Diagnostic Pathology

A case report of infantile cystic nephroblastoma

--Manuscript Draft--

Manuscript Number:	DPAT-D-18-00128R2
Full Title:	A case report of infantile cystic nephroblastoma
Article Type:	Case Report
Funding Information:	
Abstract:	Background: Nephroblastoma (NB) is a malignant embryonal neoplasm derived from nephrogenic blastemal cells. NB usually forms a solid mass, but in extremely rare cases, it may show cystic changes. Case Presentation: A six-month-old girl with a persistent high fever was found to have pyuria and bacteriuria. Ultrasonography revealed multilocular cysts in the right kidney. Right nephrectomy was performed, but the cystic wall was ruptured during surgery. An intraoperative rapid diagnosis based on peritoneal fluid cytology confirmed three components of blastemal, stromal and epithelial cells. The blastemal cells were dyshesive, with naked nuclei with scant to no cytoplasm and were the most predominantly observed cell type. The spindle-shaped stromal cells were arranged in fascicles. The epithelial cells demonstrated tubular structures. Macroscopically, the resected cystic tumor measured 80 mm in maximum diameter, and the cystic wall was thinned. Histologically, the tumor was diagnosed as cystic NB (blastemal-predominant) displaying a triphasic pattern. Hyperchromatic nuclei and apoptotic bodies were found. The clinical stage classification of Japan Wilms Tumor Study group was 3. Chemotherapy and radiotherapy were performed, and tumor recurrence and metastasis have not been observed in the eight months since the surgery. Conclusion: This is an extremely rare case of infantile cystic NB. We diagnosed the NB cells that appeared in the peritoneal fluid by intraoperative rapid cytology. A cytological examination proved to be a very useful technique for determining the clinical stage of NB. Massive tumor degeneration and necrosis should be considered as a pathogenic mechanism of cyst formation.
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Response to Reviewers:	Reviewer #1: -Page 6, lines 40-47: consider adding additional details to the cytopreparatory techniques (fluid smear, cytospin, etc? staining method - DiffQuik, rapid Pap stain, full Pap stain, etc) >We added "by rapid Papanicolaou stain". -Page 6, line 50: this paragraph seems to be missing a topic sentence. I can assume that this description is of the cytomorphology, but this is not clear from the text. Please revise for clarity. Also, consider some re-wording/critical reading for quality of language. For example, I'm not familiar with the term "reduced cell adhesion" - perhaps you mean that the cells are dyshesive? >We changed the this paragraph. "Cytologically, the blastemal cells were dyshesive, with naked nuclei with scant to no cytoplasm and were the most predominantly observed cell type. " -Page 7: regarding the histomorphology - please describe the appearance of the cyst wall. Any cyst lining cells? Please include a photomicrograph if possible. >We described about cystic wall, and take a microphotograph of the cystic wall (Fig4D). The cystic wall was lined by blastemal cells (Fig. 4D). Some solid areas were present within the cyst wall. -Page 10, line 33: Please comment on if any of the previous cases of cystic Wilms tumor also had tumor rupture (or if it is unknown, please include this information). >We added about case report of ruptured cystic nephroblastoma. "However, there were no case reports of ruptured Cystic NB." Reviewer #2: -The case report needs to be better developed with expansion of the case presentation and discussion to show that this is a unique case. Cysts, hemorrhage and necrosis are common in Wilms tumors. Sufficient description is needed to distinguish this case from a cystic partially differentiated Wilms tumor. >We described cystic partially differentiated Wilms tumor. The differential diagnosis of cystic NB is CPDNB. CPDNB is a multilocular lesion with no apparent solid or nodular mass formation. CPDNB contains mature or immature nephroblastic tissue, but conforms to the septum between cysts. Cystic NB is also a multilocular lesion but has obvious solid NB components expanding the cyst wall. -In the case presentation, the macroscopic description can be improved by a description of the cysts and solid components (expansive or within the walls of the cysts). The histology has limited detail and needs to be expanded (i.e. description of the cystic regions, location of the solid Wilms tumors components, and any necrosis). >We described about the cystic wall. "The cystic wall was lined by blastemal cells (Fig. 4D). Some solid areas were present within the cyst wall. " -In the discussion, the histologic differential diagnosis should be addressed. The cytology is emphasized in the case presentation and conclusion but is not included in the discussion. There needs to be clarification of the statement 'spontaneous regression' (page 9, line 40). Degenerating tumor cells and apoptotic cells are not evidence of regression and are a relatively common finding in Wilms tumors. This finding is not sufficient to argue that spontaneous regression occurs in Wilms tumors. >We deleted supontaneous regression. And we changed conclusion. Massive tumor degeneration and necrosis should be considered as a pathogenic mechanism of cyst formation. - Fig 1 and Fig 2 should be annotated for clarification of the important findings. >We added figure legends (Fig 1 and Fig 2) -Fig 4 could be improved with a low-power image to show cystic areas. The quality of the histology images could be improved - especially Fig 5. >We added Fig4D (Cystic wall of the tumor). And we deleted Fig. 5 (Because

