Expression of somatostatin receptors in inflammatory lesions and diagnostic value of somatostatin receptor scintigraphy in patients with ANCA-associated small vessel vasculitis

I. Neumann, S. Mirszæi¹, R. Birck², K. Osinger³, R. Waldherr⁴, H. D. Köhn¹ and F. T. Meisl

Objective. To assess the usefulness of somatostatin receptor (SSTR) scintigraphy for the evaluation of disease activity in the upper and lower respiratory tract in ANCA-associated vasculitis (AASV).

Methods. Thirty-two consecutive patients with AASV were subjected to SSTR scintigraphy as part of their initial diagnostic evaluation and follow-up. The presence of SSTRs in inflammatory lesions was evaluated with immunohistochemistry in selected cases.

Results. In AASV, specificity of SSTR scintigraphy for active vs non-active disease was 96% for pulmonary disease and 100% for ear, nose and throat (ENT) involvement, while sensitivity was 86% and 68%, respectively. Absence of previously present tracer accumulation characterized treatment responders, and treatment resistance was reflected by repeated positive scintigraphy. We could demonstrate the expression of SSTRs in lung and mucosal biopsies obtained from patients with active Wegener's granulomatosis and with microscopic polyangiitis.

Conclusion. SSTR scintigraphy is useful for the assessment of AASV, indicating disease activity, disease extent and treatment efficacy. SSTRs are expressed in both granulomatous as well as non-granulomatous AASV.

Key words: Somatostatin receptor, Scintigraphy, ANCA, Vasculitis, Immunohistochemistry, Disease activity, Radiology, Lymphocytes, Pulmonary, ENT.

The upper and lower respiratory tract are common targets in antineutrophil cytoplasmic antibody (ANCA)-associated small vessel diseases (AASV) such as Wegener’s granulomatosis (WG), microscopic polyangiitis (MPA) and the Churg–Strauss syndrome (CSS). These disease entities often occur as a pulmonary–renal syndrome, but lung manifestations may also appear independent of kidney involvement [1]. Given the wide morphological variety of pulmonary lesions found in these patients [2] combined with the often non-specific radiographic findings [3], correct diagnosis of extra-renal vasculitis can be difficult. Moreover, residual radiographic abnormalities may persist despite treatment, representing either scars or smoldering disease complicating the differentiation between active and inactive lesions. Although ANCA’s have been proven as an important diagnostic tool [4], it has to be emphasized that, especially in limited AASV, a negative ANCA result does not exclude the diagnosis of active WG or MPA [5]. Consequently, an additional non-invasive diagnostic procedure that detects active disease early in the course and registers disease extent and reflects response to treatment would be of great value.

In AASV, activated T cells are believed to play a central role in pathogenesis [6, 7], and the prominence of T cells and monocyte-macrophages has been demonstrated in lung [8], ear, nose and throat (ENT) [9] and kidney specimens of active AASV [10, 11]. Granuloma formation also suggests an important role of cell-mediated immune reactions [8, 12].

Somatostatin receptors (SSTRs) have been identified on human immune cells including mononuclear leucocytes [13] and peripheral blood lymphocytes [14], while granulocytes and red blood cells did not express SSTRs [14]. Thus, SSTR expression can be detected in vivo by SSTR scintigraphy using [111In]octreotide [15]. Octreotide is an octapeptide analogue of somatostatin that binds with high affinity to SSTR2 and SSTR5 and with lower affinity to SSTR3, while somatostatin binds to all five SSTR subtypes [16]. The usefulness of SSTR scintigraphy for the assessment of disease activity and extent in AASV has not been investigated so far. Positive findings of SSTR scintigraphy have already been reported in immune-mediated granulomatous diseases such as sarcoidosis, but only in three patients with WG [17, 18].

An imaging technique that specifically reflects the pathophysiological mechanism may be attractive in the diagnostic armamentarium of AASV.

Patients and methods

Patients

Thirty-two consecutive patients [17 males, 15 females; mean age 62±15 yr (range 34–81)] with AASV were studied. The patients’ written consent was obtained according to the declaration of Helsinki. The design of the work conforms to standards currently applied to the country of origin. Sixteen patients presented with...
new onset of disease and 16 during follow-up in our out-patient clinic. According to the Chapel Hill Nomenclature Conference, patients were classified as having WG (n = 20), MPA (n = 8) and CSS (n = 4) [19]. In 29 cases diagnosis was supported by at least one biopsy specimen, including 24 renal biopsies and 17 extra-renal biopsies (six pulmonary, five nasal, five skin, one sural nerve), while in three patients (two had limited WG and one severe CSS) histology was not available. ANCA serology identified cANCA in 21 patients and pANCA in seven patients, all in presence of their typical target antigens, proteinase-3 (PR3) and myeloperoxidase (MPO), respectively. Four patients were ANCA negative.

