

# $^{123}\text{I}$ -Vascular Endothelial Growth Factor-165 ( $^{123}\text{I}$ -VEGF<sub>165</sub>) Scintigraphy for Visualisation of Extranodal Marginal Zone B-Cell Lymphoma of the Mucosa-Associated Lymphoid Tissue (MALT) Type

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**Abstract:** *Aim:* Vascular endothelial growth factor (VEGF) and VEGF receptor are over-expressed in lymphomagenesis. The aim of this study was to evaluate the usefulness of  $^{123}\text{I}$ -VEGF<sub>165</sub> scintigraphy for imaging of B-cell lymphoma of the Mucosa-Associated Lymphoid Tissue (MALT lymphoma).

*Methods:* Three Patients (age range: 52-71 years) with histopathological verified MALT lymphoma were included in the study. Two patients had disseminated MALT-lymphoma and one patient had parotid MALT-lymphoma with involvement of cervical lymph nodes. Recombinant VEGF<sub>165</sub> was radiolabeled with  $^{123}\text{I}$  by electrophilic radioiodination using the chloramine T method. Each patient received 172 MBq (<130 pmole (< 5 µg) VEGF<sub>165</sub> per patient) of  $^{123}\text{I}$ -VEGF<sub>165</sub>. Dynamic acquisition was initiated immediately after administration and carried out until 30 minutes post injection. Whole-body images were acquired in anterior and posterior views at various times points post injection (p.i.). SPECT was performed 1 hour p.i.

*Results:* None of the patients with MALT lymphoma showed pathologically increased focal  $^{123}\text{I}$ -VEGF uptake in verified tumor lesions. No clinical adverse reaction and no side-effects after intravenous application of  $^{123}\text{I}$ -VEGF<sub>165</sub> were found.

*Conclusions:* Our results suggest that  $^{123}\text{I}$ -VEGF<sub>165</sub> scintigraphy does not visualize B-cell lymphomas of MALT type.

**Keywords:** Mucosa-associated lymphoid tissue, MALT, B-cell lymphoma,  $^{123}\text{I}$ -VEGF<sub>165</sub> scintigraphy, angiogenesis, receptor.

## INTRODUCTION

B-cell lymphoma of the mucosa-associated lymphoid tissue (MALT) type is a distinctive type of malignant B-cell lymphoma among non-Hodgkin's lymphomas [1], characterized by its presence in various extranodal tissues or organs such as the stomach, salivary gland, thyroid gland and orbital adnexa [2,3]. In a previous study, various tumor cells and tumor tissues such as primary melanomas, breast cancers and ovarian carcinomas as well as meningiomas expressed significantly higher amounts of vascular endothelial growth factor (VEGF) receptors as compared to adjacent normal tissues or peripheral blood cells [4]. The over-expression of specific binding sites for VEGF provided the rationale for the clinical use of radiolabeled VEGF for imaging of tumors [4]. A VEGF-mediated mechanism has been shown to play an important role in the expansion of MALT lymphoma tissue and VEGF receptor antibodies has a suppressive effect on MALT lymphoma growth [5]. Our previous studies [6-8] have shown that a variety of

gastrointestinal tumors and metastases as well as osteosarcomas can be visualized using  $^{123}\text{I}$ -VEGF<sub>165</sub> scan.  $^{123}\text{I}$ -VEGF<sub>165</sub> scintigraphy may be useful for visualization of tumor angiogenesis [6]. However, there is as yet no experience with  $^{123}\text{I}$ -VEGF scintigraphy in patients with MALT-lymphoma. Thus, we have performed a pilot series using  $^{123}\text{I}$ -VEGF<sub>165</sub> scintigraphy in patients with MALT lymphomas.

## MATERIALS AND METHODS

### Patients

The study protocol for administration of  $^{123}\text{I}$ -VEGF<sub>165</sub> to patients was approved by the Ethical Committee of the Medical Faculty of the University of Vienna. The study was in accordance with the Declaration of Helsinki. All patients gave written informed consent to participate in the study.

