APMIS JOURNAL OF PATHOLOGY, MICROBIOLOGY AND IMMUNOLOGY

APMIS 131: 152–160
DOI 10.1111/apm.13297

Immunohistochemical somatostatin receptor expression in insulinomas

ELINA PELTOLA,[1,2] TIINA VESTERINEN,[3,4] HELENA LEIJON,[3] PÄIVI HANNULA,[1,5] HEINI HUHTALA,[6] MARKUS MÄKINEN,[7] LASSE NIEMINEN,[8] ELINA PIRINEN,[9] MIKKO RÖNTY,[3] MIRVA SÖDERSTRÖM,[10] JOHANNA AROLA[3] and PIA JAATINEN[1,2,11]

[1]Faculty of Medicine and Health Technology, Tampere University; [2]Department of Internal Medicine, Tampere University Hospital, Tampere; [3]HUS Diagnostic Center, HUSLAB, Department of Pathology, University of Helsinki and Helsinki University Hospital; [4]Institute for Molecular Medicine Finland (FIMM), HiLIFE, University of Helsinki, Helsinki; [5]Endocrinology, Department of Internal Medicine, Tampere University Hospital; [6]Faculty of Social Sciences, Tampere University, Tampere; [7]Research Unit of Cancer and Translational Medicine, Department of Pathology, University of Oulu and Department of Pathology, Oulu University Hospital, Oulu; [8]Fimlab Laboratories, Pathology Department, Tampere University Hospital, Tampere; [9]Department of Clinical Pathology, Kuopio University Hospital, Kuopio; [10]Department of Pathology, Turku University Hospital, Turku; and [11]Division of Internal Medicine, Seinäjoki Central Hospital, Seinäjoki, Finland

Peltola E, Vesterinen T, Leijon H, Hannula P, Huhtala H, Mäkinen M, Nieminen L, Pirinen E, Rönty M, Söderström M, Arola J, Jaatinen P. Immunohistochemical somatostatin receptor expression in insulinomas. APMIS. 2023; 131: 152–160.

Insulinomas are rare pancreatic neuroendocrine tumours. Most patients can be cured with surgery, but patients with a metastatic disease show impaired survival. The aim of this study was to evaluate somatostatin receptor (SSTR) 1-5 expression in insulinomas and to correlate the expression profile with clinicopathological variables and with patient outcome. This retrospective study involved 52 insulinoma patients. After histological re-evaluation, formalin-fixed paraffin-embedded tissue samples were processed into tissue microarrays and stained immunohistochemically with monoclonal SSTR1-5 antibodies. All the 52 tumours (49 non-metastatic, 3 metastatic) expressed at least one SSTR subtype. SSTR2 was expressed most frequently (71%), followed by SSTR3 (33%), SSTR1 (27%), SSTR5 (6%) and SSTR4 (0%). SSTR3 expression was associated with a larger tumour size (median diameter 19 mm vs. 13 mm, p = 0.043), and SSTR3 and SSTR5 expression were associated with impaired overall survival [HR 3.532 (95% CI 1.106–11,277), p = 0.033, and HR 6.805 (95% CI 1.364–33.955), p = 0.019 respectively]. Most insulinomas express SSTR2, which may be utilized in diagnostic imaging, and in planning individualized treatment strategies for insulinoma patients. Further studies are needed to clarify the association between SSTR profile and overall survival.

Key words: immunohistochemistry; insulinoma; neuroendocrine tumour; pathology of tumours; somatostatin receptors.

Elina Peltola, Faculty of Medicine and Health Technology, Tampere University, P. O. Box 100, Tampere FIN-33014, Finland. e-mail: elina.peltola@tuni.fi

Elina Peltola and Tiina Vesterinen share equal first authorship.
Johanna Arola and Pia Jaatinen share equal last authorship.

INTRODUCTION

Insulinomas are rare pancreatic neuroendocrine tumours (PanNETs) secreting excessive amounts of insulin into the circulation. Hyperinsulinaemia leads to a clinical syndrome characterized by episodes of hypoglycaemia especially in the fasted state. Typical symptoms include confusion, drowsiness, and sweating [1,2]. Over 90% of insulinomas are non-metastatic and can usually be cured by surgery [3,4]. Metastatic insulinomas are extremely rare, but they are associated with a significantly impaired survival [1,5].

Somatostatin receptors (SSTRs) are expressed in several normal tissues (such as the pancreas and the gastrointestinal tract), as well as in a number of

Received 6 January 2023. Accepted 19 January 2023

neoplasms, including PanNETs [6,7] The overexpression of SSTRs in PanNETs has been utilized in the diagnostics and management of patients with a PanNET since the development of SSTR-targeted imaging and therapeutic options [6,8–10]. There are some reports on the immunohistochemical analysis of SSTR expression in insulinomas [11–14], but to the best of our knowledge, no previous studies have evaluated all the SSTRs with novel monoclonal antibodies. Thus, the objectives of this study were to evaluate immunohistochemically SSTR expression in a large series of insulinomas and to analyse the association of SSTR expression with the clinicopathological features of the disease, and with patient outcome.