	degenerating tumor cells and apoptotic cells are a relatively common finding in Wilms tumors.)

Case Report

A case report of infantile cystic nephroblastoma

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Short running head: Infantile cystic NB

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Abstract

Background: Nephroblastoma (NB) is a malignant embryonal neoplasm derived from nephrogenic blastemal cells. NB usually forms a solid mass, but in extremely rare cases, it may show cystic changes. **Case Presentation:** A six-month-old girl with persistent high fevers was found to have pyuria and bacteriuria. Ultrasonography revealed multilocular cysts in the right kidney. Right nephrectomy was performed with cyst wall rupture during surgery. An intraoperative rapid diagnosis, based on peritoneal fluid cytology, confirmed three components of blastemal, stromal, and epithelial cells. The blastemal cells were dyshesive, with scant to no cytoplasm and were the predominant cell type. The spindle-shaped stromal cells were arranged in fascicles. The epithelial cells demonstrated tubular structures. Macroscopically, the resected cystic tumor measured 80 mm in maximum diameter with a prominently thin cyst wall, but solid areas were also apparent. Histologically, the tumor was diagnosed as cystic NB (blastemal-predominant) displaying a triphasic pattern. Hyperchromatic nuclei and apoptotic bodies were found. The clinical stage classification of Japan Wilms Tumor Study group was 3. The patient was treated with chemotherapy and radiotherapy. Tumor recurrence and metastasis have not been observed in the eight months since surgery. **Conclusion:** This is an extremely rare case of infantile cystic NB. We

diagnosed the NB cells that appeared in the peritoneal fluid by intraoperative rapid cytology. Cytological examination proved to be a very useful technique for determining the clinical stage of NB. Additionally, we propose that massive tumor degeneration and necrosis be considered as a pathogenic mechanism of cyst formation in NB.

Key words: cyst, nephroblastoma (NB), infant, peritoneal fluid, cytology

Background

Wilms tumor, also known as nephroblastoma (NB) is a malignant embryonal neoplasm derived from nephrogenic blastemal cells. NB is the most common malignant renal tumor in children and 98% of cases occur under the age of 10. The mean age at diagnosis is 37 months and 43 months among males and females, respectively [1]. However, adult-onset cases have also been reported [2]. Grossly, NB usually forms a solitary and rounded-solid mass. Histologically, the three components of blastemal, epithelial, and stromal cells are mixed in various proportions.

Renal cystic tumors in children can be benign, such as cystic nephroma (multilocular cyst), and malignant homologous tumors, such as cystic partially differentiated NB (CPDNB) [3]. Furthermore, very few case of cystic NBs in infants and adults have been reported [4-6].

We herein report an extremely rare case of cystic NB in an infant and discuss its pathogenic mechanism. In addition, we also describe the cytological findings of NB that appeared in peritoneal fluid.

Case Presentation

A previously healthy six-month-old girl who was born full-term following an uncomplicated pregnancy presented with persistent high fever and was found to have pyuria and bacteriuria. Prior to this, she had no significant medical history. No obvious gross malformations were observed on physical examination nor was any pertinent family history noted. Ultrasonography revealed multilocular cysts in the right kidney; of note, no abdominal abnormalities had been observed at her four-month medical examination.