A total of three male patients (median age 61 ±10 years, age range 52-71 years) with biopsy-proven MALT lymphoma were referred to our department. One patient had disseminated MALT lymphoma involving the stomach, lachrymal glands, parotid glands and the lungs, while another patient had lymphoma in the stomach, colon, abdominal lymph nodes and the lung. Lung involvement was bilateral in both patients, with the largest deposits measuring

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5 x 3 cm in one and 2 cm in the other patient. The third patient had undergone biopsy of parotid MALT lymphoma with involvement of cervical lymph nodes. All patients with MALT type lymphoma underwent standardized staging procedures including CT-scan of thorax and abdomen, gastroscopy and colonoscopy, imaging of salivary glands and bone marrow biopsy to assess the extent of lymphoma. Evaluation of biopsy specimens was performed by a reference pathologist according to the standardized criteria as outlined in the recent WHO-classification. All patients were imaged before initiation of therapy.

### Radiopharmaceutical Preparation

Recombinant human VEGF<sub>165</sub> (PromoCell GmbH, Heidelberg, Germany) was labelled with iodine-123 by electrophilic radioiodination using Chloramine T as described previously [6,7] Briefly, 10 µg (0.26 nmol) of rhVEGF<sub>165</sub> were labelled with about 30 mCi <sup>123</sup>I-Na (Research Center Karlsruhe, Germany) and Chloramine T. After 3 minutes the reactions were stopped by addition of 4 µl sodium metabisulphite (Na<sub>2</sub>S<sub>2</sub>O<sub>5</sub>, 50 nmol), the reaction mixture was diluted with phosphate-buffered saline (PBS) containing 0.1% human serum albumin (HSA) and applied to a size-exclusion chromatography column (Sephadex G-25 M). The first <sup>123</sup>I-peak eluting from the column was collected and filtered using a steril membrane (Millex GV 0.2 µm). <sup>123</sup>I-VEGF<sub>165</sub> was routinely analyzed by paper-electrophoresis on Whatman Nr.3 MM in 0.1 M barbital buffer (pH=8.6) using a field of 300 V for 10 min and by trichloroacetic acid (TCA) precipitation.

### <sup>123</sup>I-VEGF<sub>165</sub> Scintigraphy

The study protocol included planar imaging and whole-body imaging as well as single-photon emission tomography (SPECT).

<sup>123</sup>I-VEGF<sub>165</sub> was administered as a single intravenous bolus injection over 3 minutes. The administered activity was 172 ± 29 MBq corresponding to ≤130 pmole (≤ 5 µg) of VEGF<sub>165</sub> per patient. In order to determine hemodynamic effects of VEGF, blood pressure and heart rate were monitored during tracer application and scintigraphy. The patients received 400 mg sodium perchlorate three times daily from one day before the tracer administration until three days after the injection of <sup>123</sup>I-VEGF<sub>165</sub> for thyroid blockage.

Whole-body acquisitions were performed simultaneously in anterior and posterior views with a double-headed and a large field of view camera (Millenium VG with Hawkeye, GE Medical Systems, Milwaukee, WI, USA) employing medium-energy high-resolution (MEHR) collimators (10 cm/min; matrix 256x1024 pixels). Serial whole-body images were obtained 45 min and 18 hours postinjection (p.i.). A 30-min dynamic image was recorded starting at the time of injection (matrix 128 x 128 pixels). To verify that the activity localization corresponded to the tumor lesions documented by computer tomography (CT) scans, SPECT studies in combination with CT were obtained 1 hour p.i. (60 projections over a 360 rotation, 40 s per step and a 64 x 64 pixel matrix). Planar images were acquired 40 min p.i. including anterior and posterior views of abdomen and thorax (matrix 256x256 pixels; 800 kcts preset). Additional

lateral or oblique views of above regions or other regions were obtained when necessary.

Scanning results were evaluated independently by two blinded observers using a yes-or-no system. These results were compared with conventional radiological imaging and results of endoscopies if indicated.

### Statistical Analysis

The  $\chi^2$  test was used to compare site detection by two techniques, and Student's test was used to analyse paired (same patient) and unpaired (between patients) samples. P values ≤0.05 were considered statistically significant.

## RESULTS

Patient characteristics and the <sup>123</sup>I-VEGF<sub>165</sub> imaging results are shown in Table 1.

**Table 1. Characteristics of Patients with MALT Lymphoma and the <sup>123</sup>I-VEGF<sub>165</sub> Imaging Results**

No.	Age	Gender	CT Tumor Location	VEGF Scan
1	61	m	parotid glands cervical Ln	- -
2.	71	m	lungs colon stomach abdominal Ln	- - - -
3.	52	m	parotid glands lachrymal glands stomach lungs	- - - -

-: negative; Ln: lymph node.