MATERIALS AND METHODS

Patient characteristics and tumour samples

We have previously described the incidence, clinical picture, diagnostics, treatment and long-term outcome of all adult patients diagnosed with an insulinoma in Finland during 1980–2010 (n = 79) [15,16]. The Finnish insulinoma register includes clinical information gathered from the patient record registers from all the five Finnish University Hospitals (Helsinki, Kuopio, Oulu, Tampere and Turku University Hospital), supplemented by follow-up data from the national registers (Finnish Population Register Centre, Finnish Cancer Registry, the Care Register for Health Care and Statistics Finland).

In the present study, all available formalin-fixed paraffin-embedded (FFPE) primary tumour tissue samples (n = 52) of this insulinoma cohort were obtained from the five Finnish University Hospitals through local biobanks (Helsinki Biobank, Finnish Clinical Biobank Tampere, Auria Biobank, Biobank Borealis of Northern Finland and the Biobank of Eastern Finland; Fig. 1). The characteristics of the patients are presented in Table 1. All the patients had hypoglycaemic symptoms with concomitantly measured low blood/plasma glucose levels prior to the surgery. In 40 (77%) patients, insulinoma was successfully localized preoperatively, in five (10%) patients the imaging results were uncertain, and in seven (14%) patients, all diagnosed in the 1980s or 1990s, the tumour could not be localized preoperatively. All the patients with a non-metastatic insulinoma (n = 49) were cured by the primary pancreatic surgery, except for one patient, who had a tumour recurrence almost 10 years after the primary enucleation of a single benign tumour, located in the head of the pancreas. After the reoperation, no recurrence was detected during the follow-up of this patient. The three patients with distant metastases underwent distal pancreatic resection, with hypoglycaemic symptoms progressing immediately or within 3 months after the primary surgery. Twelve patients (23%) deceased during the follow-up, but only one of them died of metastatic insulinoma.

Following the World Health Organization's (WHO's) 2019 classification of PanNETs [17], each tumour was re-evaluated on diagnostic whole slides by a pathologist with special expertize in endocrine pathology. Neuroendocrine differentiation and insulin secretion were confirmed by routine immunohistochemical staining for chromogranin A, synaptophysin and insulin.

This study was conducted in accordance with the Declaration of Helsinki. Informed consent was waived, because the Finnish Biobank Act provides a lawful basis for research use of biobanked samples. The Regional Ethics Committee of the Tampere University Hospital catchment area, the Finnish Institute for Health and Welfare, the National Supervisory Authority for Welfare and Health (Valvira, currently Fimea) and the Scientific Steering Committees of the Finnish biobanks reviewed and approved the study protocol. The University Hospitals of Helsinki, Kuopio, Oulu, Tampere and Turku, and the Finnish Population Register Centre yielded permissions for the use of data from their registers.

Tissue microarray construction

After a histological review, a fresh haematoxylin-eosin (H&E) stained slide was prepared from each FFPE tissue sample and digitized with a slide scanner. Digitized slides were uploaded onto CaseViewer (3D HISTECH, Budapest, Hungary) or NDP.view2 (Hamamatsu Photonics, Hamamatsu City, Japan) software, where the areas for the TMAs were annotated. To take into account tumour heterogeneity, two representative 1 mm cores from the middle of the tumour, as well as two cores from the tumour border were selected whenever possible, considering the tumour size. The TMAs were constructed in the biobanks using a TMA Grand Master (3D HISTECH) or Galileo TMA CK4500 (Isenet, Milan, Italy) microarrayer.

Immunohistochemistry

Fresh 3.5 μm thick tissue sections were deparaffinized and treated with heat-induced antigen retrieval before incubating with primary antibodies (Table 2). Antibody binding was visualized using a polymer-based OptiView or ultraView Universal DAB Detection Kit (Ventana Medical Systems, Inc., Tucson, AZ, USA) or EnVision Detection System (Dako, Agilent Pathology Solutions, Santa Clara, CA, USA). Automated (BenchMark ULTRA, Ventana) or semi-automated (AutoStainer, Lab Vision Corp., Fremont, CA, USA) staining instruments were used. All slides were counterstained with Mayer's haematoxylin (Dako). Appropriate positive controls were used for each antibody.

Scoring of the staining results

Immunohistochemically stained slides were digitized with a Pannoramic slide scanner (3D HISTECH). By using the CaseViewer software (3D HISTECH) for viewing the slides, H.L. and T.V. performed the scoring of SSTR expression. Immunoreactivity of the strongest stained TMA spot was scored based on both membranous and cytoplasmic staining, as described earlier (Fig. 2) [18–20].

Based on membranous SSTR staining, tumours were scored as negative (0) if no staining was observed, weak (1) if partial membranous positivity in <10% of the tumour cells was detected, and moderate (2) if partial membranous positivity was observed in ≥10% of the tumour cells. The staining was scored strong (3) if

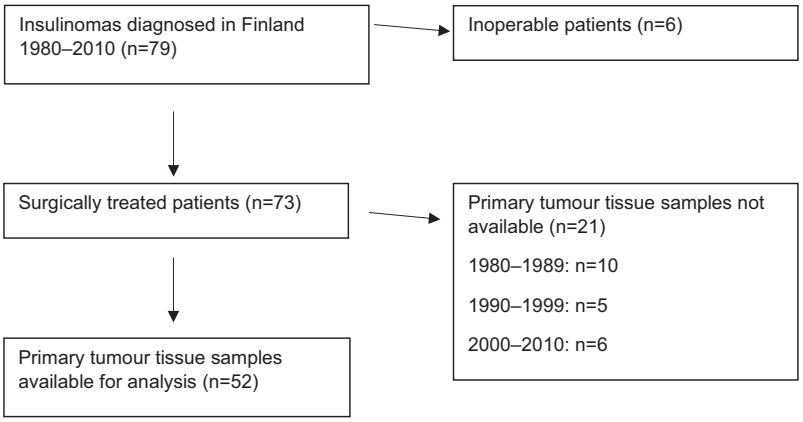

Fig. 1. Data construction for the histopathological analysis of the national insulinoma cohort.

