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EMBRYONAL RHABDOMYOSARCOMA OF THE OVARY AND FALLOPIAN TUBE: RARE NEOPLASMS ASSOCIATED WITH GERM LINE AND SOMATIC DICER1 MUTATIONS.

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Abstract:	DICER1 mutations (somatic or germline) are associated with a variety of uncommon neoplasms including cervical and genitourinary embryonal rhabdomyosarcoma (ERMS). We report a primary ovarian and two primary fallopian tube ERMS occurring in 60, 13 and 14 year-olds respectively. The three neoplasms exhibited a similar morphological appearance being polypoid and containing oedematous hypocellular areas and hypercellular foci composed of small cells with scant cytoplasm exhibiting rhabdomyoblastic differentiation (desmin, myogenin, myoD1 positive). There was cellular cartilage in all cases and extensive foci of anaplasia, eosinophilic globules and bone/ osteoid in one case each. All three neoplasms exhibited DICER1 mutations; in one of the tubal cases, the patient had a germline mutation and in the other two cases, the DICER1 mutations were somatic. Accompanying DICER1 “second hits” were identified in all cases. In two of the neoplasms, SALL4 positive glandular structures were present which we speculate may represent an unusual primitive “metaplastic” phenomenon. Our study adds to the literature on ERMS at unusual sites associated with DICER1 mutations. ERMS arising at such sites, especially when they contain cartilage or bone/ osteoid, are especially likely to be associated with DICER1 mutations. Pathologists should be aware of this since these may be the sentinel neoplasms in patients with DICER1 syndrome and confirming a germline mutation can facilitate screening of the individual and affected family members for other neoplasms which occur in this syndrome.

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Dr Stacey E Mills

Editor,

American Journal of Surgical Pathology

Dear Dr Mills,

We would be grateful if you would consider the uploaded paper "**EMBRYONAL RHABDOMYOSARCOMA OF THE OVARY AND FALLOPIAN TUBE: RARE NEOPLASMS ASSOCIATED WITH GERMLINE AND SOMATIC *DICER1* MUTATIONS**" for publication in American Journal of Surgical Pathology. We believe this is an important paper since these represent the first reports of primary fallopian tube embryonal rhabdomyosarcomas associated with *DICER1* mutation (one patient has a germline mutation, in keeping with DICER1 syndrome) and we believe the findings to be an important addition to the literature.

All the authors have seen the paper, are satisfied with its contents and approve its submission to the journal. The manuscript, or parts of it, have not been and will not be submitted elsewhere for publication.

With best wishes

Professor Glenn McCluggage
(Gynaecological Pathologist)

EMBRYONAL RHABDOMYOSARCOMA OF THE OVARY AND FALLOPIAN TUBE: RARE NEOPLASMS ASSOCIATED WITH GERM LINE AND SOMATIC *DICER1* MUTATIONS.

Short running title: *DICER1*-associated ERMS of ovary and fallopian tube.

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ABSTRACT

DICER1 mutations (somatic or germline) are associated with a variety of uncommon neoplasms including cervical and genitourinary embryonal rhabdomyosarcoma (ERMS). We report a primary ovarian and two primary fallopian tube ERMS occurring in 60, 13 and 14 year-olds respectively. The three neoplasms exhibited a similar morphological appearance being polypoid and containing oedematous hypocellular areas and hypercellular foci composed of small cells with scant cytoplasm exhibiting rhabdomyoblastic differentiation (desmin, myogenin, myoD1 positive). There was cellular cartilage in all cases and extensive foci of anaplasia, eosinophilic globules and bone/ osteoid in one case each. All three neoplasms exhibited *DICER1* mutations; in one of the tubal cases, the patient had a germline mutation and in the other two cases, the *DICER1* mutations were somatic. Accompanying *DICER1* "second hits" were identified in all cases. In two of the neoplasms, SALL4 positive glandular structures were present which we speculate may represent an unusual primitive "metaplastic" phenomenon. Our study adds to the literature on ERMS at unusual sites associated with *DICER1* mutations. ERMS arising at such sites, especially when they contain cartilage or bone/ osteoid, are especially likely to be associated with *DICER1* mutations. Pathologists should be aware of this since these may be the sentinel neoplasms in patients with *DICER1* syndrome and confirming a germline mutation can facilitate screening of the individual and affected family members for other neoplasms which occur in this syndrome.