**Disease activity**

Disease activity was evaluated by the Birmingham Vasculitis Activity Score (BVAS) [20]. Active pulmonary disease was assessed clinically by symptoms such as dyspnoea, haemoptysis or cough and was supported by a pathological CT scan and/or chest radiograph findings consisting of one or more of the following: alveolar opacities, nodules (with or without cavitation), diffuse hazy or ground-glass opacities or pleural opacities. Prominent upper airway disease including sinusitis, purulent nasal discharge, epistaxis and nasal mucoid ulceration in the presence of granulomatous inflammation suggested active WG and was supported either by characteristic histology or by granulomas detectable on CT scan or by nasal endoscopy. A history of asthma and peripheral eosinophilia of >1500/mm³ with granulomatous inflammation involving the respiratory tract led to the diagnosis of active CSS.

Remission required complete absence of clinical disease activity, including the radiographic resolution of pulmonary opacities, reduction of size of pre-existing lesions unless attributable to scarring and the absence of active ENT vasculitis verified by endoscopy and/or radiography. Diagnosis of remission was supported by a stable or improving renal function, the absence of significant haematuria and normalization of C-reactive protein.

**Somatostatin receptor scintigraphy**

In 32 patients 62 scintigraphic investigations were performed, 46 in clinically suspected or diagnosed active AAV (16 at onset of disease, 20 under suspicion of a relapse) and 16 in complete remission. Each investigation allowed images of two regions, head and neck (n = 62), and the chest (n = 62), which were evaluated separately since active disease may involve only one organ. In order to evaluate disease activity with the corresponding scintigraphic findings, at the time of SSTR scintigraphy all patients also underwent radiography of the thorax, i.e. high-resolution CT scanning and/or chest radiography, and investigation of the upper respiratory tract by radiography (CT, MRI or radiography) or nasal endoscopy.

**SSTR imaging**

Planar images from anterior and posterior view (128 × 128 matrix, 10 min/frame) were obtained of the head and neck as well as of the chest at 4, 24 and 48 h after intravenous administration of 140 MBq of [111In]DTPA-D-Phe-1-octreotide (Octreoscan®, Mallinckrodt Medical, Petten, The Netherlands) using a large-field-of-view double-head gamma camera (Helix; Elscint, Haifa, Israel) equipped with a medium-energy collimator. Prior to the 24- and 48-h images, laxatives were given in order to reduce the background activity caused by hepatobiliary elimination of the tracer. In addition, single photon emission CT (SPECT) images (64 × 64 matrix) of the chest were acquired 4, 24 and 48 h after tracer administration. Physiological uptake of [111In]octreotide includes the pituitary gland, thyroid gland, liver, spleen, kidneys and urinary bladder. Accumulation of radioactivity at an abnormal site was considered to represent SSTR binding and was described as positive only if it was present on the scintigrams of all standard imaging time points. The scintigrams were studied independently by two experts in nuclear medicine without knowledge of any other radiological findings or clinical information.

In addition, a quantitative assessment of the radioligand uptake was calculated by using the region of interest (ROI) method. ROIs were drawn in each patient on the right shoulder region and on a pulmonary region without focal pathological tracer enhancement, as the background activity, and on the area with pathological tracer uptake in the lung. For each ROI the average counts per pixel (c/p) and a ratio of areas with pathological tracer uptake to the background were calculated. In the patients without pathological tracer enhancement, a ratio of activity (c/p) in the right lower lobe to the right shoulder and to the right middle lobe was calculated.

To compare the scintigraphic findings with radiology, the images were divided into two regions, i.e. head and neck, and chest. These regions were also evaluated separately and related to the corresponding organs, lung and ENT, respectively.

**ANCA serology**

Indirect immunofluorescence was performed using antineutrophil antibody slides (INOVA Diagnostics Inc., San Diego, USA) routinely fixed with ethanol and formalin. Enzyme-linked immunosorbent assay (ELISA) (Wieslab, Lund, Sweden) was performed for antibodies to PR3 and myeloperoxidase MPO.

