Hepatic micrometastases outside macrometastases are present in all patients with ileal neuroendocrine primary tumour at the time of liver resection

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ABSTRACT

Background: Neuroendocrine tumours (NETs) in the ileum grow slowly but metastasise to the liver at an early stage. After resection of the primary tumour and mesenteric lymph nodes, selected patients with liver metastases have been operated with curative intention. Recurrence-free survival seems low, suggesting that micrometastases are present in the liver at the time of surgery. We have therefore examined whether NET metastases could be detected in perceived normal liver tissue at the time of liver resection.**Material and methods:** Liver tissue outside the macrometastases from patients (*n* = 10) operated by liver resection due to metastases from ileal NETs G1/2, were examined for NE cells by immunohistochemistry. Liver tissue from patients operated for metastatic colon cancer was used as control (*n* = 6). Groups of ≥3 NE cells ≥3 mm from macrometastases were considered micrometastases. Clinical course was recorded retrospectively.**Results:** Ten of 10 patients had micrometastases, consisting of multiple groups of NE cells. None of the control patients had NE cells in the liver tissue. After median follow-up time of 5.5 (0.8–18.7) years 6 of 10 patients had developed recurrent NET metastases detected by cross-sectional imaging. The follow-up time of the four patients without detectable metastases was 4.8 (0.8–7.5) years vs. with detectable metastases 7.9 (3.2–18.7) years.**Conclusions:** All patient had micrometastases outside macrometastases at the time of liver resection, suggesting that subsequently recurrent liver metastases develop from NET depositions in the liver already present at the time of surgery. The likelihood of curation by hepatic resection appears very low.

Keywords: Metastases ; neuroendocrine tumour ; small intestine ; surgery

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Introduction

Small intestinal neuroendocrine tumours (SI-NETs) of the jejunum and ileum derive from serotonin-producing enterochromaffin cells. The primary tumour is often small, but irrespective of primary tumour size, SI-NETs often metastasise to mesenteric lymph nodes at an early stage and the majority of patients also have liver metastases at the time of diagnosis [1,2]. Compared to other malignancies, SI-NETs have an advantageous survival, with 10-year overall, cause-specific and relative survival rates of 36%, 80% and 54%, respectively, in a large Swedish patient series [3]. As summarised in the European Neuroendocrine Tumours Society (ENETS) guideline for management of SI-NETs [4], the survival rates depend on histopathological WHO classification, Ki-67 and TNM stage. Five-year survival for all stages is reported to be 50–60%, SI-NETs with locally limited disease 80–100% or only regional lymph node involvement 70–80% and patients with metastatic disease 35–80%.

It is recommended by ENETS that patients with localised SI-NETs should be considered for surgery [5]. Whereas randomised controlled trials are lacking, observational studies suggest that resection of the primary tumour and locoregional lymph node metastases improve outcome and is associated with 5- and 10-years NET-related survival of 100% in stage I and II patients and 5-year survival of 95% and 10-year survival of 80% in stage III jejuno-ileal NETs [2,4]. Observational studies also propose that surgical removal of the primary tumour may improve survival even in metastatic disease [6], although there has been no firm consensus on this practice [7]. Even in stage IV disease, radical resection of the primary tumour, lymph nodes and liver metastases with curative intention is recommended by the ENETS [5].

However, recurrence rates after resection with curative intention are high and recurrence may be diagnosed by radiological examination in more than 80% of patients within 5 years after liver resection [8]. The proportion of patients developing detectable liver metastases increase further if followed for a sufficient period of time [9]. A study of liver tissue from patients with NETs of various origins (total n = 11, whereof three had primary SI-NET), described that NET-patients in general have small liver metastases (<2 mm) not recognised by currently available radiological imaging at the time of liver resection [10]. Recently, a larger study found that hepatic micrometastases from SI-NETs could be detected in 67% of patients when haematoxylin and eosin (H&E) stained sections were examined and that micrometastases were associated with shorter overall survival [11].