Table 1. Characteristics of the 52 insulinoma patients diagnosed in Finland between 1980 and 2010 and included in the histopathological analysis

	n (%)	Median (min–max)
Sex, women	39	(75)
Patients with distant metastases	3 (6)	
Patients with MEN1 syndrome	2 (4)	
Surgical method		
Enucleation	24	(46)
Distal resection	23	(44)
Pancreatico-duodenectomy	5	(10)
Age at surgery, years		52.7 (23.1–84.2)
Duration of follow-up after primary surgery, years		10.4 (0.2–32.4)
Tumour diameter, mm (n = 46)		15 (5–60)

circumferential membranous positivity was observed in ≥10% of the tumour cells, and intense (4) if >95% of the tumour cells had a strong, circumferential staining pattern. The following scoring system was used for cytoplasmic staining: 0, negative; 1, weak intensity; 2, moderate intensity; and 3, strong intensity. Tumours were considered SSTR positive if a membrane pattern with a score 2 or higher was observed, or if moderate or strong cytoplasmic SSTR staining was found in ≥5% of the tumour cells.

Insulin staining was considered strong if ≥50% of the neoplastic cells showed at least moderate cytoplasmic immunoreactivity. Chromogranin A and SYP were considered positive if ≥90% of the neoplastic cells showed at least moderate cytoplasmic immunoreactivity.

The proliferation index (PI), as measured with Ki-67, was analysed with deep-learning based Aiforia software (Aiforia Technologies, Helsinki, Finland), as described earlier [21]. The highest Ki-67 PI of four parallel TMA spots per tumour was used for further statistical analysis. Similarly, the highest value was used to grade the tumour according to the WHO 2019 classification of PanNETs [17]. The proliferation activity of the tumour was classified as low/grade 1 (G1) if the Ki-67 index was <3%, intermediate/G2 if the Ki-67 index was 3–20%, or high/G3 if the Ki-67 index was >20%.

Statistical analysis

The statistical analyses were conducted with the IBM SPSS Statistics for Windows, Versions 25.0, 26.0 and 27.0 (IBM Corp.). The data are presented as median (minimum–maximum) for continuous variables, and number (%) for categorical variables. Spearman correlation coefficient was calculated to analyse the correlation between tumour size and the Ki-67 index. The co-expression of SSTRs 1-5 was analysed by crosstabulation and the McNemar test. The median tumour diameter between the SSTR positive and negative tumours was compared with the Mann–Whitney *U* test. Univariate Cox regression analysis was applied to calculate the hazard ratio (HR) with the 95% confidence interval (CI), to analyse the association of SSTR expression and Ki-67 PI with overall survival. In all analyses, a two-sided p value below 0.05 was considered statistically significant.

RESULTS

Somatostatin receptor status and co-expression

All the 52 tumours showed at least weak expression of one SSTR, either on the cell membrane or in the cytoplasm, but none of the tumours expressed all five receptors. Membranous staining was observed for all receptors, although the staining for SSTR3-5 was only weak or moderate (Fig. 3A).

Based on both membranous and cytoplasmic staining, SSTR2 was expressed most frequently, followed by SSTR3, SSTR1 and SSTR5 (Fig. 3B). Only one tumour expressed SSTR4 weakly on its cell membrane and thus all the tumours were considered SSTR4 negative. Eleven (21%) of the tumours were considered negative for all SSTRs, as the intensity and/or extent of staining did not fulfil

Table 2. Features of the primary antibodies and staining protocols used for the immunohistochemistry

Antibody	Supplier (cat#)	Clone	Dilution	Incubation (min)	Pre-treatment	Detection
SSTR1	Abcam[1] (ab137083), no RRID	UMB7	1:500	45	Tris-EDTA pH 9.0	EnVision
SSTR2	Abcam (ab134152), RRID: AB_2737601	UMB1	1:300	32	CC1 std	OptiView
SSTR3	Abcam (ab137026), no RRID	UMB5	1:7000	60	Citrate pH 6.0	EnVision
SSTR4	Bio-Rad[2] (MCA5922), no RRID	sstr4	1:500	30	Citrate pH 6.0	EnVision
SSTR5	Abcam (ab109495), RRID: AB_10859946	UMB4	1:1000	30	Citrate pH 6.0	EnVision
Ki-67	Dako (M724001-2), RRID: AB_2631211	MIB-1	1:100	32	CC1 std	OptiView
Insulin	Dako (IR00261-2), no RRID	polyclonal	1:200	30	No	EnVision
Chrom A	Dako (M0869), RRID: AB_2081135	DAK-A3	1:800	32	CC1 std	ultraView
SYP	Ventana (790–4407), RRID: AB_2336016	SP11	RTU	32	CC1 mild + Protease 3 12 min	OptiView

Chrom A, chromogranin A; RTU, ready to use; SSTR, somatostatin receptor; SYP, synaptophysin.
[1]Abcam, Cambridge, UK.
[2]Bio-Rad, Hercules, California, US.

the criteria for SSTR positivity. The SSTR expression profiles of the different TMA spots were highly homogeneous: parallel TMA spots presented similar SSTR staining pattern in 94% of the tumours (n = 49). Two tumours showed heterogeneous expression of SSTR3, and one tumour showed heterogeneous expression of SSTR1.

