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Paraganglia of the Gallbladder

An Underrecognized Incidental Finding and Potential Diagnostic Pitfall

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Context.—The identification of paraganglia (PG) in the gallbladder (GB) is infrequent, and easily overlooked as it is not something routinely reported. Occasionally they may be misinterpreted as neoplastic cells, such as low-grade carcinomas, germ cell tumors, or because of their close resemblance to neuroendocrine cells, as low-grade neuroendocrine neoplasms.

Objective.—To evaluate the incidence and histological features of PG in patients that underwent cholecystectomy, and disclose the potential misinterpretation of these benign structures as clusters of neoplastic cells.

Design.—A retrospective study of cholecystectomy specimens performed over a 6-month period were reviewed for identification of PG. Immunohistochemical studies for chromogranin, synaptophysin, S100, and cytokeratin AE1/AE3 were performed in selected cases.

The gallbladder (GB) is one of the most commonly encountered specimens in the surgical pathology laboratory; however, identification of paraganglia (PG) in this organ is infrequent, and easily overlooked as it is not something routinely reported. PG are derived from the neural crest, and are widely distributed among the sympathetic and parasympathetic ganglia of the head and neck region, intra-abdominally, and within the genitourinary tract; however, their presence in the GB has rarely been documented in the literature.1–4 In this organ, they are usually located within the subserosal connective tissue, often in close proximity to vascular structures, nerves fibers, and ganglion cells. Occasionally they may be misinterpreted as small foci of neoplastic cells, such as low-grade carcinomas, germ cell tumors, or because of their close resemblance to neuroendocrine cells, as low-grade neuroendocrine neoplasms (NEN). In fact, our study was prompted by a case in which a PG of the GB was initially misinterpreted as a NEN involving the lymphatic spaces of the GB wall. The aim of our study was to evaluate the incidence and histologic features of PGs of the GB in a cohort of patients that underwent cholecystectomy, and discuss the potential misinterpretation of these benign structures as clusters of neoplastic cells.

Results.—A total of 365 GB were reviewed and in 16 cases (4.4%) PG was identified within the subserosal connective tissue of the GB wall or cystic duct adjacent to small capillaries, nerves, and ganglia. They consisted of well-demarcated, lobular structures ranging in size from 0.2 to 0.5 cm, which were predominantly composed of chief cells; with strong expression for chromogranin and synaptophysin and negative CKAE1/AE3, and a minor component of S100-positive sustentacular cells.

Conclusions.—PG is an uncommon finding with a prevalence of 4.4% in our study. Awareness of their location, histologic features, and immunohistochemical profile may help practicing pathologists to confirm their benign nature, avoid a misdiagnosis of malignancy, and prevent unnecessary diagnostic work-up and treatment.

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METHODS

In this retrospective study all cholecystectomies performed at Hartford Hospital (Hartford, CT) from March 2017 to August 2017 were examined. Formalin-fixed paraffin-embedded tissue obtained from the body and cystic duct margin of the GB (ranging from 1–3 blocks per case) was stained with hematoxylin and eosin and reviewed by 2 of the authors for the identification of PG. Immunohistochemical studies for chromogranin (CG; Clone: LK2H10; 2000 dilution; Millipore), synaptophysin (SYN; Clone: 27G12; 200 dilution; Leica), S100 (Rabbit clone; 1000 dilution; Dako), and cytokeratin AE1/AE3 (Clone: AE1/AE3; 100 dilution; Dako) were performed in select cases, and the presence of paragangliocytic tissue and its association with other pathologies were recorded. This study was conducted in accordance to the principles outlined in the Declaration of Helsinki and was approved by our local institutional review board (HHC-2020-0054).

RESULTS

A total of 365 GB were reviewed, and in 16 cases (4.4%) paragangliocytic tissue was identified. Our cohort included 5 males and 11 females, with an age range of 26 to 75 years (mean 51.4). Of 16 total cases, 12 cases showed cholecystitis with cholecystitis and 3 cases, acalculous cholecystitis. In 1
case the cholecystectomy was performed during a pancre-atoduodenectomy for pancreatic adenocarcinoma and showed normal histology.

In the majority of cases (13) only 1 paraganglia was identified; however, in 1 case 2 PG and in 2 cases 3 PG were identified, respectively (Figure 1). The PG were preferentially located within the subserosal connective tissue of the GB wall, and in 2 cases, in the cystic duct (Table). Histologically, they were well-demarcated, lobular (Zellballen) structures ranging in size from 0.2 to 0.5 cm, usually in close proximity to small capillaries, nerves, and ganglia (Figure 2). Their shape was round to elongated, and the dominant cell type was the chief cell composed of polygonal cells with round to oval dark nuclei and granular, amphophilic to clear cytoplasm (Figure 2, inset). The sustentacular cells were more difficult to identify on hematoxylin and eosin sections (Figure 3, A). Immunohistochemical stains were performed in 3 cases, including our index case that showed a strong, cytoplasmic expression for SYN (not shown) and CG (Figure 3B). In the index case (case 15) the paraganglion was misinterpreted to represent an occult NEN involving the lymphatic spaces of the GB wall, which led to a clinical work-up. However, subsequent immunostain for S100 (Figure 3, C) was positive, highlighting the sustentacular cells while cytokeratin for AE1/AE3 was negative (Figure 3, D); thereby confirming the diagnosis of paraganglion.

