The influence of Ki-67 proliferation index on Tc-99m-EDDA-HYNIC-TOC somatostatin receptor scintigraphy in patients with carcinoids

Abstract: The aim of this paper is to determine the influence of Ki-67 proliferation index on somatostatin receptor scintigraphy (SSRS) with Tc-99m-EDDA-HYNIC-TOC (Tc-99m-Tektrotyd) somatostatin analogue in patients with carcinoid tumors. Sixty-one patients (31 female, 30 male; age range: 33-76 years) were examined: 13 patients highly suspected of having a carcinoid, and 48 patients who had undergone the surgical removal of the tumor. Whole body SSRS at 4 h postinjection, spot scintigrams and SPECT of the selected regions were obtained for all patients. Tc-99m-Tektrotyd scintigraphy was classified as true positive in 26 out of 30 and true negative in 24 out of 28 patients. The sensitivity of Tc-99mTc-Tektrotyd scintigraphy was found to be as high as 94.74% in the group of patients with low mitotic index Ki67 (<2%), and it progressively decreased in patients with higher mitotic index (77.78% for Ki67 2-15% and 20% for Ki67% >20%). The likelihood of Tc-99m-Tektrotyd scan being positive when a carcinoid is present was found to be inversely proportional to the value of Ki67 proliferation index. The results showed that Tc-99m-Tektrotyd SSRS is a sensitive method for diagnosing and staging patients with well-differentiated carcinoid tumors. However, in poorly differentiated tumors with high Ki67 proliferation index, additional analyses are necessary for precise staging.

Keywords: carcinoid tumor, somatostatin receptor scintigraphy, proliferation index Ki67

DOI: 10.1515/chem-2015-0042
received February 28, 2014; accepted June 30, 2014.

1 Introduction

Neuroendocrine tumors (NETs) comprise quite a varied group of neoplasms which originate from endocrine cells and are distributed throughout the digestive or respiratory tracts, islets of the pancreas, adrenal medulla and sympathetic ganglia, as well as in the C cells of the thyroid gland. Different subtypes of NETs can develop in almost all organs and tissues, anywhere in the human body [1,2]. Carcinoid tumors are the subtype of NETs, accounting for less than 1% of all malignancies [2]. The majority of carcinoids arise in the gastroenteropancreatic and bronchopulmonary system [3]. Carcinoids are usually small, slow-growing tumors, the metastases of which are related to the site and the size of the primary lesion.

These tumors differ from other malignancies in the following characteristics: low incidence, specific histological composition, low proliferation rate, hypersecretion of biologically active substances, as well as the unique property of excessive somatostatin receptor expression [1]. A very important characteristic of these cells is their ability to synthesize biogenic amines and peptide hormones, which results in extremely diverse clinical features.

When the presence of carcinoid is confirmed, it is vital to determine its differentiation and proliferative activity. Based on the degree of differentiation, the World Health Organization has classified NETs as well and poorly differentiated [4]. The NET proliferative activity is a very important indicator of the tumor aggressiveness, and it...
is determined by the number of mitoses per unit area, or alternatively with the help of Ki67 proliferation marker of the cell nucleus, which marks every individual cell cycle [5].

Localizing the disease and determining the degree to which it has spread is of great importance for the process of choosing the appropriate therapy, which is why the diagnosis of NETs includes various visualization techniques, including echosonography, computerised tomography, magnetic resonance imaging, positron emission tomography, endoscopic ultrasound and different nuclear medicine visualization methods [6,7]. Over 90% of carcinoids and other NETs are characterized by excessive expression of somatostatin receptors, especially the sstr2 subtype, which is the molecular basis of their \textit{in vivo} localization and the clinical application of somatostatin analogue in the treatment of these tumors. Five subtypes of somatostatin receptors residing in the cell membranes of NETs have been discovered so far (sstr 1-5), the sstr2 subtype [8,9] being the most common one.