In the classification positive/negative, SSTR2 expression was associated with SSTR3 ($p < 0.001$) and SSTR5 ($p < 0.001$) expression: all SSTR3 and SSTR5 positive tumours were also SSTR2 positive. Similarly, SSTR1 expression was associated with SSTR2 expression ($p < 0.001$), since 71% of the SSTR1 positive tumours were also positive for SSTR2.

Somatostatin receptors, tumour size and Ki-67 PI

SSTR3 expression correlated with tumour size. Tumours expressing SSTR3 were significantly larger than tumours lacking expression of SSTR3 [19 (5–60) mm vs 13 (5–40) mm, $p = 0.043$]. No association with tumour size was observed for other SSTRs (data not shown).

The expression of SSTRs did not correlate with tumour proliferation, except for SSTR1. The median Ki-67 PI was 0.4 (range 0.1–16.1). No significant correlation was found between the tumour size and the Ki-67 index ($r = 0.238$, $p = 0.111$). In tumours expressing SSTR1, the median Ki-67 PI was minimally higher than in tumours lacking SSTR1 expression [0.7 (0.2–2.6) vs 0.3 (0.1–16.1) respectively, $p = 0.024$]. No significant difference was found in the Ki-67 PI between tumours expressing or lacking expression of SSTR 2–5 (data not shown).

When graded according to the WHO 2019 classification of PanNETs, 50 (96%) of the tumours were classified as G1 and two (4%) as G2, with the majority of the G1 tumours (77%) having a Ki-67 index lower than 1%. The two G2 tumours (one metastatic and the other non-metastatic) expressed SSTR2 and lacked expression of SSTR1 and SSTR4-5. SSTR3 was expressed only in the metastatic G2 tumour.

Somatostatin receptors and insulin expression

Immunohistochemical staining for insulin was considered strong in 49 (94%) insulinomas and it was associated with SSTR expression. All three insulinomas, in which the insulin staining was considered weak, were considered positive for SSTR1 and SSTR2, but negative for SSTR5, compared to the expression of 22% for SSTR1, 69% for SSTR2 and 6% for SSTR5 in insulinomas with strong insulin expression. The difference in insulin staining between the tumours expressing SSTR1 and those lacking SSTR1 expression was statistically significant ($p = 0.016$, Fisher Exact test). SSTR3 was expressed in 33% of both groups of tumours (insulin staining strong or weak). Staining for insulin was considered strong in 98% of the non-metastatic tumours, but in only one of the three metastatic insulinomas (33%). The difference in insulin staining between the non-metastatic and metastatic insulinomas was statistically significant ($p = 0.007$, Fisher's exact test).

Somatostatin receptors and metastatic or MEN1-related insulinoma

All of the three metastatic insulinomas showed intense membranous expression of SSTR2 and no or only weak membranous expression of SSTR3–5. Regarding SSTR1, one tumour was

