CASE REPORT

Major hyperghrelinemia in advanced well-differentiated neuroendocrine carcinomas: report of three cases

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Abstract

Objective: We aimed to gain insight into the functional consequences of ghrelin overproduction in patients with neuroendocrine tumors and its relations with disease characteristics and evolution.

Design: We retrospectively analyzed three cases of neuroendocrine carcinomas associated with very high levels of circulating ghrelin.

Methods: Between February and October 2007, serum ghrelin levels were determined in all patients with well-differentiated endocrine carcinoma referred to our center (n = 72). Three patients were found to have circulating ghrelin levels >10-fold the upper limit of normal. The clinical, biochemical, and pathological characteristics of these three patients were reviewed. The ratio between circulating acyl and total ghrelin was determined, and tumor tissue expression of ghrelin was assayed by immunohistochemistry.

Results: The three patients had massive hyperghrelinemia (respectively 49 028, 63 711, and 101 996 pg/ml), with <10% of acyl ghrelin. The corresponding primary tumors were located in the pancreas, rectum, and gallbladder; all were metastatic. There was no acromegaly; there was a decrease in appetite; and body mass index was low. Serum GH levels were only slightly increased and serum IGF1 levels were normal. Immunoreactive ghrelin was detected in the tumor tissue in the two cases in which tissue material was available. All three patients died before 12 months after the diagnosis of hyperghrelinemia.

Conclusion: Well-differentiated neuroendocrine carcinomas of various origins may produce markedly high levels of circulating ghrelin, without evidence of clinical or functional consequences.

European Journal of Endocrinology 161 639–645

Introduction

Ghrelin is a 28-amino acid hormone with multiple physiological functions, including the stimulation of GH release, the stimulation of appetite, and the regulation of gastric motility and gastric acid secretion (1, 2). Ghrelin exerts its orexigenic action through the GH secretagogue receptor type 1a (GHS-R1a, recently renamed GRLN) (1). Ghrelin is predominantly produced by the endocrine cells of the gastric oxyntic mucosa but has also been detected in endocrine cells of many other organs, including the small intestine, the pancreas, the lungs, the thyroid, the adrenal gland, the hypothalamus, and the pituitary (3). Ghrelin exists in two major forms: n-octanoyl-ghrelin (acyl ghrelin) and des-n-octanoyl ghrelin (desacyl ghrelin). Octanoylation, mediated by a specific enzyme, ghrelin O-acyltransferase (GOAT), is essential for the binding of ghrelin to GHS-R1a; however, although desacyl ghrelin is not able to bind to GHS-R1a, it may have some biological activities and may even be an antagonist of the orexigenic effects of acyl ghrelin (4).

Various types of neuroendocrine tumors are able to synthesize ghrelin. Ghrelin expression is particularly frequent in the endocrine tumors of the stomach (5), but has also been detected in endocrine tumors originating from other sites, including the pancreas, the lungs, the pituitary, the thyroid, and the parathyroids (6–11). Despite the frequent detection of ghrelin-producing cells in tumor tissue, circulating levels of the hormone are usually normal or only mildly elevated; moreover, they seem not to be correlated with the amount of peptide synthesized by tumor cells (5, 10, 12, 13). In some rare cases, very high levels of
circulating ghrelin have been observed in patients with neuroendocrine tumors (12, 14). In only one of these cases, of gastric origin (14), it has been possible to suspect the existence of functional consequences of ghrelin overproduction, such as appetite stimulation. In another case, of pancreatic origin (12), the high circulating levels of ghrelin seem not to be associated with any functional or biological symptom, raising doubts about the functioning nature of the tumor.

Because of the scarcity of the available information, the incidence and significance of hyperghrelinemia in patients with neuroendocrine carcinomas remain poorly documented. We were therefore prompted to report three additional cases of neuroendocrine carcinomas with massive hyperghrelinemia in order to evaluate the possible clinical and biological consequences of ghrelin overproduction and analyze the relationship between ghrelin overproduction and tumor characteristics and evolution.