Somatostatin receptor scintigraphy (SSRS) is an important method in functional imaging, as it can detect occult NETs and their metastases. The receptor imaging method was introduced in 1991, following the discovery and the subsequent clinical application of In-111-DTPA-D-Phe-octreotide (OctreoScan), which is considered a gold standard in the detection of NETs, especially carcinoids [10,11].

Increased monoamine metabolism present in NETs enables their visualization by means of I-123-MIBG labeled noradrenaline analogue via intercellular uptake by secretory vesicles, which makes this radiopharmaceutical a good alternative for SSRS [12,13]. Recent years have seen the development of other radiopharmaceuticals suitable for detecting NETs [14-16], but under certain circumstances, F-18-FDG PET can also be of diagnostic importance in NET patients with high proliferation index [17-19].

Unfavorable characteristics of somatostatin analogues labeled with In-111, as well as the still limited application of positron emission tomography and positron radiopharmaceuticals which bind with somatostatin receptors, have triggered extensive research aimed at discovering somatostatin receptor-avid radiopharmaceuticals, which can be labeled with Technetium-99m - a widely available isotope with ideal physical characteristics. The aim of this paper is to determine the influence of Ki-67 proliferation index on SSRS with Tc-99m-EDDA-HYNIC-TOC (Tc-99m-Tektrotyd) somatostatin analogue in patients with carcinoid tumors.

2 Experimental procedure

2.1 Patients

Between 2007 and 2013, 61 consecutive patients (31 female, 30 male; age range: 29-77 years; mean age: 57.9±13.4 years) were referred to our institution for somatostatin receptor scintigraphy. Thirteen out of 61 patients were highly suspected of having a carcinoid tumor and 48 had histologically proven NETs and had previously undergone surgery to have them removed.

Extensive imaging procedures were performed on patients depending on their diagnosis, including CT, MRI, ECHO, endoscopic ultrasound, colonoscopy, bronchoscopy, F-18-FDG-PET, and Tc-99m-MDP, I131-MIBG, respectively.

Patients were advised to use laxatives and follow a light diet one day before the scanning, so that optimal abdominal imaging could be obtained. On the day of the examination, the patients were asked to fast until the end of the acquisition.

2.2 Imaging

The studies were performed using a dual-head, large field of view “ROTA” and “e.cam” gamma camera (Siemens) equipped with parallel, low-energy, high resolution collimators. The ICON and e.soft operating systems were used.

All patients underwent a whole-body scan in the anterior and posterior projections (256×1.024 matrix, 6 cm min$^{-1}$) and single-photon emission computed tomography (SPECT) of the suspicious region (abdomen and/or the chest). Ten minute planar spot images of the selected regions were collected in 128×128 computer matrix. SPECT was performed using a 360° noncircular orbit (180° for each head), step and shoot mode, at 30 s per view. The acquired data were collected in a 128×128 computer matrix and reconstructed using filtered back projections with a Butterworth filter (cut-off 0.6 cycles/pixel, order 5).

The HYNIC-[D-Phe$^1$,Tyr$^3$-Octreotide] was prepared according to the manufacturer’s instructions under aseptic conditions (Tektrotyd, POLATOM, Poland). High specific activity of freshly eluted technetium-99m from the 99 Mo/99mTc generator (INS Vinca, Belgrade, Serbia) was added to the kit so that maximum activity in 1 mL did not exceed 1.5 GBq. After the reconstruction of the radiopharmaceutical, 740 MBq activity and 8 µg of octreotide were administered to the patients.
The radiochemical purity of the applied radiopharmaceutical was higher than 95%, which was tested by means of thin-layer chromatography.

The interpretation of the scans was qualitative, and any focal activity out of the normal radiopharmaceutical distribution was considered positive. The scans were analyzed by two qualified nuclear medicine specialists.

2.3 Immunohistochemical evaluation of Ki67

Four-millimeter-thick tissue sections were incubated with monoclonal mouse antihuman Ki67 antigen (Dako). The sections were counterstained with hematoxylin, and the number of positive tumor nuclei per 100 tumor cells was counted [20].