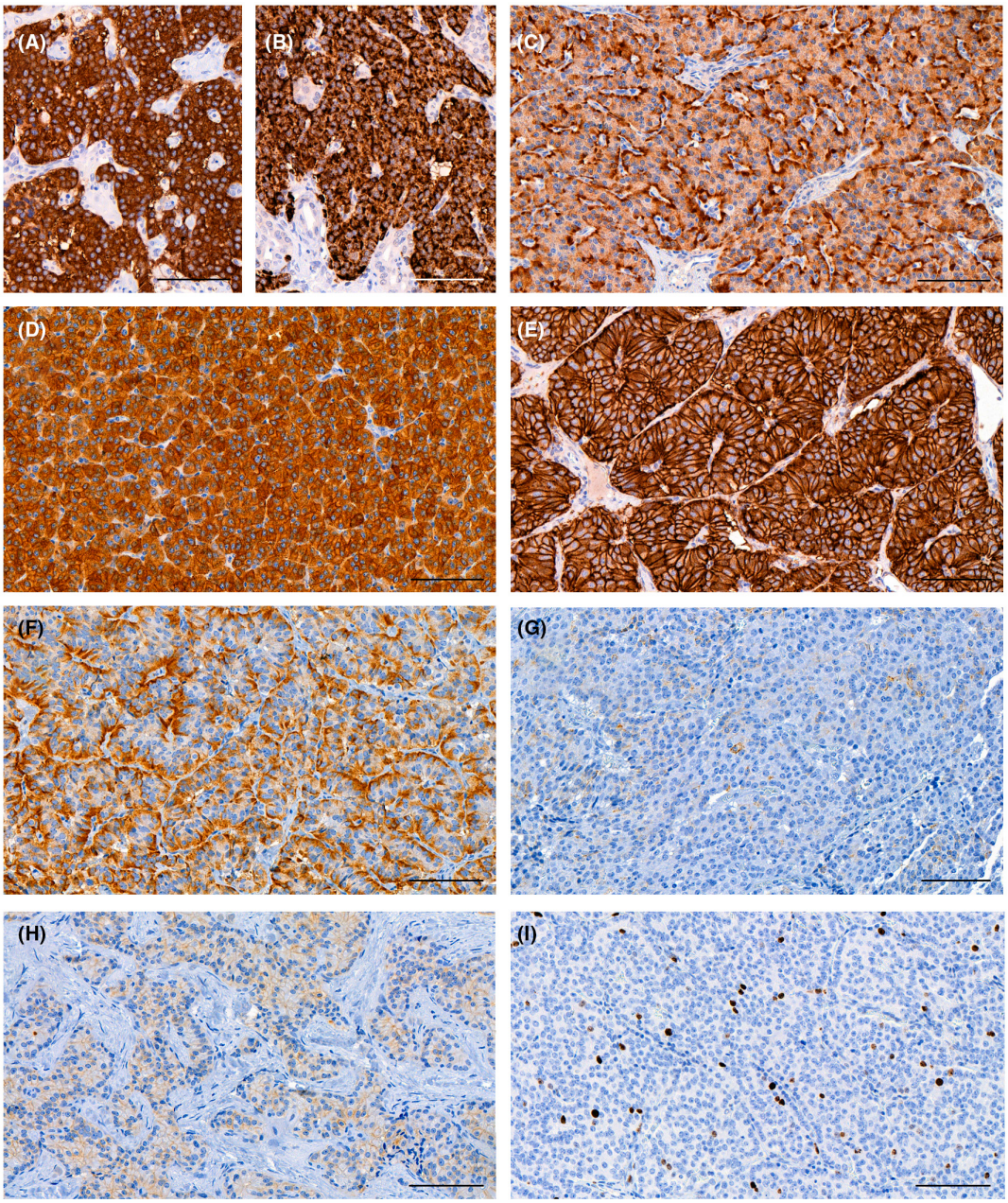

Fig. 2. Evaluating and scoring of the immunohistochemical stainings. (A) Positive chromogranin A (B) synaptophysin and (C) insulin staining. (D) Somatostatin receptor (SSTR) 1 membranous (memb) score 3 and cytoplasmic (ctpl) score 3 in >95% of tumour cells. (E) SSTR2 memb 4 and ctpl 2 in >95% of tumour cells. (F) SSTR3 memb 1 and ctpl 3 in 75% of tumour cells. (G) SSTR4 memb 1 and ctpl 2 in <1% of tumour cells. (H) SSTR5 memb 2 and ctpl 1 in 50% of tumour cells. (I) Ki-67 proliferation index 2%. Images were obtained from digitized slides with Aiforia software. Scale bar 100 μm, original magnification 20×.

negative, one moderately positive and one strongly positive. When evaluated on the basis of both membranous and cytoplasmic expression, SSTR1 was expressed in 67%, SSTR2 in 100%, SSTR3 in 67%, SSTR4 in 0% and SSTR5 in 0% of metastatic insulinomas, compared to 25%, 69%, 31%, 0% and 6% in non-metastatic insulinomas respectively.

Both insulinomas associated with the MEN1 syndrome were non-metastatic, G2 tumours that expressed SSTR2 and lacked expression of SSTR3-5. One of the MEN1-related insulinomas expressed

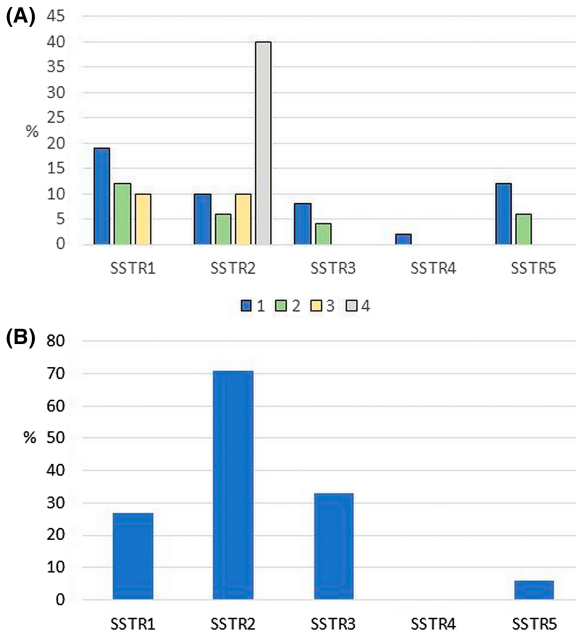

Fig. 3. Expression of somatostatin receptors (SSTR) 1-5 in 52 insulinomas. (A) Percentage of tumours showing any level of membranous expression (1 = weak, 2 = moderate, 3 = strong, 4 = intense) (B) Percentage of tumours considered positive based on both membranous and cytoplasmic SSTR expression.

SSTR1 and weak staining for insulin. The other MEN1-related insulinoma lacked expression of SSTR1 but showed strong insulin expression.