Key words: ovary, fallopian tube, embryonal rhabdomyosarcoma, *DICER1*.

INTRODUCTION

DICER1 syndrome is a hereditary cancer predisposition syndrome characterised by deleterious germline *DICER1* mutations which can result in a variety of "hyperplastic" disorders and uncommon neoplasms, including lung cysts, thyroid multinodular goitre, various benign and malignant thyroid neoplasms, pleuropulmonary blastoma, cystic nephroma, anaplastic sarcoma of the kidney, ciliary body medulloepithelioma, nasal chondromesenchymal hamartoma, pituitary blastoma, pineoblastoma, ovarian Sertoli-Leydig cell tumour (SLCT) and embryonal rhabdomyosarcoma (ERMS) of the cervix and genitourinary tract (1). More recently, primary intracranial sarcomas have been described, further expanding the spectrum of *DICER1*-associated tumours (2,3). The neoplasms predominantly occur in children, adolescents or young adults. Macrocephaly is present in a significant number of cases. The germline pathogenic variants in the *DICER1* gene are typically inherited in an autosomal dominant pattern but may arise de novo in the germline or in a somatic mosaic distribution (1). Some of the tumours associated with this syndrome, for example SLCT and ERMS, also occur sporadically and are associated with hotspot somatic *DICER1* mutations (4-6). In this report, we describe three *DICER1* mutated ERMS involving the fallopian tube and ovary; these are extremely rare primary sites for these neoplasms.

CASE REPORTS

CASE 1

A 60-year-old woman presented with lower abdominal pain and difficulty in voiding urine and she was found on CT scan to have a large pelvic mass. Serum CA125 and CA19.9 were within the normal range. Her past medical history included IgG multiple myeloma from which she had been in remission for 11 years following an autologous stem cell transplant. There was no known personal or family history of *DICER1*-related neoplasms. At operation, she was found to have a left ovarian mass with involvement of the pelvic peritoneum. She underwent total abdominal hysterectomy, bilateral salpingo-oophorectomy, infracolic omentectomy, partial pelvic peritonectomy and appendicectomy.

There was tumour recurrence at the vaginal vault three months postoperatively and the patient was commenced on IVA (ifosfamide, vincristine and actinomycin D) chemotherapy with GCSF support. Dose reduction and ultimately cessation of vincristine was required

because of peripheral neuropathy but partial response was achieved. Maintenance cyclophosphamide, with consolidation irradiation to the main pelvic mass, is currently being considered.

Pathological Findings

The left ovary contained a 28 cm mass with a ruptured capsule. Sectioning revealed a solid and oedematous appearance with areas of haemorrhage. The uterus, both fallopian tubes, right ovary, omentum and appendix were grossly normal. The specimen of pelvic peritoneum measured 5cm in length and contained tumour deposits.

Histology showed a low power polypoid architecture. The polypoid nodules contained stromal cores lined focally by squamous epithelium (without skin appendage structures) and focally by primitive appearing glandular epithelium with a low degree of mitotic activity and extensive cytoplasmic vacuolation. Occasional glands were also present within the stroma. For the most part, the stroma was hypocellular and oedematous but there were also multiple cellular aggregates of small blue tumour cells with scant cytoplasm and obvious mitotic activity. There was prominent interstitial haemorrhage in both the hypocellular and cellular areas. Small foci of obvious skeletal muscle differentiation were present with cells with abundant eosinophilic cytoplasm and cross striations. Focally, there was condensation of the cellular tumour cell aggregates around the epithelium, in keeping with a cambium layer. Multiple foci of cytologically bland cellular cartilage were also present as well as focal bone/ osteoid formation. In addition, involving about 20% of the neoplasm, there was a different morphological appearance with a cellular monotonous population of mitotically active moderately atypical spindle cells. No teratomatous elements were present.

Immunohistochemically the small blue cells exhibited focal, but quite widespread, nuclear staining with myogenin and myoD1 and cytoplasmic immunoreactivity with desmin. S100, oestrogen receptor (ER), alpha-fetoprotein (AFP) and glial fibrillary acidic protein (GFAP) were negative. The monotonous spindle cell component was diffusely positive with desmin and there was focal nuclear staining with myogenin and myoD1. S100, ER, AFP, GFAP and SALL4 were negative. There was focal nuclear staining within the glandular epithelial component with SALL4 and a few of the small blue tumour cells were also positive.