Patients and methods

Selection of patients

Between February and October 2007, serum ghrelin levels were measured in 72 consecutive patients with well-differentiated endocrine carcinoma, referred to the outpatient clinic of the Department of Digestive Oncology of Hôpital Edouard Herriot, who had given their informed consent to the study. The site of the primary tumor was: small intestine (n = 33), rectum (n = 2), pancreas (n = 27), lungs (n = 4), gallbladder (n = 2), renal pelvis (n = 1), ovary (n = 1); two cases presented with liver metastases from an unknown primary tumor. A total of 65 patients were metastatic. Three out of these 72 patients were found to have circulating ghrelin levels > 10-fold the upper limit of the normal range of our laboratory. They form the basis of the present report.

Clinical and pathological data

Complete clinical charts were available for the three patients. The following information were collected: age, sex, presentation, delay from the initial diagnosis, and treatments received.

In one patient (case 1), the histological diagnosis of endocrine carcinoma was made from a guided biopsy of a liver metastasis; no sample from the primary tumor, located late in the course of the disease, was available for histological study. In the other two cases (cases 2 and 3), the diagnosis was made after the surgical resection of the primary tumor. Tissue material was available for review and for complementary immunohistochemical studies, only for cases 1 and 3; it was not possible to retrieve archival tissue material from case 2. No frozen tissue has been preserved for any patient.

The following information was collected from the original pathological reports and/or from the review of the available tissue material by two pathologists (V H, J Y S): morphological differentiation, WHO classification (15), mitotic index, Ki67 index, histological grade, and tumour–node–metastasis (TNM) stage. According to European Neuroendocrine Tumour Society (ENETS) recommendations (16, 17). All three patients were maintained under follow-up after the diagnosis of hyperghrelinemia; during follow-up, clinical and biological information were regularly collected.

Serum ghrelin assays and other hormonal and biochemical investigations

For determination of serum ghrelin levels, blood samples were collected after overnight fasting and immediately centrifugated. Serum aliquots were immediately frozen and stored at −20 °C until use. Serum total ghrelin levels were measured with a commercial RIA kit (Linco Research, St Louis, MO, USA), using a specific antibody binding to the sequence of 14-amino acid forming the C-terminal part of the mature ghrelin peptide; according to the manufacturer’s data, the cross-reactivity is <0.1% M desoctanoyl ghrelin. The normal range, as determined in the laboratory in 42 young healthy subjects without any gastrointestinal disease, was 696–1467 pg/ml.

In addition, serum active ghrelin was determined with a single-site commercial RIA kit (Linco Research) in the three patients with hyperghrelinemia. We compared these values with those obtained in 20 patients with neuroendocrine tumors associated with normal total ghrelin levels, selected at random in our own series and for whom serum has been preserved in appropriate conditions.

The serum levels of GH, insulin-like growth factor 1 (IGF1), chromogranin A, and gastroenteropancreatic hormones (gastrin, vasoactive intestinal peptide, pancreatic polypeptide, somatostatin, insulin, and glucagon) were determined in the same blood samples by using commercial RIAs validated for diagnostic purposes (hGH-RIACT, IGF1-RIACT, CgA and GASK PR: CisBio International, Gif-sur-Yvette, France; EURIA-VIP, EURIA-PP, EURIA-somatostatin: Eurodiagnostica, Malmö, Sweden; INS-IRMA: Biosource, Nivelles, Belgium; and RIAZEN glucagon: ZenTech, Angleur, Belgium). Serum serotonin levels were assessed by HPLC separation with fluorescence detection.