Somatostatin receptors, SSTR imaging and somatostatin analogue treatment

None of the patients (diagnosed in 1980–2010) underwent SSTR PET/CT imaging, but 12 patients had undergone octreotide scintigraphy, with insulinoma being visualized in two (17%) of them. There was no statistically significant association between the expression of SSTR1-5 and tumour visibility on octreotide scintigraphy (data not shown). Of the two visible tumours, both showed intense membranous and strong cytoplasmic staining for SSTR2. In addition, one of the visible tumours expressed SSTR1, and the other expressed SSTR3. On the other hand, six tumours were not visible on preoperative octreotide scintigraphy, despite immunohistochemical expression of SSTR2.

All three patients with a metastatic insulinoma received somatostatin analogue (SSA) treatment pre- and/or postoperatively. The response to SSA treatment was poor in two of them, but in one patient, the insulinoma with liver and lung metastases was successfully controlled with postoperative SSA treatment, and the patient was alive at the end of the follow-up, over 6 years after the pancreatic surgery. The tumour of this patient expressed SSTR1-3 but lacked expression of SSTR4 and SSTR5. In addition to the patients with metastatic insulinomas, three patients with a non-metastatic insulinoma were treated with a SSA preoperatively: one patient with a SSTR1-5 negative insulinoma did not respond to the treatment (no relief of hypoglycaemia), and for the other two patients, data on the response to treatment was not available.

Somatostatin receptors and patient outcome

The expression of SSTR3 and the expression of SSTR5 were associated with impaired overall survival in univariate Cox regression analysis [HR 3.532 (95% CI 1.106–11,277), p = 0.033 and HR 6.805 (95% CI 1.364–33.955), p = 0.019 respectively]. Regarding SSTR1 and SSTR2, no significant difference was found in the overall survival of patients with SSTR positive versus SSTR negative insulinomas (data not shown). Ki-67 PI was associated with a decreased overall survival [HR 1.220 (95% CI 1.041–1.430), p = 0.014].

DISCUSSION

In this study we analysed the immunohistochemical SSTR1-5 profile of 49 non-metastatic and three metastatic insulinomas and studied their association with clinicopathological variables. SSTR2 was expressed most commonly, followed by SSTR3 and SSTR1. Three tumours were positive for SSTR5, and no tumour was considered positive for SSTR4. This study is unique since we evaluated all five SSTRs with commercially available monoclonal primary antibodies. In addition, our cohort of 52 insulinomas is the second largest of all reported studies [11–14].

In previous studies with the same primary antibody clone as ours, 57% [11] and 83% [22] of the tumours in 65 and 18 insulinoma patients respectively, showed SSTR2 expression, which is in line with our findings. On the other hand, in three other series of 36, 17 and 16 insulinomas, 58%, 41% and 13% expressed SSTR2 respectively [12–14]. Varying results have also been reported for the expression of SSTR1 (25–31%), SSTR3 (19–78%), SSTR4 (88%) and SSTR5 (19–88%), compared to 27%, 33%, 0% and 6% in the present study respectively [12–14]. Different scoring criteria and primary antibodies have possibly affected the results. Recently, Yu *et al.* [23] reported heterogeneity of the SSTR2 expression in 43% of 100 gastroenteropancreatic

NETs. This is in contrast to our findings, as the SSTR expression profiles of the parallel TMA spots in our study were highly homogeneous.

In the present study, the expression of both SSTR3 and SSTR5 were associated with impaired overall survival. The expression of SSTR3 was also associated with a slightly larger tumour size, but not with the Ki-67 PI or a metastatic disease. The expression of SSTR1 was associated with weaker immunohistochemical staining for insulin (moderate or stronger cytoplasmic immunoreactivity in <50% of the neoplastic cells), and with a slightly higher Ki-67 PI compared to tumours lacking SSTR1 expression (0.7% vs 0.3% respectively). This minimal difference in the KI-67 PI can be considered clinically insignificant, as all the SSTR1 positive insulinomas were still low-grade tumours, with a Ki-67 PI of less than 3%. In contrast to our results, Watanabe *et al.* [22] found an inverse correlation between SSTR2 immunoreactivity and Ki-67 PI. They used the HER2 scoring, where score 3 corresponds to our membranous staining pattern score 4 (intense), and found no HER2 score 3 G2 insulinomas, while our G2 tumours showed intense membranous staining for SSTR2.

All the three metastatic insulinomas in our study showed an intense membranous expression of SSTR2 but no expression of SSTR4 or SSTR5. Similarly to us, Andreassen *et al.* [11] reported immunohistochemical expression of SSTR2 in all insulinomas having distant metastases. The weak staining for insulin in metastatic insulinomas was also in line with the findings of Andreassen *et al.* [11] who suggested that a negative staining for insulin and proinsulin could be signs of poor differentiation and thereby associated with malignant behaviour.

Information on the SSTR expression profile of insulinomas can be utilized in planning SSTR-targeted imaging and treatment strategies, especially for insulinoma patients with a metastatic disease. Due to the retrospective design dating back to the 1980s, only a few patients had undergone SSTR imaging, and for that reason we were not able to properly study the association between the SSTR expression and the visibility of the tumour in SSTR-targeted imaging. In previous studies on 15 or 17 patients, all the insulinomas visible on octreotide scintigraphy also showed immunohistochemical expression of SSTR2, but not all SSTR2-expressing tumours were visible on octreotide scintigraphy [11,13]. Recent studies on gastroenteropancreatic neuroendocrine neoplasms indicated that the results of SSTR PET/CT imaging significantly correlate with the immunohistochemical expression of SSTR2 [18,23]. To our knowledge, no studies have yet investigated the association between the SSTR PET/CT imaging and the immunohistochemical expression of SSTR2 in patients with an insulinoma.