All the three patients had normal level of blood lactate dehydrogenase (LDH) (165 U/L, 186 U/L and 178 U/L respectively. Normal range is smaller than 248 U/L).

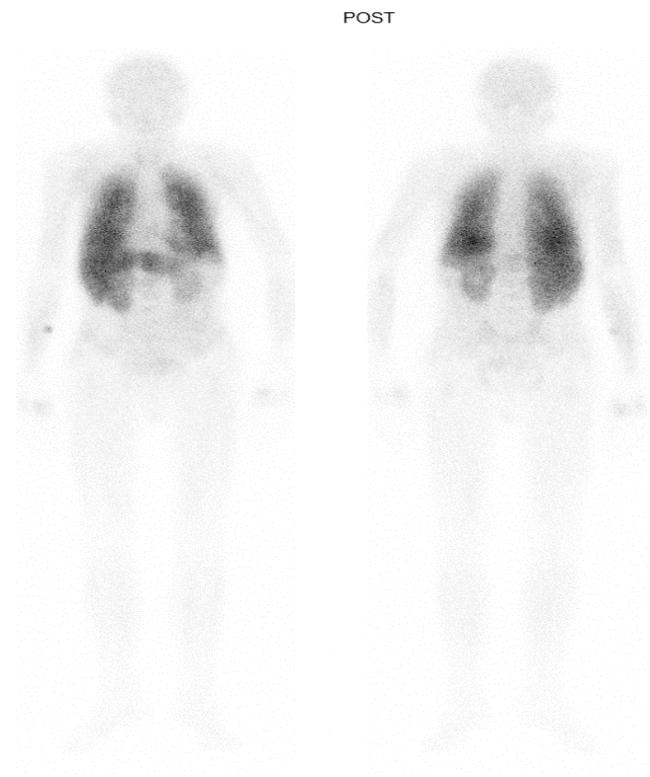
Three patients with MALT lymphoma underwent <sup>123</sup>I-VEGF<sub>165</sub> scanning. After intravenous injection, <sup>123</sup>I-VEGF<sub>165</sub> showed a first-pass effect in the lungs. Whole-body measurements revealed a very high uptake in the lungs (Fig. 1). In all patients, MALT lymphomas were not visualized by dynamic, planar, whole-body and SPECT scanning at different time points after injection of <sup>123</sup>I-VEGF<sub>165</sub>. The uptake ratios between MALT lymphoma and background ranged from 0.99 to 1.04.

Imaging of patient No. 2, who had MALT lymphoma in both lungs, stomach and colon as well as abdominal lymph node involvement is shown in Fig. (1). None of the tumor lesions could be visualized by <sup>123</sup>I-VEGF<sub>165</sub>. The histopathological picture is shown in Fig. (2).

## DISCUSSION

Marginal zone lymphoma (MZL) as defined by the most recent classification of lymphoid malignancies is a subgroup of indolent B-cell non-Hodgkin's lymphoma (NHL) thought to originating from mature B-cells corresponding to cells of

the 'marginal zone' of the secondary lymphoid follicle. It is the third most common type of NHL, and extranodal marginal zone B-cell lymphoma of the mucosa associated lymphoid tissue (MALT-lymphoma) accounts for 50–70% of all MZL [2,3]. Though recent data have questioned a common origin of MALT-lymphoma, splenic- and nodal marginal zone B-cell lymphoma, further data on this topic are needed and for the time being, these lymphomas are thought to constitute a spectrum of diseases arising from marginal zone cells.