DISCUSSION

Our study confirms, although uncommon, PG can be identified on routine sections of the GB if a careful and diligent search for these structures is performed and, if necessary, confirmed by immunohistochemistry. Because of their close resemblance to neuroendocrine cells and the lack of awareness of their existence in this location, they may cause an erroneous interpretation as a low-grade NEN.4 Kuo et al1 was the first to report the presence of normal PGs in the wall of the human GB. Since then, only a few additional studies2–4 mostly in the form of case reports, have confirmed their presence in this organ. In a small but interesting study, Fine and Raju,3 identified 1 to 5 PGs in each of the 9 of 10 surgically resected GB after they had been subserially sectioned. In this study, the authors submitted multiple pieces of tissue for a total of 8 to 10 paraffin blocks per case, and when no PGs were identified in the initial hematoxylin and eosin levels, sectioning of the block was repeated until PGs were found or all tissue was completely exhausted. Interestingly, the number of sections made to obtain these results varied from 96 to 166 per case. They concluded the low frequency of encountering PGs in the GB was due to their scarcity in this location.3

Similar to other sites, PG of the GB have a lobular arrangement composed of 2 cell types, imparting the appearance of organoid “Zellballen” structure.1,2 The predominant and most often recognized cell type is the chief cell demonstrating round or oval nuclei and vesicular, granular amphophilic or clear cytoplasm.2 These cells are usually positive for CG and SYN and are negative for cytokeratins.2 In 1 study, they were also found to be immunoreactive for tyrosine hydroxylase and dopamine β-hydroxylase.5 The other cell type component, often less readily identified on hematoxylin and eosin stain, is the supporting sustentacular cell that displays a more spindled or oval hyperchromatic nucleus, and poorly defined vacuolar cytoplasm.2,3 These cells are highlighted by positive immunostaining for S100.2

Since the first case report of paraganglioma of the GB6 and the subsequent identification of paragangliotic tissue in...
the subserosal connective tissue of the GB wall,\(^1\) a few additional case reports of both entities have been described in the literature.\(^2-4,7-9\) However, to our knowledge, this represents the largest study on PG of the GB performed in recent years. Our findings show the prevalence of PG of the GB in routine surgical pathology practice (4.4 %) in which 1 to 3 sections are submitted.

In a review of the literature, we identified several case reports in which PG, especially when observed at unusual sites, were misinterpreted as either metastatic or primary carcinomas.\(^10\) In the urogenital tract, Makinen and Nickels\(^12\) reported 2 cases of clear cell renal cell carcinoma and 2 testicular germ cell tumors with clear cell features, in which the PGs in sympathetic ganglia, removed as part of a retroperitoneal resection, caused misinterpretation as metastatic carcinomas. Additionally, Clevenger et al\(^13\) observed retroperitoneal paraganglia in dissected lymph nodes mimicking metastatic germ cell tumors. Furthermore, the same group reported on the potential pitfalls related to the use of OCT4 in this setting.\(^13\)

In fact, it is well known, that strong nuclear staining for OCT4 is very useful in the diagnosis of seminoma, embryonal carcinoma, and intratubular germ cell neoplasia with high sensitivity and specificity.\(^14\) Furthermore, OCT4 is the marker of choice in identifying metastases of these tumors in particular, including in retroperitoneal lymph nodes. However, Clevenger et al\(^13\) observed that when retroperitoneal PG are stained with certain monoclonal antibodies for OCT4 (Cell Marque) they may show a diffuse intense cytoplasmatic staining causing a potential misdiagnosis of metastatic granulosa cell tumors. Awareness of this finding is especially important in preventing a misdiagnosis of metastatic germ cell tumors when using the above-mentioned monoclonal antibody for OCTs in retroperito-

**Figure 3.** Paraganglia: Index case. The paraganglia are composed of cells with round prominent nuclei, and abundant clear cytoplasm organized into nested “Zellballen” pattern (A) showing immunoreactivity for chromogranin (B). S100 highlights a minor component of sustentacular cells (arrow, [C]) with negative immunoreactivity to cytokeratin AE1/AE3 (D). (Hematoxylin-eosin [A], ×400 all figures).
In light of this report, during our study, we retrospectively stained 5 of 16 cases of PG of the GB (data not included in the results) with a mouse monoclonal antibody for OCT4 (Clone: SEMGC; 10 dilution; Biocare Medical) and found no staining in any of the 5 PG examined. Finally, PG have also been described in the periprostatic adipose tissue, where they may show features that overlap with hypernephroid variant of prostatic carcinoma. However, in these cases, the use of immunohistochemical studies for prostate-specific antigen, SYN, CG, cytokeratins AE1/AE3, and S100 can help in avoiding this potential diagnostic pitfall.15–18

In addition, because of their location in the GB subserosa, PG may be misdiagnosed as either peritoneal dissemination of carcinoma, or if they are observed in cases of GB carcinoma, as infiltration of a GB carcinoma into the subserosal connective tissue; however, their immunohistochemical profile (defined by SYN, CG, and S100 positivity with cytokeratins AE1/AE3 negativity) can help in differentiating these lesions. To our knowledge, our report is the first in which PG of GB were misdiagnosed as lymphovascular involvement by a NEN adding to the list of potential pitfalls that may arise in the interpretation of normal PG in unusual locations. According to Young et al19 the features that may help to differentiate paraganglionic tissue from neoplastic lesions are (1) their intimate association with capillaries, vessels, and nerves, (2) lack of desmoplastic stromal reaction associated with infiltrating carcinoma, and (3) an immunohistochemical profile demonstrating positivity for CG, SYN (in chief cells), S100 (in sustentacular cells), and lack of expression for cytokeratins.

Conclusions
In the routine surgical pathology evaluation of GB, PG is an uncommon finding with a prevalence of 4.4% in our study. Awareness of their location, histologic features, and immunohistochemical profile (SYN, CG, and S100 positive/cytokeratins AE1/AE3 negative) may help the practicing pathologist to confirm their benign nature, avoid a misdiagnosis of malignancy, and prevent unnecessary additional diagnostic work-up and treatment.

References