A diagnosis of ERMS was made. Figure 1 shows representative photomicrographs of the neoplasm and figure 2 illustrates the immunophenotype.

Metastatic tumour, identical to the monotonous spindle cell component described above, was present on the serosal surface of the right tube and in the pelvic peritoneum. No pathological abnormality was identified within the uterus, left fallopian tube, right ovary, omentum or appendix.

CASE 2

A 13-year-old girl presented with abdominal pain. Abdominal CT scan showed a left adnexal mass and she underwent exploratory laparotomy where a tortuous and haemorrhagic left fallopian tube with an attached mass was identified, suggestive of torsion. Left partial salpingectomy was performed. There was no known personal or family history of *DICER1*-associated neoplasms. No follow-up is available.

Pathological Findings

The specimen measured 9 cm in maximum dimension and comprised a red, dusky mass attached to a 3 cm segment of fallopian tube. The entire mass and fallopian tube were submitted for histological examination.

Histology of the fallopian tube showed haemorrhage, vascular congestion and oedema, in keeping with torsion. The sections from the mass showed a polypoid lesion focally lined by squamous epithelium (without skin appendage structures) and focally by primitive appearing glandular epithelium with cytoplasmic vacuolation. Some of these glandular structures were also present within the core of the lesion. The underlying stroma was composed of hypocellular oedematous areas and multiple cellular aggregates; there was prominent interstitial haemorrhage. The cellular aggregates were composed of small round blue tumour cells with hyperchromatic nuclei and scant cytoplasm; in these areas, there was prominent mitotic activity. No cells with cross striations were identified. Focally, there was condensation of the cellular tumour cells around the epithelium, in keeping with a cambium layer. Many foci of cytologically bland cellular cartilage were present. No teratomatous elements were present.

Immunohistochemistry showed focal positive staining of the small blue tumour cells with desmin, myogenin and myoD1. There was quite widespread weak positive nuclear staining with SALL4 within the glandular elements.

A diagnosis of ERMS was made.

CASE 3

A 14-year-old girl presented with a two-week history of abdominal pain, urinary incontinence and fluid leakage per vagina. An ultrasound and CT scan revealed a large complex pelvic and abdominal mass, more prominently involving the right side. There was no known personal or family history of *DICER1*-related neoplasms.

Intraoperatively, three discrete masses were identified. One involved the lower abdomen and omentum, one the pelvic cul de sac and one arose from the right fallopian tube, which was considered to be the primary. Both ovaries, the left fallopian tube and the uterus were grossly normal. The patient underwent total resection of all tumour masses and postoperative PET/CT showed no evidence of disease elsewhere. She received abdominopelvic radiation and multiagent chemotherapy. There was no evidence of residual or recurrent disease 25 months after diagnosis.

Pathological Findings

The tumours from the abdomen, pelvis and right fallopian tube were received as separate specimens measuring between 10 and 15cm in maximum dimension. Gross examination revealed solid and cystic masses with multifocal areas of haemorrhage and necrosis. A 2 cm portion of fimbriated tube was present attached to the right tubal mass.

Histologically, the three tumour masses showed similar features with a low-power polypoid architecture. The polypoid nodules were lined by glandular epithelium which focally comprised primitive appearing glands with cytoplasmic vacuolation. No squamous epithelium was present. Some of these glandular structures were also present within the core of the lesion. Hypocellular oedematous areas were interspersed with many cellular aggregates composed of small round blue tumour cells with hyperchromatic nuclei and scant cytoplasm. Focally, there was condensation of the cellular tumour cells around the epithelium, in keeping

with a cambium layer. Expansive areas composed predominantly of mitotically active spindle cells were also present. Areas of "anaplasia" were present multifocally with markedly atypical nuclei, including multinucleate forms, and abundant mitotic activity, including atypical mitoses. Cells with cross striations were not identified. Many variably sized eosinophilic globules were present. Foci of cellular cartilage were also present, including areas with significant cytological atypia. There were areas of necrosis and interstitial haemorrhage. No teratomatous elements were present. The segment of fallopian tube attached to one of the masses was histologically normal but was undermined by tumour.