2.4 Statistical analysis

The data were analyzed using SPSS software, version 20.0 (IBM SPSS Inc.). Descriptive values were presented. Pearson’s correlation was used to test the liner association between two variables. P values of less than 0.05 were considered significant.

3 Results

The distribution of carcinoid tumors according to location is presented in Table 1. In the majority of cases (79%), Tc-99m-Tektrotyd scan was performed during the follow-up of patients previously operated on. Other patients were scanned due to high clinical suspicions of having a carcinoid tumor, or after the carcinoid was histologically proven by biopsy. The most frequent localizations of the carcinoids in the post-operative group of patients were the small bowel (33%) and the lung (19%). Carcinoid tumors were confirmed by histopathology in 59 out of 61 patients. They were positive in 11 out of 13 patients who were highly suspected of having a NET and investigated pre-operatively. Two out of 61 patients were excluded after they were proven to be NET negative.

The results of comparing the somatostatin scan results with the final diagnosis are presented in Table 2. The somatostatin scan was found negative in 24 out of 28 patients with the proven absence of either primary carcinoid tumors or metastases (85.7%), whereas Tc-99m-Tektrotyd scintigraphy was classified as true positive in 26 out of 30 (86.6%). Primary tumors were detected in 7 patients, metastases in 14 out of 20, and both primary tumors and metastases in 5 patients. Concordance between the final diagnosis and SSRS results was observed in 50 out of 61 patients (81.9%), so that the Pearson’s correlation was found as significant (R 0.60, p < 0.001). The sensitivity, specificity, positive predictive value, and negative predictive value of Tc-99m-Tektrotyd scan were 78.79%, 84.62%, 86.67% and 75.86%, respectively.

Somatostatin receptor scintigraphy failed to detect the presence of the disease in seven patients, while in four the results came back as false positive.

Table 1: Clinical status and localization of carcinoid tumors.

<table>
<thead>
<tr>
<th>Location</th>
<th>Pre-operative</th>
<th>Post-operative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung carcinoid</td>
<td>5</td>
<td>9</td>
<td>14</td>
</tr>
<tr>
<td>Stomach carcinoid</td>
<td>2 (3*)</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Pancreatic carcinoid</td>
<td>1 (2*)</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Duodenal carcinoid</td>
<td>1</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Rectal carcinoid</td>
<td>0</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Colon carcinoid</td>
<td>0</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Ileal carcinoid</td>
<td>0</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>Carcinoid of the ceacum</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Carcinoid of the appendix</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Liver carcinoid</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><em><em>11 (13</em>)</em>*</td>
<td><strong>48</strong></td>
<td><strong>59</strong></td>
</tr>
</tbody>
</table>

*Diagnosis of NET excluded in one of the patients from the group

<table>
<thead>
<tr>
<th>Final diagnosis</th>
<th>Tc-99m Tektrotyd scan result</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>No disease</td>
<td>24</td>
<td>4</td>
</tr>
<tr>
<td>Primary tumor</td>
<td>-</td>
<td>7</td>
</tr>
<tr>
<td>Metastases</td>
<td>6</td>
<td>14</td>
</tr>
<tr>
<td>Primary tumor and metastases</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>31</strong></td>
<td><strong>30</strong></td>
</tr>
</tbody>
</table>

Table 3 shows the findings of SSRS in relation to carcinoid differentiation status. The most frequent carcinoid forms demonstrated by immunohistochemical analysis were well differentiated neuroendocrine tumors with low proliferation mitotic index Ki67 of < 2%, found in 39 patients (66%). Fourteen patients were diagnosed with well differentiated neuroendocrine carcinoma and...
6 patients were diagnosed with poorly differentiated neuroendocrine carcinoma. The sensitivity of Tc-99mTc-Tektrotyd scintigraphy was found to be as high as 94.74% in the group of patients with low mitotic index Ki67 (< 2%), and it progressively decreased in patients with higher mitotic index (77.78% for Ki67 2-15% and 20% for Ki67% > 20%). The likelihood of Tc-99m-Tektrotyd scan being positive when a carcinoid is present was found to be inversely proportional to the value of Ki67 proliferation index (Fig. 1). Fig. 2 shows patients with well differentiated neuroendocrine carcinoma (Ki67 8.5%) with intense Tc-99m-Tektrotyd uptake in both primary pancreatic tumor and liver metastases. However, uptake might not be visible even in cases of huge carcinoids when Ki67 proliferation index is 2-15% (Fig. 3). As many as 4 out of 7 patients with false negative SSRS results were those with the highest proliferation index Ki67 (> 15%).