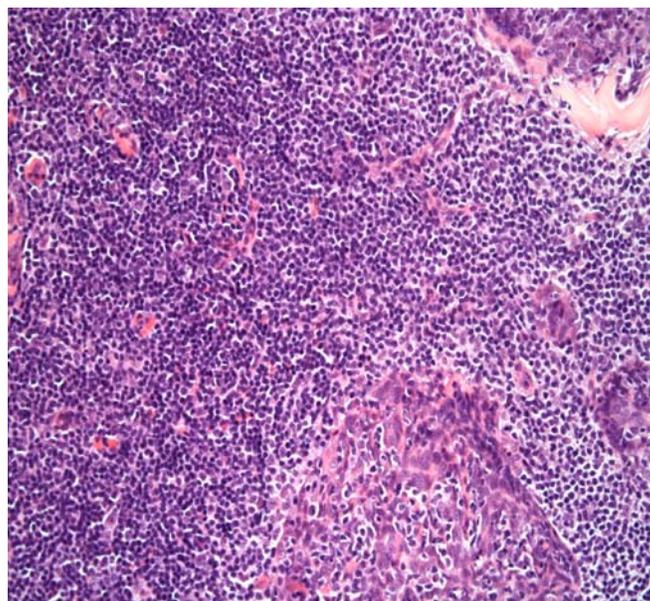


**Fig. (1).** Whole-body <sup>123</sup>I-VEGF<sub>165</sub> scintigraphy (A: anterior scan; B: posterior scan) 45 min after injection of 185 MBq <sup>123</sup>I-VEGF<sub>165</sub> in a patient with MALT-lymphoma in both lungs, stomach and colon as well as abdominal lymph nodes. Intense diffuse uptake in lungs is observed. No pathological local tracer-accumulation is found in the tumor regions.

Previous study [6-8] results from our institution have demonstrated that there are no clinical adverse reaction and no side-effects after intravenous application of <sup>123</sup>I-VEGF<sub>165</sub> and that iodination of VEGF did not affect the functional properties of VEGF.

In these studies, specific binding of <sup>123</sup>I-VEGF<sub>165</sub> to a variety of human tumor cell lines and primary tumors was found as compared to peripheral blood cells and non-neoplastic tissues *in vitro*, providing the basis for development of VEGF receptor scintigraphy using radiolabelled VEGF. Scanning with <sup>123</sup>I-VEGF<sub>165</sub> can visualize gastrointestinal tumors and metastases expressing receptors for VEGF<sub>165</sub>, and also specific binding of <sup>123</sup>I-VEGF<sub>165</sub> to pancreatic tumor cells and tissues has been demonstrated. It has been reported that VEGF may play an important role in the expansion of MALT lymphoma and antibodies against VEGF receptor may have a suppressive effect on MALT lymphoma growth [5]. Therefore, the

results of the study [5] have led us to initiate a clinical pilot series evaluating the role of <sup>123</sup>I-VEGF<sub>165</sub> in patients with MALT lymphoma.



**Fig. (2).** Histology of a MALT-lymphoma. Infiltration with small centrocyte-like cells typical for MALT lymphoma.

However, the results of present study were disappointing as none of the patients with MALT lymphoma demonstrated pathologically increased focal <sup>123</sup>I-VEGF<sub>165</sub> uptake within the verified tumor lesions. Several reasons may offer an explanation for the negative <sup>123</sup>I-VEGF<sub>165</sub> results. Firstly, it has been reported that the most negative <sup>123</sup>I-VEGF<sub>165</sub> scanning results were found in tumors and metastases with a maximum median diameter of <2 cm [5,6]. In our present study, the most tumor diameters ranged from 0.5 to 2 cm. Therefore, one might speculate that patients with larger tumors might show positive results. MALT-lymphomas, however, especially if located in the gastrointestinal tract, are characterized by mucosal/submucosal spread sometimes without forming larger tumor deposits, therefore remaining below the detection threshold of the method. Secondly, another explanation maybe the blockade of tumor VEGF receptors by locally produced ligands in MALT lymphomas. Last but not least, an additional explanation is that VEGF receptor expression in the tumor tissue was relatively low compared with that in the surrounding normal tissue, especially in lung-lesions. In fact, one of the major shortcomings of <sup>123</sup>I-VEGF<sub>165</sub> is its inability to visualize small pulmonary tumor lesions due to the intensive uptake in the lungs [7]. Previous studies have suggested that VEGF expression in lymphoma is associated with poor prognosis [9, 10]. The degree of angiogenesis might vary in different lymphoma subtypes [10]. The patients in our present study had indolent MALT lymphoma and belong to low grade lymphoma. It might be also another explanation that negative <sup>123</sup>I-VEGF<sub>165</sub> scintigraphy results were obtained. Although no patients in our study showed pathologically increased focal <sup>123</sup>I-VEGF<sub>165</sub> uptake in MALT lymphoma, our previous study have shown that many MALT lymphomas express somatostatin receptors (SSR) which can be visualized by using SSR-scintigraphy [11].

**CONCLUSION**

Our preliminary results suggest that  $^{123}\text{I}$ -VEGF<sub>165</sub> scintigraphy does not visualize B-cell lymphomas of MALT type.

**CONFLICT OF INTEREST**

The authors confirm that this article content has no conflict of interest.

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