Several non-surgical treatment modalities improve the overall prognosis of patients with metastatic SI-NET, as well as of patients with disease recurrence after surgery. Somatostatin analogues are the mainstay of SI-NET treatment and have anti-tumour as well as symptom-reducing effects and prolong progression-free survival [12,13]. In addition, Peptide Receptor Radionuclide Therapy (PRRT) increases progression free survival rates and may also increase overall survival [14,15]. Therapy directed against focal liver metastases, such as embolisation or radiofrequency ablation of liver metastases, also has a role in selected patients [4,16]. Considering the mentioned treatment modalities, the rationale for surgical resection of SI-NET liver metastasis could be re-evaluated if it was demonstrated that most patients have multiple hepatic micrometastases at the time of liver resection.

In the current study, we have examined if patients with SI-NET have hepatic NE micrometastases outside macrometastases at the time of liver resection.

Methods

Patients

Patients with SI-NET liver metastasis were identified in the archives of Department of Pathology, St Olav's Hospital – Trondheim University Hospital, Trondheim, Norway, searching the SNOMED codes T64xxx (small intestinal tumour), M824xx (NET) and T56xxx (liver tumour) during the 20-year period from 1998 to 2018. Eleven patients who had undergone liver resection due to SI-NET metastases were identified, whereof 10 patients had available liver tissue. Consent was been obtained from each patient still alive after full explanation of the purpose and nature of all procedures used. The study protocol was approved by the Regional Committee for Medical and Health Research Ethics (2018/318).

Patient characteristics and diagnostic imaging procedures before liver surgery were obtained from the medical records. Treatment intention (palliative or curative) as judged at the time of liver surgery as well as the type of resection (R0, R1 or R2) were recorded. Postoperative clinical course was followed until November 2018. Disease recurrence was recorded as the date of a cross-sectional imaging procedure with the conclusion of likely disease recurrence.

Histopathology and immunohistochemistry

All tissue blocks from each patient having undergone liver resection for SI-NET were examined (range 1–10 blocks). Sections were made from formalin fixed and paraffin embedded tissue samples of liver tissue before immunohistochemical (IHC) labelling of the general neuroendocrine markers chromogranin A (CgA) and synaptophysin. Before IHC examinations, sections were deparaffinized, rehydrated and underwent antigen retrieval in buffer pH 9 for 15 minutes in a microwave oven at 160W. Sections were incubated with antibodies against chromogranin A (CgA) (M0869, Dako, Glostrup, Denmark. Dilution 1:1000, incubation 1 h in room temperature), synaptophysin (M7315, Dako, 1:200, incubation 1 h at room temperature, Mouse linker SM804 for 15 minutes) and Ki-67 immunolabelling (M7240, Dako, dilution 1: 100, incubation 1 h in room temperature). The immunoreactions were visualised using an EnVision-HRP kit with DAB+ (K5007, Dako).

Liver tissue from patients operated for metastatic colorectal cancer was used as control tissue ($n = 6$) and immunolabelled with antibodies against CgA and synaptophysin. Groups of ≥3 NE cells located ≥3 mm from macrometastases were considered micrometastases. The distance from macrometastases to the micrometastases furthest away from the macrometastases in each patient was measured.

The number of Ki-67 positive cells pr 500 tumour cells were counted in the primary tumours as well as in the liver metastases and the tumours were subsequently graded as G1 or G2.

Statistical analyses

Descriptive data are presented as frequency (*n* (percentage)) or median (range), as appropriate. Data were analysed using IBM SPSS Statistics version 21.0 (IBM Corporation, Armonk, NY, USA).

Results

Patient and tumour characteristics

Patient and tumour characteristics, as well as type of liver resection and preoperative imaging, are presented in Table1. All patients had a primary NET located to the ileum graded as G1 or G2 with Ki67 up to 5% in hotspots. The liver metastases were all G1 or G2 with Ki67 up to 6% in hotspots. Four patients had a single liver metastasis, six patients had multiple metastases.

Table 1. Demographic and disease characteristics of patients with ileal neuroendocrine tumours operated with resection of liver metastases.