**Immunohistochemistry**

Immunohistochemical analysis for SSTR2A and SSTR3 was performed on three open lung biopsies in active disease obtained from two patients with WG and one patient with MPA as well as on nasal and lung tissue of one autopsy case with WG. Paraffin-embedded tissue sections (4 mm) mounted on glass slides were deparaffinized in xylene and rehydrated in a graded series of ethanol. After rinsing in phosphate-buffered saline (PBS), sections were microwaved in 0.1M citric acid (pH 6.0) for 4.5 min at 600 W. Subsequently, after washing in PBS and blocking of endogenous peroxidase activity, sections were incubated with anti-SSTR2A (Biotrend, Cologne, Germany) or anti-SSTR3 (Gramsch Laboratories, Schwabhausen, Germany) or anti-CD68 (DAKO, Hamburg, Germany) antibodies. A positive immune reaction was visualized with AEC (3-amino-9-ethyl carbazole). The paraffin-embedded tissue samples were generously provided by Dr Bo¨hm, Department of Pathology, Baumgartner Höhe, Vienna and Dr Hartleb, Department of Pathology, Wilhelminenspital, Vienna.

**Statistical analysis**

Data are shown as mean ± S.D. Student’s t-test or ANOVA with Bonferroni option were used to compare means as appropriate. A P value of <0.05 was considered to be significant.

**Results**

**Scintigraphic results**

Representative scintigraphic findings obtained in patients with active disease compared with those in remission are illustrated.
in Figs 1–3. Quantitative assessment of ROIs revealed significant differences between scintigraphic findings in active disease and during remission (Table 1).

**Scintigraphy of the chest.** Overall, 62 investigations of the chest were performed. Thirty one of 32 positive scans were true positive. Interestingly, in one patient with WG, pericardial effusion due to vasculitis was visualized (Fig. 1A). The only false-positive scintigram was obtained in a patient with large pulmonary infiltrates due to cytomegalovirus pneumonia, but clinically in remission with respect to vasculitis. Twenty five of 30 negative scans were true negative. In five patients no tracer accumulation was visible despite active pulmonary disease; however, in these patients immunosuppressive therapy with cyclophosphamide and high doses of corticosteroids or methotrexate (n = 1) had been initiated at least 2 weeks (2–16 weeks) before SSTR scintigraphy was performed. Interestingly, in two patients who underwent SSTR scintigraphy under suspicion of active disease, the scintigraphic images were positive even before (6 and 9 weeks) the lesions became visible on the CT scan. In AASV, for pulmonary disease, SSTR scintigraphy revealed a sensitivity of 86% and a specificity of 96% with a positive predictive value of 97% for active disease (Table 2).

**Scintigraphy of ENT.** In analogy, 62 investigations were performed of the head and neck in order to detect ENT involvement. Of 20 positive scans all were true positive. Fifteen reflected newly diagnosed active disease and five positive investigations were obtained in persistent destructive sinusitis (four in WG, one in MPA). Thirty-three negative scans confirmed remission being true negative. However, nine scans were negative despite clinically active ENT disease being false negative. Six of these patients had received high dosages of immunosuppressive therapy for active pulmonary disease shortly before SSTR scintigraphy was performed. Interestingly, in three of our four patients with CSS, active ENT lesions were not detectable by scintigraphy. Thus, for ENT involvement, SSTR scintigraphy
exhibited a sensitivity of 68% and a specificity of 100% with a positive predictive value of 100% (Table 2). Sensitivity, specificity and predictive values for each vasculitic syndrome are given in Table 3.

Repeat scintigraphy was performed in nine patients within one therapeutic course. In seven patients (nos 1–7 of Table 4) who responded to therapy and achieved remission, previously present tracer accumulation disappeared, while scans remained positive in two patients with poor treatment response (nos 8 and 9 of Table 4).

**Radiological correlation with SSTR scintigraphy.** The following various radiological findings were recognized by SSTR scintigraphy: 14 infiltrates without cavitation (eight in WG, four in MPA, two in CSS), five infiltrates with central cavitation (four in WG, one in MPA), eight infiltrates with ground-glass attenuation (two in WG, five in MPA, one in CSS), six interstitial opacities (two in MPA, four in CSS), seven nodules without cavitation (all in WG) and seven nodules with central cavitation (all WG).