Abdominal contrast computed tomography (CT) revealed a multilocular cystic mass accompanied by septal wall formation pressing on the normal kidney parenchyma (Fig. 1). The tumor had not directly infiltrated the renal pelvis and there was no coexistence of hydronephrosis. Renal dysplasia was ruled out due to the presence of adjacent normal kidney parenchyma. Because the cystic septa was thickened, cystic NB was deemed the most likely entity, preoperatively. One month later, right nephrectomy was performed. Unfortunately, the cyst wall ruptured during surgery. An intraoperative cytological evaluation by rapid Papanicolaou staining of peritoneal fluid confirmed three components of blastemal, stromal, and epithelial cells. The findings were interpreted on-site as NB (Fig. 2).

Cytologically, the blastemal cells were dyshesive, exhibiting naked nuclei with scant to absent cytoplasm and were the most predominant cell type. The spindle-shaped stromal cells were arranged in fascicles, showing a fibroblast-like configuration. And epithelial cells formed tubular structures. Macroscopically, the resected cystic tumor measured 80 mm in maximum diameter involving the upper pole to middle portion of the right kidney (Fig. 3A) and the cystic wall was predominantly thin. A portion of the cystic wall was ruptured by the surgical procedure and was associated with hemorrhage in the surrounding tissues. On the cut surface of the tumor, the cystic septum within the tumor had disappeared (Fig. 3B). Instead, hemorrhage and muddy, degenerative necrotic tumor tissue was found within the cystic space. Solid areas were observed within the cyst walls.

Histologically, the tumor was diagnosed as cystic NB (blastemal-predominant) displaying a triphasic pattern. The blastemal cells showed a solid growth pattern, whereas the epithelial cells showed tubular structures (Fig. 4A and 4B). The spindle-shaped stromal cells resembled embryonic mesenchyme, displaying differentiation into smooth muscles and fibroblasts (Fig. 4C). The cystic wall was lined by blastemal cells (Fig. 4D). Some solid areas were present within the cyst wall. There were no anaplastic cells or atypical mitoses. Degenerated hyperchromatic nuclei and apoptotic bodies were found in some areas.

Immunohistochemically, the blastemal cells and epithelial cells were positive for WT1 (WT49, Leica, diluted 1:1), but the stromal cells were negative. The Ki-67 (MIB-1, BioGenex, diluted 1:30) labeling index of the tumor was as high as 90%, while the degenerated necrotic tumor cells showed decreased staining properties. Nephrogenic rest, a precursor lesion of NB, was not identified in the remaining renal parenchyma. The clinical stage classification of the Japanese Wilms Tumor Study (JWiTS) group was 3. The patient was treated with chemotherapy and radiotherapy and tumor recurrence and metastasis have not been observed in the eight months since surgery.

Discussion

This case report describes an extremely rare case of cystic NB that developed in an infant with no family history or malformation. Furthermore, we describe the cytological findings of the tumor observed intraoperatively involving the peritoneal fluid.

The differential diagnosis of cystic NB includes CPDNB. CPDNB also exhibits multiloculated cysts, as in this case, however these lesions should not have apparent solid or nodular mass formation. CPDNB contains mature or immature nephroblastic tissue, but conforms to the septum between cysts. In contrast, cystic NB has solid NB components expanding the cyst wall, which were easily identified in this case.

To our knowledge, only three cases of NB with cyst formation have been reported in the English literature [4-6]. However, there were no case reports of ruptured cystic NB. One case was an infantile case (9.5 months old) and the other 2 cases were in adults (36 and 30 years old). The mechanism underlying the cyst formation is not well understood. However, the following two possible mechanisms have been proposed: 1) formation due to tumor degeneration and necrosis and 2) formation due to the tumor infiltrating into the renal pelvis, leading to urine inflow inside the tumor. In the present case, there was no extensive coagulative necrosis or thrombus formation, nor any apparent perforation between the tumor

and the renal pelvis. Although there have been no case reports of spontaneous regression of NB, nephrogenic rest, which is a precursor lesion of NB, exhibits spontaneous regression and eventual scarring as obsolescent rest [7,8]. In our case, there was no nephrogenic rest or scar tissue in the remaining renal parenchyma. Therefore, it was thought that cyst formation may have been caused by massive tumor degeneration and necrosis of unknown etiology rather than spontaneous regression, given the presence of degenerated tumor cells and apoptotic bodies.