### Table 3: Tc-99m-Tektrotyd scan results in relation to carcinoid differentiation.

<table>
<thead>
<tr>
<th>Tc-99m-Tektrotyd scan results</th>
<th>Carcinoid differentiation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ki67 &lt; 2%</td>
</tr>
<tr>
<td>True positive</td>
<td>18</td>
</tr>
<tr>
<td>True negative</td>
<td>16</td>
</tr>
<tr>
<td>False positive</td>
<td>4</td>
</tr>
<tr>
<td>False negative</td>
<td>1</td>
</tr>
</tbody>
</table>

Figure 1: Sensitivity of Tc-99m-Tektrotyd scan in relation to proliferation index Ki67.

**Figure 2:** Well differentiated neuroendocrine carcinoma. Nonfunctional pancreatic carcinoid with liver metastases (Ki67 8.5%). A: Tc-99m-Tektrotyd SPECT demonstrates high tracer uptake of primary pancreatic lesions (dotted black arrows) and in liver metastases (solid black arrow). B: CT transversal section shows pancreatic lesion (dotted white arrow) and low attenuation liver metastases (solid white arrow).

### 4 Discussion

The presence of molecular biomarkers on the NET cells enables functional visualization of the tumors by means of SPECT and PET imaging, as well as their treatment with peptide receptor radionuclide therapy. Excessive
Somatostatin receptor scintigraphy in patients with carcinoids

343

expression of somatostatin receptors, especially the subtype 2 receptors, is typical of NETs [8,9], even though other biomarkers characterized by excessive expression, such as dopamine receptors or molecules involved in monoamine processes, can be equally as suitable a target [10].

Somatostatin receptor scintigraphy, a reliable, non-invasive method of medical imaging used for the detection of tumors with excessive somatostatin receptor expression, has been irreplaceable ever since its introduction into the clinical practice two decades ago. Furthermore, In-111-octreotide has become the gold standard in diagnosing NETs in various locations [21-28]. However, poor physical properties of In-111 have triggered intense efforts to synthesize somatostatin analogues which can be labeled with Tc-99m, for the purpose of achieving better image quality, smaller expositional radiation doses per patient, and ready availability [29,30].

This is how octreotide derivates which can be labeled with Tc-99m-pertechnetate were synthesized, including Tc-99m-Na-(hydroxynitrile)-Tyr3-octreotide (HYNIC-TOC, Tectrotyd), which yields excellent image quality in NET patients [31-35]. Comparative studies have shown that HYNIC-TOC labeled with Tc-99m ensures a better detection of lesions in comparison to In-111-Octreotide, and also that it decreases the chances of false positive findings [31,32]. Apart from the appropriate pharmacokinetics, better spatial resolution and availability, the advantage of Tc-99m labeled somatostatin analogue is the imaging which can be finished over the course of one day, which makes this radiopharmaceutical suitable for routine use.

However it was found that the degree of NET differentiation may influence some types of functional imaging. Ki67 proliferation index is determined per 100 tumor cells per unit area and is used for classifying NETs as well differentiated tumors (Ki67 < 2%), well differentiated carcinomas (Ki67 2-15%), and poorly differentiated carcinomas (Ki67% > 15%). Well differentiated NETs are characterized by diffuse and intensive expression with 2 neuroendocrine tissue markers – synaptophysin and chromogranin A, whereas poorly differentiated neuroendocrine carcinomas can typically be labeled with synaptophysin only [33]. The degree of differentiation and the Ki67 proliferation index have great importance in the choice of diagnostic modality most suitable for capturing the degree of spread of NETs.