	All patients	No clinical recurrence	Clinical recurrence
Number of patients (*n*)	10	4	6
Gender (Male/Female)	6/4	2/2	4/2
Age at diagnosis (years), *median (range)*	56.4 (38.2–70.4)	59.5 (38.2–70.4)	54.4 (44.3–67.2)
Primary tumour			
Size (cm), *median (range)*	3.0 (1.4–5.0)	2.9 (1.4–3.5)	3.0 (2.0–5.0)
Grade G1/G2 (*n*/*n*)	7/2	3/1	4/1
Liver surgery			
Number of liver metastases			

	All patients	No clinical recurrence	Clinical recurrence
Single	4	3	1
Multiple	6	1	5
Largest metastasis (cm), (range)	17.0 (0.9–17.0)	17.0 (0.9–17.0)	5.0 (1.5–5.6)
Grade G1/G2 (*n*/*n*)	7/3	3/1	4/2
Operation type			
Hemihepatectomy	2	0	2
Lobectomy	5	1	4
Wedge resection	3	3	0
Resection margin R0/R1/R2 (*n*/*n*/*n*)	5/2/3	1/1/2	4/1/1
Preoperative imaging			
CT	10	4	6
Octreotide scintigraphy	8	3	5
MR	8	3	5
Disease recurrence			
Recurrence		4	6
Micrometastasis	10	4	6
Follow-up time after liver resection (years), *median (range)*	5.5 (0.8–18.7)	4.9 (0.8–7.5)	7.9 (3.2–18.7)
Time to recurrence (years), *median (range)*			2.4 (1.7–5.8)

Micrometastases

All 10 patients had micrometastases outside macrometastases in the available liver tissue, whereas none of the control patients had NE cells in the examined liver tissue. Images of micrometastases are presented in Figures 1–3. Two patients did not have micrometastases in the first section from the available tissue blocks, but micrometastases were revealed after two additional sections from the same tissue blocks were immunolabelled and examined. Thus, all patients had multiple micrometastases with a varying distance from the larger metastases, ranging from 3.5 to 14 mm where macrometastases and micrometastases were present in the same section. Two patients also had embedded liver tissue not containing macrometastases, but still containing micrometastases. The median ~~distance between macrometastases and the detected~~ maximal ~~measured~~ distance between macrometastases and micrometastases in each patient ~~micrometastases~~ was 10 mm.

Figure 1. Immunohistochemical labelling of chromogranin A positive micrometastases outside macrometastases in resected liver tissue. The distance between the micrometastasis and macrometastasis was 3.1 mm.

Figure 2. Immunohistochemical labelling of chromogranin A positive micrometastases with numerous chromogranin A positive cells surrounding a portal field.

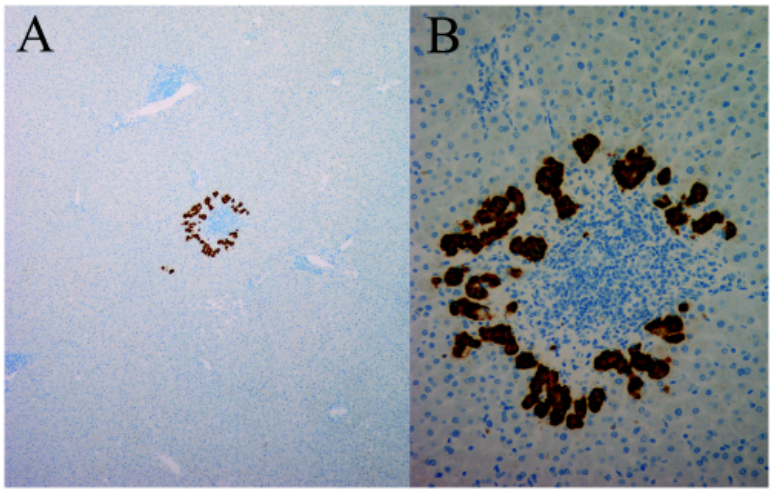
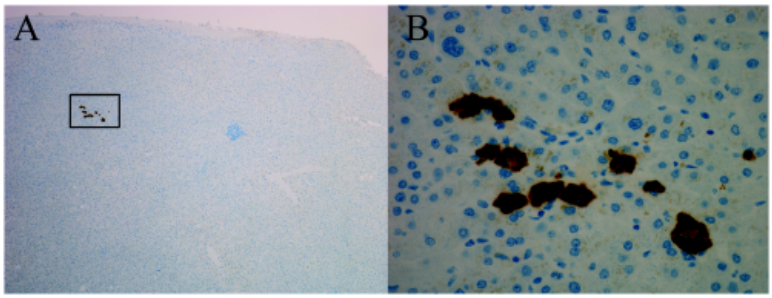


Figure 3. Immunohistochemical labelling of chromogranin A positive micrometastases with small clusters of chromogranin A positive cells close to the liver capsule (A), also seen with higher magnification in B.