Immunohistochemistry showed the small blue tumour cells and the spindle cells to be focally positive for desmin, myogenin, myoD1 and SALL4. SALL4 was negative in the primitive glandular component.

A diagnosis of ERMS was made. Figure 3 shows representative photomicrographs of the neoplasm.

Germline and Somatic Sequencing of *DICER1*

Representative formalin fixed paraffin embedded (FFPE) blocks or unstained sections (with corresponding haematoxylin and eosin slides marked, if necessary, for areas containing tumour and normal tissue) were used. DNA was extracted using a commercially available QIAamp DNA FFPE Tissue Kit (Qiagen, Toronto, ON, Canada) according to the manufacturer's instructions. The extracted DNA was quantified by photo-spectrometric methods. For cases 1 and 2, tumour DNA was screened for *DICER1* mutations using a custom-design Fluidigm access array (7). The Fluidigm access array specifically targets the exons, exon-intron boundaries, and 3′UTR of *DICER1* gene. Library preparation and next-generation sequencing was performed at McGill University and Genome Québec Innovation Centre on an Illumina MiSeq instrument. Sequenced reads were aligned to the human reference sequence UCSC build hg19 (NCBI build 37) using Burrows-Wheeler Aligner (8). Freebayes tool in Galaxy (https://usegalaxy.org/) was utilized for variant calling using default parameters except that at least 10 reads of coverage were required to process the site and variants were called when the mutation frequency was ≥10%. Variants were annotated using wANNOVAR (http://wannovar.wglab.org/). Integrative Genomics Viewer software (IGV version 2.4; http://www.broadinstitute.org/igv/) was used to visualize the data. All variants identified were confirmed by Sanger sequencing. In addition, the sequence encoding for *DICER1* RNase IIIb

domain was PCR-amplified and Sanger sequenced in tumor DNA samples. DNA extracted from lymphocytes from whole blood (case 1) and normal FFPE tissue (cases 2 and 3) was used to determine the germline origin of the variants. Germline testing for case 1 was performed at Royal Marsden Hospital, London and for case 3 was carried out using an Invitae DICER1 Syndrome Test (https://www.invitae.com/). Somatic testing for case 3 was done at Resource Path (https://www.resourcepath.net/dicer1-syndrome/). The rest of the analyses were performed in Dr. Foulkes' laboratory at McGill University.

DICER1 Mutation Results

In case 1, the ovarian ERMS was found to harbour a *DICER1* hotspot mutation (c.5428G>C, p.D1810H, figure 4A) coupled with loss of heterozygosity (LOH) (figure 4A). Germline testing did not reveal an underlying pathogenic constitutional mutation.

In case 2, the fallopian tube ERMS harboured a deletion of two nucleotides in exon 24 of *DICER1* (c.5315_5316del; p.F1772fs, figure 4B) and a typical *DICER1* missense hotspot mutation in the RNAse IIIb coding region (c.5428G>T, p.D1810Y, figure 4C). Using DNA extracted from normal tissue, the deletion was confirmed to be of germline origin.

In case 3, the fallopian tube ERMS harboured biallelic *DICER1* mutations, one loss of function and a hotspot RNase IIIb mutation. The predicted loss of function mutation was a complex 15 bp deletion comprising AG nucleotides at the 3' end of intron 17 (canonical 3' splice site) and extending 13 nucleotides into exon 18 (c.2805-2_2817deletionAGATATCGCAATTTT; figure XX). The RNase IIIb mutation was a typical missense hotpot mutation (c.5428G>C, p.D1810H), with a variant allele frequency of 53%. Germline testing did not reveal an underlying pathogenic constitutional mutation.

DISCUSSION

The human *DICER1* locus is located on chromosome 14q32.13 (1). Pathogenic germline *DICER1* variants or characteristic RNase IIIB hotspot somatic mutations are key mutational events in *DICER1*-associated neoplasms. We report three *DICER1*-associated ERMS, two fallopian tube neoplasms involving young girls and the other an ovarian neoplasm occurring in a postmenopausal woman; these are extremely unusual sites for ERMS. One of the patients

with fallopian tube ERMS had a germline *DICER1* mutation (DICER 1 syndrome) whereas in the other two cases the *DICER1* mutations were of somatic origin. This represents the second reported ovarian ERMS associated with a *DICER1* mutation and, as far as we are aware, the first reported examples of primary fallopian tube *DICER1*-associated ERMS.