**Immunohistochemistry**

Immunohistochemical analysis of the lung revealed SSTR2α and SSTR3 expression on monocytes–macrophages and giant cells surrounding granulomas and occasionally in the centre of the granulomatous reactions (Fig. 4A and B). Furthermore, in WG and also in MPA, some perivascular and peribronchial mononuclear infiltrates were positive for SSTR2α and even more for SSTR3 (Fig. 5A and B), but no positive staining was found on endothelial cells. In both diseases SSTR2α and SSTR3 were strongly expressed on alveolar macrophages. In addition, in MPA, mononuclear cells within the inflamed parenchyma were also strongly positive for SSTR2α. There were only a few eosinophils in the infiltrates and they were negative for SSTR. Nasal mucosa was only available from an autopsy of a patient with WG demonstrating SSTR3 and SSTR2α expression in the basal cell layer of the nasal mucosa and on a few mononuclear inflammatory cells.

**Discussion**

In AASV, diagnosis and evaluation of disease activity in the respiratory tract is challenging. Endoscopic biopsy of the upper respiratory tract often fails to reveal adequate diagnosis and transthoracic needle biopsy engenders a complication rate of about 15% [21]. Once quiescence of disease is achieved the duration of treatment still remains a controversial issue, but
careful assessment of subclinical disease is mandatory for adequate therapeutic management.

This is the first report on systematic evaluation of SSTR scintigraphy for disease activity in AASV. Specificity and sensitivity for pulmonary vasculitis were 96 and 86%, and for ENT involvement 100 and 68%, respectively. In patients who responded to therapy and went into full remission, repeat SSTR scintigraphy demonstrated the absence of previously present tracer.
accumulation. In contrast, patients with aggressive disease who responded poorly to immunosuppressive therapy remained positive at repeat scintigraphy. Thus, in AAVS, SSTR expression appeared to be related to active disease or remission. Persisting abnormalities on SSTR scintigraphy in conjunction with radiological normalization may therefore indicate ongoing disease activity. Radiological findings representing fibrosis or scars without SSTR-positive cells will result in negative scintigraphy. This was demonstrated in one of our patients with a long history of granulomatous destructive disease with bronchopulmonary stents and multiple scars in whom disease activity was very difficult to assess clinically and by conventional radiology. Visualization of pulmonary inflammation by SSTR scintigraphy led to the correct diagnosis of active vasculitis, and scintigraphy also became negative after successful immunosuppressive treatment.

While specificity for active vs non-active disease was excellent for all three vasculitic syndromes, sensitivity was lower for CSS. Since the initiation of immunosuppressive therapy before SSTR scintigraphy appeared to affect the expression of SSTR, it is possible that corticosteroids required for the treatment of asthma in CSS may have contributed to the lower sensitivity in this subgroup.

Patients with AAVS may present with non-specific symptoms before organ manifestations become evident [1]. Prognosis depends to a great extent on early diagnosis and the rapid institution of effective therapy [1]. SSTR scintigraphy was able to recognize early active pulmonary inflammation even before it became visible on conventional radiography. Thus, the finding of increased tracer uptake in individual patients without corresponding radiographic findings may prompt the performance of more frequent check-ups and therefore contribute to an earlier diagnosis of relapses. Since visualization of the kidneys represents mainly metabolism of the radiopharmaceutical, $^{[111}\text{In}]$octreotide is not suitable for the detection of active renal disease [15].

The whole wide spectrum of radiological findings attributable to vasculitic lung disease could be visualized by SSTR scintigraphy. Pathological correlates included not only sites of granulomatous inflammation but also infiltrates with and without ground-glass attenuation and interstitial opacities as seen in MPA. Noteworthy, in one of our patients with quiescent WG, SSTR scintigraphy strongly reflected cytomegalovirus pneumonia, where mainly mononuclear cells are involved, and became completely negative 2 weeks after antiviral treatment. This case was also the only ‘false-positive’ pulmonary scintigraphy in our study.

Activated T cells play a crucial role in the pathogenesis of AAVS [6, 7] and inflammatory lesions are characterized by dense infiltrates of lymphocytes and monocytes–macrophages [8–11]. There is increasing evidence that SSTR2 and SSTR3 expression is up-regulated in activated T cells and peripheral blood mononuclear cells [13, 22, 23]. From the binding property of octreotide on the SSTR it can be assumed that positive findings reflect this up-regulated SSTR expression.