Disease recurrence

The median follow-up time for all 10 patients was 5.5 years (range 0.8–18.7 years). Six of the patients had recurrence of NET metastases detectable by cross-sectional imaging and all had disease recurrence including metastases in the liver. Their mean follow-up time was 7.9 years (range 3.2–18.7 years). The follow-up time of the four patients without detectable metastases was 4.9 years (range 0.8–7.5 years). Recurrence of disease at cross-sectional imaging was not associated with the resection status of the macrometastases (Table 1).

Discussion

We have found that all patients with hepatic metastases from SI-NETs also have micrometastases outside the resected macrometastases when sections immunolabelled for neuroendocrine markers were examined. The findings suggest that hepatic micrometastases are present in the liver at the time of liver resection and that macroscopic liver metastases will become evident eventually. In fact, long-term follow-up of operated SI-NET patients have revealed that nearly all seem to develop recurrent disease if followed for a sufficient period of time (>20 years) [9]. However, specific data for SI-NETs often lack in studies reporting hepatic recurrence after resection of liver metastases, but in well differentiated NETs in general, the recurrence rate after 10 years has been reported to be 75% [17], suggesting that this may be a common phenomenon in NETs. It was recently reported that hepatic micrometastases were found in 67% of 42 patients with SI-NET when already available H&E-stained sections were re-examined. Considering the small size of micrometastases, we find it very likely that a higher proportion of the patients would be positive if further tissues sections of all perceived negative patients had been made. Additionally, immunolabelling of NE markers may increase the sensitivity when searching for NE micrometastases.

The observed dormancy of NETs may in part be explained by a low rate of proliferation, often with a Ki67 < 1%, and it may take many years before micrometastases become visible (>3 mm) at cross sectional imaging. However, also in other types of cancer there may be years to decades of latency before disease recurrence can be detected. The

concept of cancer dormancy has developed over time [18] and although its mechanisms are incompletely understood [19], cellular dormancy, angiogenic dormancy as well as immunosurveillance have been suggested to be important. A better understanding of dormancy as a phenomenon could prove to be of particular importance for NET patients. Immunological destruction of smaller groups of cells have been described in other types of cancer [20] and one could speculate that micrometastases from NETs could be eliminated by this mechanism. However, avoiding immune destruction is considered a hallmark of cancer [21] and the clinical course of NET patient cohorts, where recurrence most often occurs, suggests that the immune system by itself does not eradicate all NET cells. One could further speculate that highly differentiated NET cells express markers that initiate immunological destruction less frequently than many other cancers.

However, regardless of hepatic micrometastases, patients should be offered liver resection if there is evidence that surgery may increase the overall survival. Resection of liver metastases is considered to increase survival and debulking surgery is therefore recommended [5,7]. Although the evidence to support such practice was assessed as very weak by a NET expert group, it was recommended that liver resection should be considered the first choice for patients with completely resectable G1 or G2 liver metastases and no resectable extrahepatic disease [7]. Clearly, the best surgical approach to SI-NET patients will still be debated. A recent analysis of the United States SEER data found that although surgical resection seemed to improve survival, radical resection was not more favourable than local resection in patients with SI-NETs [22]. Several research groups have analysed their liver resections in NET-patients overall and found that R-status did not significantly affect survival [17,23] and increasing resection margins would most likely not affect survival. Such studies may be interpreted as indirect evidence that attempts of radical resections are ineffective in improving overall survival, and the lack of benefit may reflect NET biology as well as widespread use of non-surgical treatment modalities including PRRT.

The overall survival in SI-NETs is high, whereas long-term disease-free survival is very low. Reporting overall survival rates is robust and clinically relevant at first sight. However, in order to understand tumour biology, reporting disease-free survival is more informative, particularly in patients with SI-NET stage I and II who have high overall survival.

In conclusion, hepatic micrometastases outside macrometastases are found in the liver of all SI-NET patients undergoing liver resection for such metastases. It is likely that recurrences evident later in the course of their disease develop from such micrometastases.

Disclosure statement

The authors have no conflicts of interest to declare.

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