass radiolabeled antibody construct directed against CEA (cT84.66). They demonstrated tumor targeting in colorectal cancer and a faster clearance in comparison with intact antibodies, making it appropriate for further evaluation as an imaging and therapeutic agent. The conclusion was that  $^{123}\text{I}$ , because of its favorable physical characteristics, allows easy performance of SPECT, which is the advantage, but has much shorter half life which doesn't allow acquisition of the delayed images, an issue that has been emphasized as very important in this type of investigation [8-10], and is much more expensive in comparison to  $^{131}\text{I}$ .

Some investigators tried to compare the results obtained by PET and immunoscintigraphy. According to Ito et al. [17] the usefulness of PET and immunoscintigraphy (by means of  $^{131}\text{I}$  or  $^{111}\text{In}$  anti-CEA monoclonal antibody) was confirmed for the diagnosis of recurrent colorectal cancer. They concluded that, although PET reflects the biological character of the tumor and makes a more accurate diagnosis when combined with regular CT and MRI, this technique cannot provide the specificity of an antibody-based functional imaging agent, and cannot help select patients for antibody-based therapy.

However, Willkomm et al. [18] point out that both FDG PET and immunoscintigraphy are suitable for the diagnosis of local recurrence of colorectal carcinoma, but that FDG PET alone is clearly superior in the detection of distant metastases (liver, bone, lung) and lymph node involvement. However, considering that PET has an increasingly important role in the diagnosis, staging, and monitoring response to treatment in a variety of cancers, recent efforts have focused on immuno-PET, which uses antibody-based radiotracers to image tumors based on the expression of tumor-associated antigens. This way, the specificity allowed by antibody targeting should improve both tumor detection and provide information related to primary and metastatic lesions that will guide therapeutic decisions. Advances in antibody engineering are providing the tools to develop antibody-based molecules with pharmacokinetic properties optimized for use as immuno-PET radiotracers [19].

According to Florio et al. [20] application of RIGS demands close cooperation between nuclear medicine physicians and surgeons. It can be performed using colloid radiotracers, monoclonal antibodies or various non-immunological tracers. The way and time of application of the radiopharmaceutical can also be variable. It is being used for radioguided occult lesion localization and sentinel lymph node biopsy, as well as for pre- and intraoperative staging of primary and recurrent colorectal cancers. This method gives a chance for an optimal radical surgical and oncological treatment in localized and recurrent cancer. According to Lechner et al. [21], in 30% of the cases this method led to an up-staging of the disease. Furthermore, metastatic spread to lymph nodes was not always very close to the primary tumor. According to these investigators, this is the way to precisely identify even very small tumor deposits, leading to accurate staging even during operation. RIGS is found to be particularly useful in recurrences and in small tumor deposits which are difficult to localize. According to Sun et al. [22] RIGS with  $^{125}\text{I}$ -labeled anti-TAG-72 antibody provides opportunities for intraoperative cancer detection of both big and small tumors. Kim et al. [23] reported that RIGS with two  $^{125}\text{I}$ -labeled anti-CEA antibodies facilitated accurate detection and removal of occult cancer foci in colorectal cancer. Similarly, Hladik et al. [24] and Mery et al. [25] emphasized the value of immunoscintigraphy and RIGS in order to improve the pre- and intraoperative localization of colorectal cancer lesions. However, all of the authors emphasize the importance of further research and prospective studies in order to estimate the precise usefulness of this methodology and its impact on survival parameters [20-25].

The preclinical data presented are compelling, and it is evident that antibody-based molecular imag-

ing tracers will have important future role in the diagnosis and management of cancer and other diseases [6]. Some authors pointed out that soon SPECT/CT, PET/CT and RIGS with the development of new radiopharmaceuticals/antibodies may enable patient selection for radioimmunotherapy [25,26]. Because of the fact that  $^{131}\text{I}$  is also a beta emitting radionuclide currently used in radionuclide therapy of various diseases, (hyperthyroidism, thyroid carcinoma) it is necessary to emphasize that  $^{131}\text{I}$  labeled antibodies can have the best potential to be used not only for diagnosis but also and for radioimmunotherapy.

## Conclusion

The main advantages of immunoscintigraphy consist of the ability to estimate tumor tissue viability, as well as whole body imaging at the same time. Therefore, immunoscintigraphy is a very suitable method for staging colorectal cancer patients before and during their follow up after surgery, and a big help in the choice of appropriate and due-time therapy. Furthermore, the hybrid SPECT/CT, PET/CT, RIGS and radioimmunotherapy open a new field for application of radiolabeled antibodies.

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