Clinical and biometrical data

The following clinical and biometrical data were collected at the time of measurement of circulating ghrelin levels: weight, body mass index (BMI), and clinical signs of acromegaly. A nutritional inquiry was performed as well.
Tissue immunodetection of ghrelin

Only a limited number of immunohistochemical investigations was possible on the archival tissue material available for study. No frozen tissue was available for biochemical or molecular studies. Immunohistochemical detection of ghrelin was performed on archival material. Sections of Bouin’s fluid or formalin-fixed, paraffin-embedded tumor tissue were pretreated for antigen unmasking (35 min in Tris-citrate pH 7.3 at 98°C), then incubated with mouse antihuman ghrelin antibody, recognizing both the preprohormone and the mature hormonal products (Abcam, Cambridge, UK), diluted at 1:500 at room temperature for 30 min. The reaction product was visualized using the Envision + system with diaminobenzidine as a chromogen (Dako, Glostrup, Denmark).

Results

The clinical, biochemical, and pathological features of the three patients are summarized in Table 1.

Clinical and pathological features of the patients

One patient (patient 1) was a 37-year-old male; the other patients (patients 2 and 3) were female, aged respectively 49 and 52 years old. The delay between the initial diagnosis of endocrine carcinoma and hyperghrelinemia was respectively, 9.1, 2.2 and 3.3 years.

The primary site of the endocrine tumor was the pancreas in one patient (case 1), the rectum in another (case 2), and the gallbladder in the last patient (case 3). Two tumors (cases 2 and 3) were nonfunctioning; the last one (case 1) was a gastrinoma revealed by a typical Zollinger–Ellison syndrome and requiring long-term treatment with proton pump inhibitors (160 mg/day).

At pathological examination, all three tumors were classified as well-differentiated endocrine carcinomas (Fig. 1a), according to the WHO criteria (15). Their endocrine nature was demonstrated by the coexpression of chromogranin A and synaptophysin by tumor cells; gastrin and serotonin were detected by immunohistochemistry in tumor tissue samples from patient 1 and 3 respectively. All three tumors were classified as grade 2 according to ENETS recommendations (16, 17).

All three patients had liver metastases at presentation. The corresponding TNM stages, according to ENETS proposals (16, 17), were T1N1M1 for patient 1 and T3N1M1 for patient 2; patient 3 was classified as T4N1M1 according to the TNM classification of gallbladder cancer. All three patients were stage IV.

Table 1 Hormonal, pathological and clinical parameters in the three patients with overt hyperghrelinemia.

<table>
<thead>
<tr>
<th>Reference range</th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
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<tbody>
<tr>
<td>Gender</td>
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<td>Female</td>
<td>Female</td>
</tr>
<tr>
<td>Age at diagnosis of hyperghrelinemia (years)</td>
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<td>49</td>
<td>52</td>
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<tr>
<td>Total serum ghrelin (pg/ml)</td>
<td>696–1467</td>
<td>49 028</td>
<td>63 711</td>
</tr>
<tr>
<td>Active serum ghrelin (pg/ml)</td>
<td>847</td>
<td>149</td>
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<tr>
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<td></td>
<td></td>
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<td>Rectum</td>
<td>Gallbladder</td>
</tr>
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<td>Well differentiated carcinoma</td>
<td>Well differentiated carcinoma</td>
</tr>
<tr>
<td>TNM staging</td>
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<td>T3N1M1</td>
<td>T4N1M1</td>
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<td>+</td>
<td>+</td>
</tr>
<tr>
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<td>16</td>
<td>23</td>
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<tr>
<td>BMI (kg/m²)</td>
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</tr>
<tr>
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<tr>
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<td>Hormonal features</td>
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<tr>
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<td>11 950</td>
<td>140</td>
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<td>16 533</td>
<td>ND</td>
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<td>ND</td>
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<tr>
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<td>ND</td>
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<td>ND</td>
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<tr>
<td>IGF1 (ng/ml)</td>
<td>80–197</td>
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<td>88</td>
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</table>
Plasma levels of total ghrelin and acyl ghrelin

The three patients had markedly elevated serum levels of total ghrelin, as compared to the normal range of our laboratory (696–1467 pg/ml): respectively 49,028 (patient 1), 63,711 (patient 2) and 101,996 (patient 3) pg/ml. The presence of interfering serum antighrelin antibodies was excluded by precipitation with polyethylene glycol. In one patient (case 1), serial determinations were available (Fig. 2): i) in an additional sample obtained 2 months after the first determination, serum ghrelin levels remained comparable; ii) in a cryopreserved serum sample obtained 8 months before the first determination, circulating levels of total ghrelin were 7560 pg/ml; at this time, the patient was treated by somatostatin analogs.