NB may be the cause of abdominal pain, anemia, and shock accompanying tumor rupture. According to the staging system of the JWiTS and the National Wilms Tumor Study (NWTS), diffuse peritoneal contamination associated with spillage of NB cells before and during surgery is classified as stage 3 [9-11]. The NWTS has also reported that spillage of NB cells does not constitute a risk factor after three-drug chemotherapy and whole-abdomen radiotherapy [12]. In addition, the Children's Oncology Group reported that the incidence of intraoperative tumor spillage increased to 11.9%, and the odds ratio reached 2.183 in patients with a maximum tumor diameter of ≥12 cm compared to those with <12 cm [13]. In the present case, the maximum tumor diameter was 8 cm, but there was intraoperative tumor rupture associated with tumor cells in the peritoneal fluid. We propose that cystic changes in

NB may be a risk factor for tumor rupture. After surgery, the patient received chemotherapy and radiation therapy according to the protocol of the NWTS. Because of the patient's favorable histologic features (lack of anaplasia) and excellent response to therapy, this case is expected to have a good prognosis.

Conclusions

We present an extremely rare case of infantile cystic NB. The diagnosis was able to be made on-site by intraoperative rapid cytology of peritoneal fluid. This technique of cytological examination proved to be very useful for determining the clinical stage of NB. Additionally, massive tumor degeneration and necrosis should be considered as a possible mechanism of cyst formation in NB.

Abbreviations

NB	Nephroblastoma
CPDNB	Cystic partially differentiated nephroblastoma
CT	Computed tomography
JWiTS	Japanese Wilms Tumor Study

NWTS National Wilms Tumor Study

Declarations

Ethics approval and consent to participate.

Not applicable.

Consent for publication

Written informed consent was obtained from the patient's parents for the publication of this case report and any accompanying images. A copy of the signed consent is available for review by the editor of this journal.

Availability of Data and Materials

The dataset supporting the findings and conclusions of this case report is included within the article.

Funding

None.

Competing Interests

The authors declare no conflicts of interest in association with this study.

Authors' Contributions

NK and SY participated in the conception of the study and writing of the manuscript. MT, MY, CS, MN, BB, XG, CF and AS performed the clinical imaging and/or pathological/cytological/immunohistochemical interpretation of this lesion. All of the authors have read and approved the final manuscript.

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FIGURE LEGENDS

Figure 1. Enhanced abdominal CT. Multilocular cystic mass located in the right kidney. The septal wall of the tumor was thickened. Multiple solid areas were identified within the tumor.

Figure 2. Cytology of the peritoneal fluid. Three components of blastemal, stromal and epithelial cells were seen. The blastemal cells showed decreased cell adhesion (arrow), the stromal cells showed a fibroblast-like configuration (arrow head), and the epithelial cells showed a tubular structure (inset) (Papanicolaou staining, x400).

Figure 3. Gross findings of the resected right kidney. A: The tumor was localized from the upper pole to the middle portion. B: The cystic septum within the tumor had disappeared. Some solid areas were seen (arrow head).

Figure 4. Histological findings of the tumor. A: Blastemal cells (H&E staining, x400). B: Epithelial cells (H&E staining, x400). C: Stromal cells (H&E staining, x400). D: Low-powered view of cystic wall of the tumor (H&E staining, x40).