In the present study we demonstrated the expression of SSTR2A as well as of SSTR3 by immunohistochemistry of active lung disease in both WG and MPA. SSTR2A and SSTR3 were found on mononuclear cells within granulomatous inflammation and in mononuclear cells surrounding granulomas in WG. In addition, we found a strong positivity for both receptor types within perivascular and peribronchial cellular infiltrates in both WG and MPA. Alveolar macrophages and, especially in the case of MPA, mononuclear cells in the inflamed parenchyma also highly expressed SSTR2A and SSTR3. Our findings suggest that these immune-competent cells are the morphological correlate of the scintigraphic findings observed in active disease. We found no SSTR2A and SSTR3 expression on endothelial cells of capillaries as observed in rheumatoid arthritis [24]. Thus, it remains to be elucidated if endothelial cells in AAVS are targets for other subtypes of SSTR.

Other imaging modalities such as leucocyte and granulocyte scintigraphy have also been useful for detecting unsuspected sites of vasculitis, especially in WG [25–27]. Nevertheless, lymphocytes may be damaged owing to their high sensitivity to ionizing radiation [28] and granulocytes may be activated as a result of manipulation during in vitro cell labelling [25], limiting these techniques. The visualization of inflammatory processes with FDG-positron emission tomography (PET) in various forms of vasculitis seems promising for diagnosing and monitoring disease activity [29, 30] and, recently, successful imaging of immune-mediated inflammation was demonstrated by employing radiolabelled anti-E-selectin Fab fragment targeting endothelial cells [31]. However, enhanced FDG uptake will be seen in all cells with high metabolic requirements as observed in inflammation and infection [32], and leucocyte and granulocyte scintigraphy are also methods of choice in the detection of infection.

There is little experience with SSTR scintigraphy in infection. With respect to experimental data, this method is not recommended for detection of bacterial inflammation [33]. Although not systematically investigated, in our experience, chronic infection as seen in patients with severe WG showed only low tracer uptake compared with active disease in the same patient. This was the case in a patient with multiple bronchiectasis and persisting Pseudomonas and Staphylococcus aureus in repeated bronchoalveolar lavage, and also in a patient with chronic purulent sinitis which was successfully treated with antibiotics without escalation of immunosuppression. Six patients with a positive scintigram and active AAVS received antibiotics because concomitant infection could not be ruled out. These manifestations included osteomyelitis of the maxilla ($n = 2$), chronic obstructive bronchitis ($n = 2$), bronchiectasis ($n = 1$) and the suspicion of pneumonia ($n = 1$). In contrast, 10 patients referred to our hospital because of pulmonary disease unresponsive to antibiotics had a positive SSTR scan and responded to immunosuppressive therapy after cessation of antibiotic therapy indicating that positive scans in these patients reflected active disease rather than infection.

However, the cellular pattern in infection is a dynamic process. Predominant granulocytes in acute infection will not be reflected by SSTR scintigraphy [14], but considerable $^{[111}\text{In}]$octreotide uptake within the course of infection cannot be excluded and may be time dependent.

SSTR scintigraphy has been employed successfully in the visualization of other immune-mediated diseases such as rheumatoid arthritis and Graves’ disease [34, 35]. We could confirm positive scintigraphic findings in granulomatous diseases such as tuberculosis and sarcoidosis [17, 18]. In addition, we were able to demonstrate positive scans in single patients with Goodpasture syndrome, systemic lupus erythematosus and mixed connective tissue disease, while scintigraphy for pulmonary oedema was negative (data not shown).

In conclusion, our data provide evidence for the expression of SSTRs in active lesions of patients with AAVS. SSTR scintigraphy appears as a complementary or alternative diagnostic instrument visualizing the pathological correlates of active vasculitis. It is a non-invasive and direct imaging technique, providing adequate information on the extent of the disease activity and also reflecting the response to treatment. This may be of advantage especially in difficult cases with a long history of AAVS, frequent relapses and many scars, where radiological differentiation of active and chronic lesions may be difficult. This imaging technique may also identify previously unknown disease localizations. All of these properties may facilitate and improve the clinical assessment of patients with AAVS leading to an earlier and more precise diagnosis. The possible relevance of SSTR expression in the pathophysiology of AAVS and also therapeutic implications remain to be elucidated.
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Conflict of interest

The authors have declared no conflicts of interest.

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