This study had some limitations. The major limitation was the small number of patients with a metastatic disease or a disease-related death, despite the long follow-up. As only three patients had a metastatic disease, we were not able to analyse the potential difference in the SSTR expression between metastatic and non-metastatic insulinomas, nor could we analyse the association between SSTR profile and long-term survival in patients with a metastatic insulinoma. Since this was a retrospective study on patients diagnosed in the 1980s – early 2000s, PET/CT imaging using radiolabelled SSTR ligands were not available for the diagnostics of this patient cohort. Thus, we were unable to compare the SSTR immunohistochemical expression with data from the modern SSTR imaging. As only a few patients of the cohort used preoperative SSA treatment, we could not properly analyse the association between the SSTR expression and response to SSA treatment, either.

Considering the rarity of insulinomas, however, this national series of 52 insulinomas with the comprehensive follow-up data of the patients is regarded as valuable. Since all the tumours were re-evaluated by an experienced endocrine pathologist, and monoclonal primary antibodies and optimized protocols for immunohistochemistry were used, the results of this study can be considered reliable [24].

CONCLUSION

In conclusion, most insulinomas express SSTR2, while the expression of SSTR4-5 is rare. Larger studies are needed to analyse the SSTR expression of metastatic insulinomas, and to study the impact of the SSTR profile on the results of SSTR-targeted imaging and therapies, and on the prognosis of patients with an insulinoma.

We thank our colleagues Tapani Ebeling, Hanna Hämäläinen, Saara Metso, Leena Moilanen, Pirjo Nuutila, Antti Piiroinen, Camilla Schalin-Jäntti, Minna Soinio, Fia Sundelin, and Mirja Tiikkainen for good collaboration in collecting the insulinoma register data from the patient records in Helsinki, Kuopio, Oulu, Tampere and Turku University Hospital catchment areas. We acknowledge Helsinki Biobank (Helsinki, Finland), Auria Biobank (Turku, Finland), Biobank of Eastern Finland (Kuopio, Finland), Borealis Biobank (Oulu, Finland), Finnish Clinical Biobank Tampere (Tampere, Finland) and Kuopio University Hospital as the providers of the tumour

samples. We thank Jenni Niinimäki and Eija Heiliö for their excellent technical assistance, and Esko Väyrynen for revising the language of the manuscript.

FUNDING INFORMATION

This work was supported by the Emil Aaltonen Foundation, the Finnish Cancer Foundation, the Helsinki University Hospital Research Fund, the Liv och Hälsa Foundation, the Medical Research Fund of Seinäjoki Central Hospital, and the Research Funding provided by Seinäjoki City, Tampere University and Tampere University Hospital.

CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this study.