Fig.1

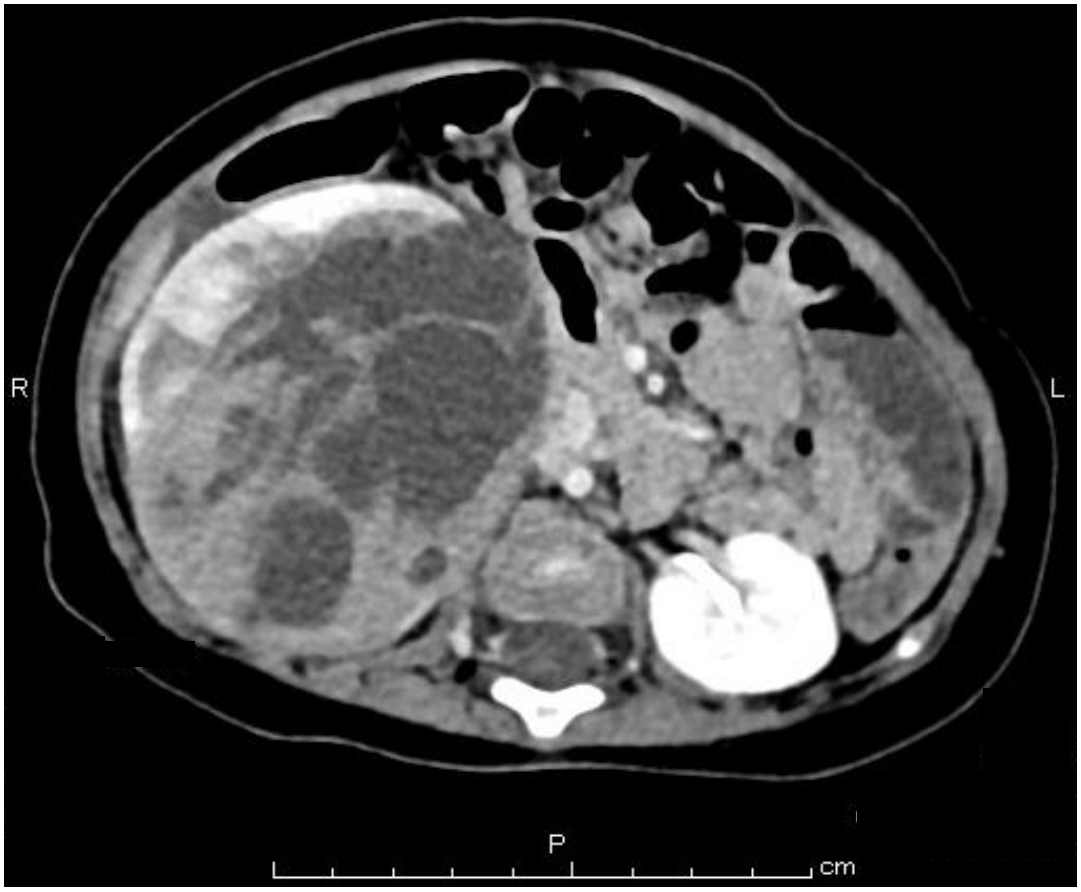


Fig. 2

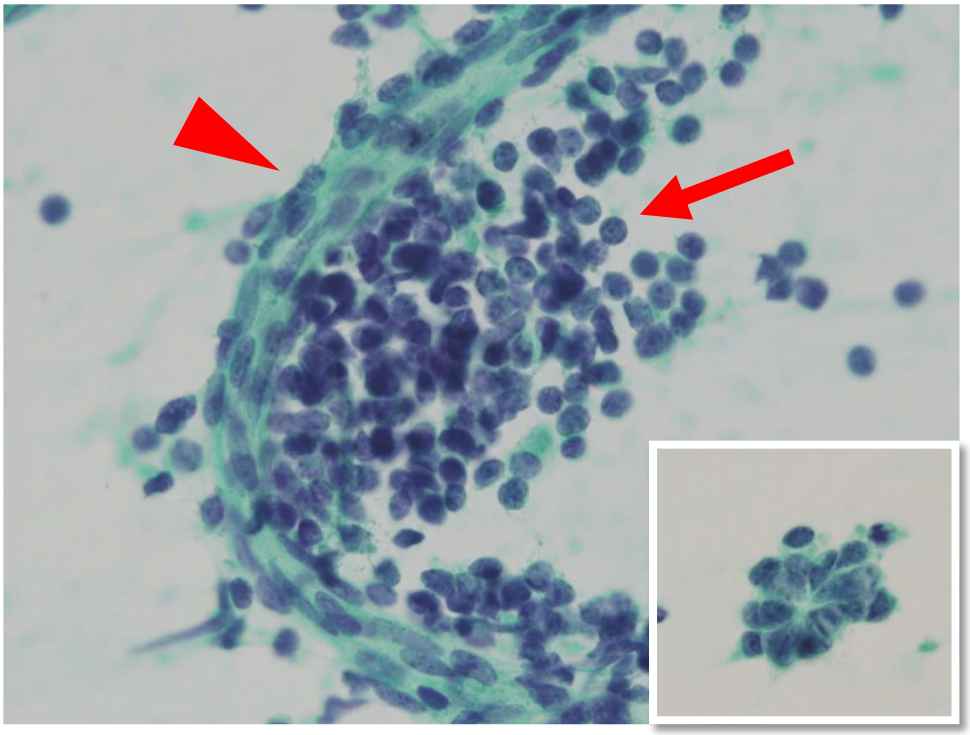


Fig. 3