A review of the literature revealed only 3 prior reports of primary fallopian tube rhabdomyosarcoma (9-11). These were of embryonal type (1 case), alveolar type (1 case) and the other was described as having an epithelioid appearance; the ERMS occurred in a 17-year-old (9). *DICER1* mutation analysis was not undertaken in any of these neoplasms. Regarding the published cases of primary ovarian rhabdomyosarcoma, in many cases it is difficult to ascertain the morphological subtype and some have been associated with other neoplasms such as teratoma, dysgerminoma, clear cell carcinoma, adenosarcoma and SLCT (discussed below) (12-19). However, cases of primary pure ovarian ERMS have been reported (20-23). The presence of a *DICER1* mutation has been investigated in only a single prior case of ovarian ERMS in a 6-year-old girl (23). The tumour harboured two *DICER1* mutations; the girl subsequently developed cystic nephroma and thyroid multinodular goitre, in keeping with DICER1 syndrome. Molecular sequencing of the patient's germline DNA revealed a deleterious mutation, c.1196_1197dupAG, in exon 8 of *DICER1*. This mutation was confirmed in the tumour, which also contained an acquired somatic mutation. The cystic nephroma contained a *DICER1* hotspot mutation different from that in the ovarian ERMS. In addition, a *DICER1*-associated ovarian sarcoma has been reported in a 5-year-old girl with a germline mutation (5). Morphologically this was described as being composed of undifferentiated small round blue cells, spindle cells, large bizarre pleomorphic cells and islands of malignant cartilage; desmin and myogenin were positive confirming rhabdomyoblastic differentiation. While this tumour would appear likely from the description to represent an ERMS, the authors did not use that term (5).

The largest reported series of primary ovarian rhabdomyosarcoma is by Nielsen et al who in 1998 (before the association of ERMS and *DICER1* was established) reported 13 cases in patients aged 7 to 79 years (22). The neoplasms comprised 11 ERMS and 2 alveolar rhabdomyosarcomas. Schultz and colleagues reported a 10-year-old girl who developed a "poorly differentiated ovarian sarcoma with limited myogenic differentiation" and 4 years later developed SLCT in the contralateral ovary (24). Panagiotou and colleagues reported a 10-year-old girl who underwent oophorectomy and partial salpingectomy for retiform SLCT;

she presented 3-4 months later with ERMS in the region of the residual fallopian tube which the authors suggested might have been derived from a heterologous mesenchymal component within the SLCT which was not identified in the original specimen (25). The *DICER1* mutation status of these tumours was not established, although the patient reported by Schultz et al had another *DICER1*-related neoplasm (no details provided). As discussed, rare ovarian rhabdomyosarcomas, including ERMS, have been reported arising in association with other neoplasms, including SLCT where they are thought to arise from heterologous skeletal muscle elements (12-19, 26); given the association of SLCT with *DICER1* mutations, those ERMS arising in SLCT are likely to be *DICER1*-related.

In the three ERMS we report, the neoplasms contained foci of cellular cartilage and in 1 (case 3), this was cytologically atypical. This is interesting since many *DICER1*- associated ERMS at various sites contain cartilage, which is otherwise relatively uncommon in ERMS. For example, cervical ERMS often contain cartilage (27, 28). We recently reported a series of 19 uterine ERMS (mainly cervical) and 18 of these were associated with *DICER1* mutations (6 germline, 12 somatic); cartilage was present in 10 of 19 (53%) cases (27). The single previously reported ovarian ERMS associated with *DICER1* mutation also contained cartilage (23). As such, when dealing with an ERMS containing cartilage, a *DICER1*-related neoplasm should always be considered and appropriate confirmatory studies undertaken. Establishing a morphological diagnosis of ERMS may be difficult with a wide range of differential diagnoses, including benign lesions and other malignant neoplasms (especially adenosarcoma), and the presence of cartilage may be a clue to the diagnosis.