We then determined the proportion of circulating acyl ghrelin in the three patients. The serum levels were respectively 847 (patient 1), 149 (patient 2), and 106 (patient 3) pg/ml. The ratio between acyl and total ghrelin was therefore respectively: 1.73, 0.23, and 0.1, as compared with ratios comprised between 2.7 and 10.1 in our control group. The determination of acyl ghrelin in the cryopreserved sample stored for patient 1 was considered not reliable because of the storage conditions.

Tissue expression of ghrelin

Tissue material for immunohistochemical detection of ghrelin was available for cases 1 and 3 only. In the small amount of tumor tissue still available for patient 1, very rare tumor cells were found positive. In patient 3, ghrelin was detected in both the primary tumor and in the liver metastasis available for study; in the primary tumor, only scattered positive cells were present: they were heterogeneously and focally distributed and represented about 10% of the total neoplastic cell population (Fig. 1b); in contrast, in the liver metastasis, the number of positive cells was much higher, amounting up to 50% of tumor cells (Fig. 1c).

Correlations with clinical and biological parameters

Clinical features

At the time of diagnosis of hyperghrelinemia, two patients (cases 1 and 2) had decreased BMI (respectively 17 and 16 kg/m²) whereas, in the remaining patient (case 3), BMI was within the normal range (23 kg/m²). All patients had decreased appetite. No patient had clinical signs suggestive of acromegaly (Table 1).

At the time of diagnosis of hyperghrelinemia, one patient (case 3) was receiving somatostatin analogs. Another patient (case 1) was under proton pump inhibitor treatment; somatostatin analogs had been discontinued 7 months before the diagnosis of hyperghrelinemia. The third patient (case 2) received no treatment.

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Figure 1 Histological and immunohistochemical features in case 3. (a) Endocrine tumor cells infiltrate the whole thickness of the gallbladder wall. By immunohistochemistry, scattered ghrelin-positive cells are visible in the primary tumor; their distribution is heterogeneous and focal; (b) representing about 10% of the total neoplastic cell population. (c) In the part of the tumor invading the adjacent liver, the proportion of ghrelin-positive cells is about 50%. Original magnifications: (a), ×120; (b and c), ×380.

Figure 2 Evolution of serum levels of ghrelin, gastrin, and chromogranin A in patient 1, in relation to the clinical course.
Biochemical characteristics Because of the possible interactions between active ghrelin and GH, we determined the circulating levels of GH in the three patients. Serum GH levels were moderately increased in patient 1 (54.7 mU/l for a normal <1.5); in the other two patients, they were only very mildly elevated. We verified that the circulating levels of IGF1 were in the normal range for all three patients.

All three patients presented alterations in the circulating levels of endocrine markers and hormones. Serum chromogranin A levels were markedly elevated in all three patients. Patient 1 presented markedly increased serum levels of gastrin (Fig. 2) and pancreatic polypeptide. Patient 3 presented elevated serum levels of pancreatic polypeptide and serotonin.

Clinical course Before the diagnosis of hyperghrelinemia, all three patients had received various courses of chemotherapy; two (cases 1 and 3) had received somatostatin analogs and one (case 1) had received interferon alpha. No response to the various treatment attempts has been observed.