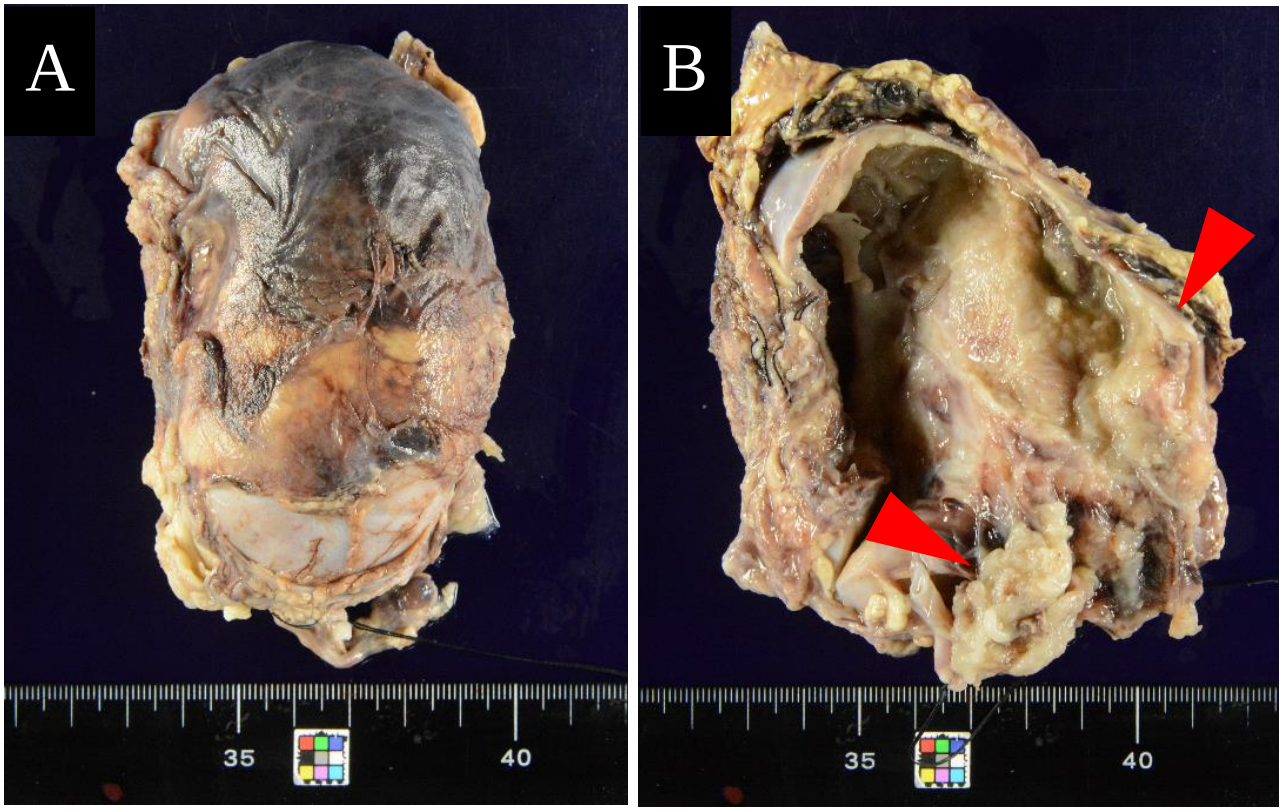
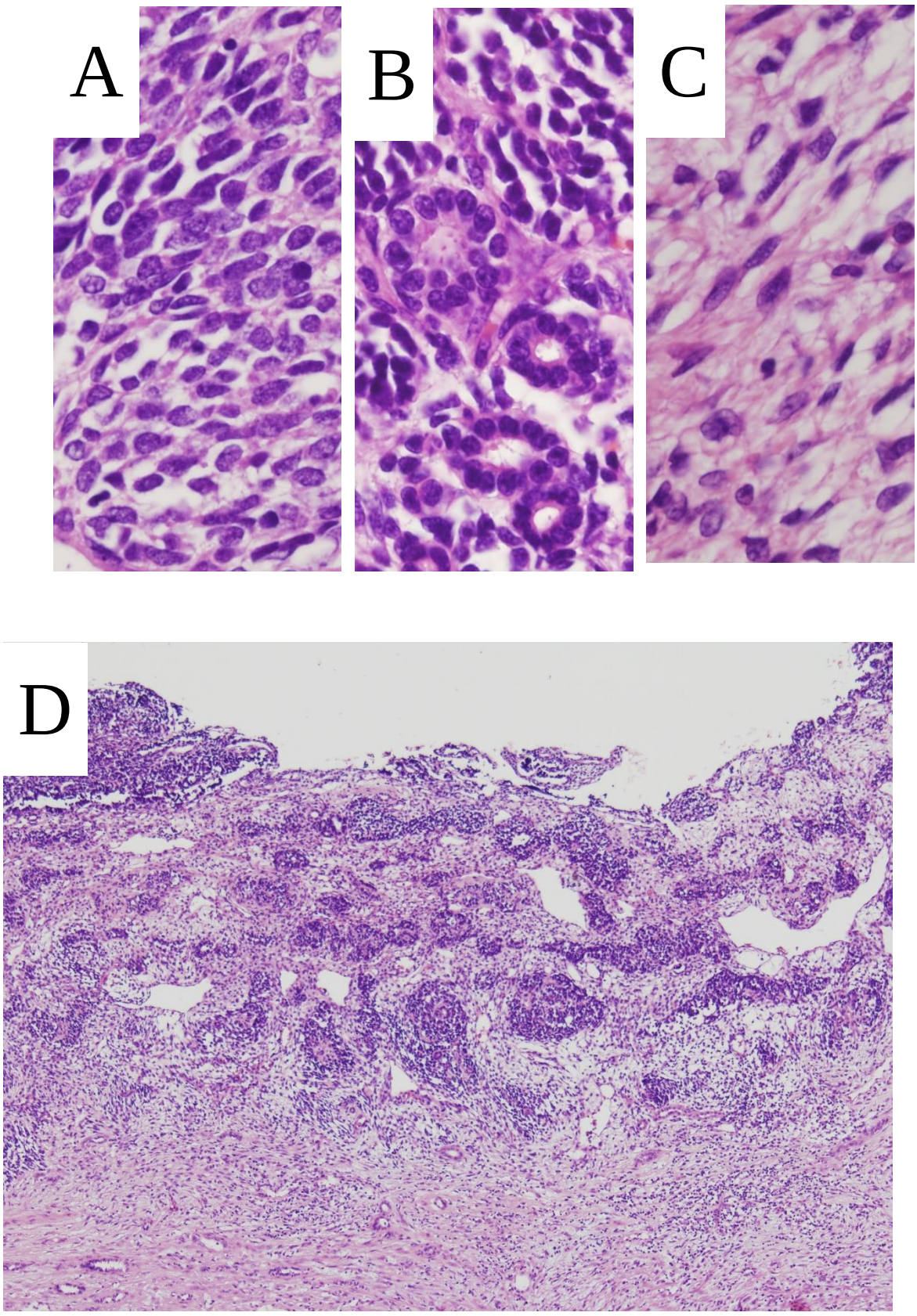


Figure 4

Click here to access/download;Figure;E11804066 Kurose 20180413 cystic NB Fig4.docx



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