REFERENCES

1. Jensen RT, Cadiot G, Brandi ML, de Herder WW, Kaltsas G, Komminoth P, et al. ENETS consensus guidelines for the management of patients with digestive neuroendocrine neoplasms: functional pancreatic endocrine tumor syndromes. Neuroendocrinology. 2012;95:98–119. https://doi.org/10.1159/000335591
2. Cryer PE, Axelrod L, Grossman AB, Heller SR, Montori VM, Seaquist ER, et al. Evaluation and management of adult hypoglycemic disorders: an endocrine society clinical practice guideline. J Clin Endocrinol Metabol. 2009;94:709–28. https://doi.org/10.1210/jc.2008-1410
3. Mehrabi A, Fischer L, Hafezi M, Dirlewanger A, Grenacher L, Diener MK, et al. A systematic review of localization, surgical treatment options, and outcome of insulinoma. Pancreas. 2014;43:675–86. https://doi.org/10.1097/MPA.0000000000000110
4. Falconi M, Eriksson B, Kaltsas G, Bartsch DK, Capdevila J, Caplin M, et al. ENETS consensus guidelines update for the management of patients with functional pancreatic neuroendocrine tumors and non-functional pancreatic neuroendocrine tumors. Neuroendocrinology. 2016;103:153–71. https://doi.org/10.1159/000443171
5. Veltroni A, Cosaro E, Spada F, Fazio N, Faggiano A, Colao A, et al. Clinico–pathological features, treatments and survival of malignant insulinomas: a multicenter study. Eur J Endocrinol. 2020;182:439–46. https://doi.org/10.1530/EJE-19-0989
6. del Olmo-Garcia MI, Prado-Wohlwend S, Andres A, Soriano JM, Bello P, Merino-Torres JF. Somatostatin and somatostatin receptors: from signaling to clinical applications in neuroendocrine neoplasms. Biomedicine. 2021;9:1810. https://doi.org/10.3390/biomedicines9121810
7. Priyadashini S, Allison DB, Chauhan A. Comprehensive assessment of somatostatin receptors in various neoplasms: a systematic review. Pharmaceutics. 2022;14:1394. https://doi.org/10.3390/pharmaceutics14071394
8. Sundin A, Arnold R, Baudin E, Cwikla JB, Eriksson B, Fanti S, et al. ENETS consensus guidelines for the standards of care in neuroendocrine tumors: radiological, nuclear medicine and hybrid imaging. Neuroendocrinology. 2017;105:212–44. https://doi.org/10.1159/000471879
9. Hicks RJ, Kwekkeboom DJ, Krenning E, Bodei L, Grozinsky-Glasberg S, Arnold R, et al. ENETS consensus guidelines for the standards of care in neuroendocrine neoplasms: peptide receptor radionuclide therapy with radiolabelled somatostatin analogues. Neuroendocrinology. 2017;105:295–309. https://doi.org/10.1159/000475526
10. Hu Y, Ye Z, Wang F, Qin Y, Xu X, Yu X, et al. Role of somatostatin receptor in pancreatic neuroendocrine tumor development, diagnosis, and therapy. Front Endocrinol. 2021;12:679000. https://doi.org/10.3389/fendo.2021.679000
11. Andreassen M, Ilett E, Wiese D, Slater EP, Klose M, Hansen CP, et al. Surgical management, preoperative tumor localization, and histopathology of 80 patients operated on for insulinoma. J Clin Endocrinol Metabol. 2019;104:6129–38. https://doi.org/10.1210/jc.2019-01204
12. Portela-Gomes GM, Stridsberg M, Grimelius L, Rorstad O, Janson ET. Differential expression of the five somatostatin receptor subtypes in human benign and malignant insulinomas – predominance of receptor subtype 4. Endocr Pathol. 2007;18:79–85. https://doi.org/10.1007/s12022-007-0014-8
13. Vezzosi D, Bennet A, Rochaix P, Courbon F, Selves J, Pradere B, et al. Octreotide in insulinoma patients: efficacy on hypoglycemia, relationships with Octreoscan scintigraphy and immunostaining with anti-sst2A and anti-sst5 antibodies. Eur J Endocrinol. 2005;152:757–67. https://doi.org/10.1530/eje.1.01901
14. Kulaksiz H, Eissele R, Rössler D, Schulz S, Höllt V, Cetin Y, et al. Identification of somatostatin receptor subtypes 1, 2A, 3, and 5 in neuroendocrine tumours with subtype specific antibodies. Gut. 2002;50:52–60. https://doi.org/10.1136/gut.50.1.52
15. Peltola E, Hannula P, Huhtala H, Metso S, Kiviniemi U, Vornanen M, et al. Characteristics and outcomes of 79 patients with an insulinoma: a Nationwide retrospective study in Finland. Int J Endocrinol. 2018;2018:1–10. https://doi.org/10.1155/2018/2059481
16. Peltola E, Hannula P, Huhtala H, Metso S, Sand J, Laukkarinen J, et al. Long-term morbidity and mortality in patients diagnosed with an insulinoma. Eur J Endocrinol. 2021;185:577–86.
17. Digestive System Tumours. WHO classification of Tumours (5th ed.). WHO classification of Tumours editorial board. Lyon: IARC Press; 2019 ISBN 978-92-832-4499-8.
18. Majala S, Vesterinen T, Seppänen H, Mustonen H, Sundström J, Schalin-Jäntti C, et al. Correlation of somatostatin receptor 1–5 expression, [68Ga]Ga-DOTANOC, [18F]F-FDG PET/CT and clinical outcome in a prospective cohort of pancreatic neuroendocrine neoplasms. Cancer. 2022;14:162. https://doi.org/10.3390/cancers14010162
19. Elston MS, Meyer-Rochow GY, Conaglen HM, Clarkson A, Clifton-Bligh RJ, Conaglen JV, et al.

Increased SSTR2A and SSTR3 expression in succinate dehydrogenase–deficient pheochromocytomas and paragangliomas. Hum Pathol. 2015;46:390–6. https://doi.org/10.1016/j.humpath.2014.11.012
20. Körner M, Waser B, Schonbrunn A, Perren A, Reubi JC. Somatostatin receptor subtype 2A immunohistochemistry using a new monoclonal antibody selects tumors suitable for In vivo somatostatin receptor targeting. Am J Surg Pathol. 2012;36:242–52. https://doi.org/10.1097/PAS.0b013e31823d07f3
21. Vesterinen T, Säilä J, Blom S, Pennanen M, Leijon H, Arola J. Automated assessment of Ki-67 proliferation index in neuroendocrine tumors by deep learning. APMIS. 2022;130:11–20. https://doi.org/10.1111/apm.13190
22. Watanabe H, Ide R, Yamazaki Y, Fujishima F, Kasajima A, Yazdani S, et al. Quantitative digital image analysis of somatostatin receptor 2 immunohistochemistry in pancreatic neuroendocrine tumors. Med Mol Morphol. 2021;54:324–36. https://doi.org/10.1007/s00795-021-00294-6
23. Yu J, Cao F, Zhao X, Xie Q, Lu M, Li J, et al. Correlation and comparison of somatostatin receptor type 2 immunohistochemical scoring systems with ^{68}Ga-DOTATATE positron emission tomography/computed tomography imaging in gastroenteropancreatic neuroendocrine neoplasms. Neuroendocrinology. 2022;112:358–69. https://doi.org/10.1159/000517530
24. Remes SM, Leijon HL, Vesterinen TJ, Arola JT, Haglund CH. Immunohistochemical expression of somatostatin receptor subtypes in a panel of neuroendocrine neoplasias. J Histochem Cytochem. 2019;67:735–43. https://doi.org/10.1369/0022155419856900