At the time of diagnosis of hyperghrelinemia, all three patients were in an advanced and progressive stage of the disease. After the diagnosis, one received no further treatment and two (cases 1 and 2) were enrolled in a clinical trial during which they received everolimus; no response was observed in either of the patients.

The pattern of evolution is well exemplified in patient 1 (Fig. 2). The diagnosis of massive hyperghrelinemia was made at a time in which liver metastases showed a rapid increase in size, associated with a sharp rise in the serum levels of endocrine tumor markers, such as chromogranin A and gastrin (Fig. 2). At the same period, BMI strongly decreased and the patient progressively became cachectic. The patient died 5 months after the diagnosis of hyperghrelinemia and 9.6 years after the initial diagnosis. In the same way, the two other patients died shortly after the diagnosis of hyperghrelinemia: 12 months for patient 2 and 2 months for patient 3. The total duration of evolution after the initial diagnosis of neuroendocrine tumor was respectively 3.2 and 3.5 years.

Discussion

We report three additional cases of well-differentiated neuroendocrine carcinomas of digestive origin associated with marked hyperghrelinemia: one case originated from the pancreas, one from the rectum, and one from the gallbladder. In these patients, serum levels of ghrelin were extremely high, of more than 49 000 pg/ml for a normal range between 696 and 1467 pg/ml.

So far, only two cases of neuroendocrine digestive tumors have been reported in the literature as ‘ghrelinomas’ because of their association with extremely high serum levels of ghrelin: one was of gastric origin (with serum ghrelin levels of 2100 μg/l) (14) and the other of pancreatic origin (with serum ghrelin levels of 12 000 pM) (12). In addition, in a series of 31 patients with endocrine pancreatic tumors (10), five patients have been reported to present mildly elevated serum ghrelin levels.

In our patients, as in the two previous cases of ‘ghrelinomas’ reported so far (12, 14), the source of ghrelin was likely to be the tumor tissue. We were able to demonstrate the presence of ghrelin-positive tumor cells in the two cases for which tumor tissue material was available. Taken together, these observations underline that marked or even massive hyperghrelinemia may be associated with a large spectrum of digestive neuroendocrine tumors, likely as a result of an ectopic secretion (3).

To classify the three cases observed in our series as ghrelinomas would require the demonstration of clinical and/or functional consequences of ghrelin overproduction. In our cases, there was no evidence for clinical and/or biological effects of ghrelin overproduction: i) BMI was normal or low; ii) appetite was not increased; and iii) only one patient had slightly elevated serum GH levels and none had elevated IGF1 levels. Among the two previous cases reported in the literature as ‘ghrelinomas’, only one presented with clinical and metabolic features in keeping with a diagnosis of functioning tumor (14): interestingly, this tumor originated from the stomach, the major source of production of ghrelin in the normal body. In contrast, the other case previously reported as ‘ghrelinoma’, of pancreatic origin, was not associated with clinical or biological signs suggestive of functional consequences of ghrelin overproduction (12).

Ghrelin exists under two forms: acyl ghrelin, able to bind to GHS-R1a, and desacyl ghrelin, unable to bind to the specific receptor (1). In the only case reported so far as a functioning gastric ‘ghrelinoma’, it was possible to demonstrate the presence of high circulating levels of acyl ghrelin (14). In contrast, in our patients, most of the circulating ghrelin was not acylated and the ratio acyl/total ghrelin was very low compared with the controls, even if the use of a single-site commercial RIA assay, with high specificity according to the manufacturer’s data and our own data, may have resulted in a lower sensitivity of the determination of active ghrelin. This suggests the presence of very high serum levels of desacyl ghrelin in our patients, even if we could not definitely exclude the presence of circulating degradation products of mature ghrelin peptides. Several mechanisms may therefore be hypothesized to explain the absence of functional consequences of ghrelin overproduction in our patients, such as: i) competition between desacyl ghrelin and acyl ghrelin, as previously suggested on experimental grounds (4, 18–21) and ii) interference with other mechanisms contributing to the regulation of appetite.
and the induction of cachexia in patients with advanced cancer, such as inflammatory cytokines and adipokines. The limited amount of tumor tissue available for study in our patients and the absence of frozen tissue prevented the possibility to analyze in depth the expression and metabolism of ghrelin in tumor tissues and to evaluate the expression of its specific receptors. In particular, it would have been of interest to test whether functional GHS-R was expressed in tumor cells and whether the downstream signaling pathway associated with GHS-R was normal. In the same way, in the present work, we could not assess whether an abnormal expression of GOAT in tumor tissue may account for the low levels of acyl ghrelin detected in our patients. The recent availability of antibodies directed against acyl ghrelin and GOAT (22, 23), and adapted for immunohistochemical studies makes it possible to plan further studies to investigate the metabolism of ghrelin in endocrine tumor tissues.