A study by Binderup et al. compared findings obtained by somatostatin receptor scintigraphy, F18-FDG PET and I123-MIBG in patients with NETs [34]. The results pointed to significant differences in sensitivity between SSRS and F18-FDG PET in NET detection, depending on the values of Ki67 proliferation index. In tumors with Ki67 over 15%, the F18-FDG sensitivity amounted to 92%, in comparison to 69% and only 46% for SSRS and I123-MIBG, respectively.

Figure 3: Well differentiated neuroendocrine carcinoma. Lung carcinoid with liver metastasis (Ki 67-8%). A: MRI demonstrates huge right lung lesion (dotted white arrow) with large liver metastasis (solid white arrow). B: Sequential spot Tc-99m-Tektrotyd scan of thoracal and abdominal regions shows absence of tracer accumulation in the lung lesions (dotted black arrow) and liver “cold” lesions (solid black arrow).
[34]. The authors concluded that the F18-FDG PET should be the method of choice for NETs with proliferation index above 15%, as other methods either cannot visualize NETs with high aggressiveness, or tend to underestimate the number of detected lesions [34].

In this paper, the negative findings of Tc-99m Tektrotyd somatostatin receptor scintigraphy correlated with findings obtained by means of other methods in 24 patients, whereas the disease was confirmed by positive findings in 26 patients. In 7 patients there were no pathological changes on the scan, even though they had previously been confirmed by means of other visualization techniques, whereas 4 patients showed false positive changes.

In comparison to the principal diagnosis, the overall sensitivity of the Tc-99m Tektrotyd scan per patient amounted to 79%, whereas specificity, positive predictive value and negative predictive value amounted to 84%, 87% and 76%, respectively. The findings obtained by means of radioisotope imaging were mostly concurrent with the principal diagnosis (Pearson R 0.60, p < 0.001).

By analyzing the findings of SSRS in relation to the proliferation index value, we concluded that sensitivity was high (95%) in the well-differentiated NET group with Ki67 proliferation index lower than 2%, but that specificity was lower (80%). The positive predictive value of the scans amounted to 82%, whereas negative predictive value was 94%. Lower specificity was the cause of false positive results in 4 patients: in 3 patients false positive results were obtained after ileum surgery and in 1 after the large intestine carcinoid surgery.

In the well-differentiated NET group with Ki67 proliferation index between 2-15%, Tc-99m-Tektrotyd imaging detected 7 out of 9 patients with carcinoids, and the scans came back normal in all 5 who did not have the disease. Imaging sensitivity in this NET group was 78%, whereas specificity was as high as 100%. However, in the poorly-differentiated NET group with Ki67 proliferation index higher that 15%, only 1 out of 5 carcinoid positive findings was detected, i.e. the sensitivity of somatostatin receptor imaging in highly aggressive NETs was calculated at 20%. The results highlighted the following: the higher the proliferation index of carcinoids, the lower the sensitivity of SSRS.

This paper analyzed the influence of proliferation index values on Tc-99m-Tektrotyd imaging findings, so different sensitivity of the Tc-99m Tektrotyd imaging in groups with different proliferation indexes can be explained as the influence of differentiation and proliferation on somatostatin receptor expression, and consequently on the ability to bind somatostatin analogue. This claim can be supported by the results of our study, which show false negative findings with Tc-99m-Tektrotyd in as many as 4 out of 7 patients in which Ki67 proliferation index was over 15%.

5 Conclusion

The results point to the fact that Tc-99m-Tektrotyd somatostatin receptor scintigraphy is a sensitive and specific method for diagnosing and staging patients with well-differentiated NETs and carcinomas. It can be applied in clinical practice for pre-operative evaluation, as well as recurrent disease or distant metastases localization. However, with poorly-differentiated NETs, pathological lesions can be overlooked, so additional analyses are required in cases of tumors with a high Ki67 proliferation index.

Acknowledgment: This work has been supported by the Ministry of Science and Technological Development of the Republic of Serbia, project no. 43011 and 45015.

References