The endocrine tumors presented by our three patients had several features in common: as in the previous cases reported in the literature (12, 14), they were well differentiated, metastatic, progressive, and in an advanced stage of evolution. This suggests that large tumor masses are required to produce significant peptide overproduction. This is in line with the fact that ghrelin-positive cells are usually rare in tumor tissue (6, 10, 12, 24). Moreover, the secretion of ghrelin, like that of other hormones, may be modulated by some of the treatments taken by patients with neuroendocrine tumors. In particular, somatostatin analogs are likely to inhibit ghrelin secretion, as suggested by concurrent clinical and experimental data (25–27). One of our patients (case 3) was still treated with somatostatin analogs when the diagnosis of hyperghrelinemia was made: we cannot exclude that the circulating levels of the hormone, while very high, may have been decreased by the treatment. In another patient (case 1), the dramatic increase in the circulating levels of ghrelin (from 7560 to 49 028 pg/ml) was observed 8 months after the discontinuation of somatostatin analogs: we cannot therefore exclude that this may have contributed to the subsequent emergence of massive hyperghrelinemia.

In contrast to somatostatin analogs, proton pump inhibitors, taken by one of our patients, may be associated with increased serum levels of ghrelin. It is conceivable that the inhibition of gastric acid secretion may induce a compensatory increase in ghrelin production by the stomach. However, it remains to be demonstrated that this hyperplasia may be responsible for a significant increase in circulating hormone levels; such an effect has been described in only one previous study in humans, only in its abstract form (28) and not in the corresponding final publication (13). Even in this case, the elevations reported were mild and much lower than the levels found in our patient.

In one of our patients, hyperghrelinemia was detected during the late evolution of a typical gastrinoma. Several similar observations have been reported in the literature, including a case of pancreatic glucagonoma (29) and one of an ACTH-producing lung carcinoid (30). In addition, several previous immunohistochemical studies have shown that low amounts of ghrelin-producing cells may be found in association with the major hormone-producing population in many examples of functioning endocrine tumors (10). It is therefore conceivable that a ghrelin-producing clone may expand late in the course of the disease. From a clinical point of view, this suggests that the secondary emergence of a ghrelin-producing clone in a neuroendocrine carcinoma may be a sign of advanced disease. However, this point could not be definitely assessed in our patients, since only one patient had serial measurements of circulating ghrelin.

In conclusion, we report three cases of metastatic, well-differentiated neuroendocrine carcinomas of various origins with markedly high levels of circulating ghrelin. However, there was no evidence of clinical or functional consequences of this marked hyperghrelinemia. It is therefore difficult to classify these cases as ‘ghrelinomas’: our experience rather suggests that ectopic ghrelin production may be a late or secondary event in a subset of well-differentiated endocrine carcinomas of various origins.

Declaration of interest
We declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding
This research did not receive any specific grant from any funding agency in the public, commercial, or not-for-profit sector.

Acknowledgements
We are grateful to F Claustrat and V Raverot for helping in biochemical analysis, and to N Picchetti-Mayer and N Elbaz for providing archival tissue material.

References