Warren et al reported three *DICER1*- associated sarcomas, namely an ovarian sarcoma (germline) in a 5-year-old girl, a peritoneal sarcoma (somatic) in a 16-year-old girl and an intracranial sarcoma (somatic) in a 4-year-old boy (5). The authors undertook a comprehensive review of the literature of 83 *DICER1*-associated sarcomas and suggested that *DICER1*-associated sarcomas, regardless of their site of origin, exhibit a characteristic histological appearance, including the presence of undifferentiated small round blue cells, poorly differentiated spindle cells, large bizarre pleomorphic cells (anaplasia), common rhabdomyoblastic and cartilaginous differentiation and rare bone/ osteoid formation; they noted that the morphological features resembled pleuropulmonary blastoma (5). The authors suggested that a sarcoma with this histological appearance should raise the possibility of a

pathogenic *DICER1* mutation warranting a detailed review of the family history and *DICER1* mutation analysis. Bone/ osteoid was present in 1 of the cases we report (case 1); this is uncommon in ERMS and, as suggested by Warren et al, should raise the possibility of a *DICER1*-associated neoplasm (5). Anaplastic foci, characterised by focal collections of cells with large pleomorphic nuclei, were present in one of our cases (case 3); these are also uncommon in ERMS (28, 29) and should result in consideration of a *DICER1*-related neoplasm. In case 3, there were abundant eosinophilic globules akin to those recently described in primary intracranial sarcomas with *DICER1* mutations (2,3).

An interesting fact is that a significant majority of *DICER1*-associated neoplasms arise in females. In the review of 83 *DICER1*-associated sarcomas by Warren et al, 62 occurred in females and 21 in males; the median age of sarcoma diagnosis was 12 years but with a very wide range of 0-92 years (5). This illustrates that *DICER1*-associated neoplasms may occur in older patients; the patient with the ovarian neoplasm in our series was aged 60. de Kock et al recently undertook a review of all *DICER1* genetic alterations reported in articles published before 31st January 2019 (1). There was a large female bias amongst the affected patients with neoplasms; of these 537 patients, 414 (77%) were females and 123 (23%) were males. The female bias was attributed to the high number of gynaecological neoplasms and thyroid disorders in females.

A novel observation in the tumours we report is that primitive appearing glands containing cytoplasmic vacuoles were present. In two of the cases, the glands exhibited nuclear immunoreactivity with SALL4. We feel that these glands may be an integral component of the neoplasms (rather than entrapped) and it is possible that this represents another morphological feature characteristic of *DICER1*-associated ERMS. Glands are often a feature of *DICER1*-associated ERMS at other sites, such as the cervix, but, as far as we are aware, immunoreactivity of these elements with SALL4 has not been previously documented. The reason for SALL4 immunoreactivity is unknown but this is a transcription factor that plays an essential role in maintaining self-renewal and pluripotency of embryonic stem cells (30,31). In fully differentiated cells, SALL4 expression is down-regulated or silenced. Accumulating evidence suggests that SALL4 expression is reactivated in some cancers (30,31) and we speculate that the SALL4 positive glands may exhibit a stem cell phenotype. As discussed, it may be that these do not comprise native entrapped glandular structures but rather they may

represent some form of unusual primitive "metaplastic" phenomenon. This is not without parallels in other tumours; for example, "metaplastic" glands may be found in other mesenchymal neoplasms, such as cardiac myxoma (32). The glands also raise the possibility of an adenosarcoma but while the distinction between ERMS and adenosarcoma exhibiting rhabdomyoblastic differentiation may be difficult, we feel the cases we report represent the former rather than the latter neoplasm. One of us (WGM) has occasionally observed similar primitive appearing glandular structures in *DICER1*-related ERMS at other sites, such as the cervix (unpublished observations). SALL4 was also focally positive in the neoplastic cells in cases 1 and 3; this has been reported previously in occasional cases of rhabdomyosarcoma (33).

These neoplasms potentially exhibit aggressive behaviour, as exemplified by case 1 where metastatic tumour involved the serosal surface of the contralateral tube and the pelvic peritoneum and the tumour recurred three months postoperatively. In case 3, two metastatic tumour masses were present elsewhere within the pelvis and abdomen. In cases 1 and 3, the primary ovarian neoplasm focally exhibited "overgrowth" of a monotonous cellular malignant spindle cell neoplasm which was positive with skeletal muscle markers myogenin and myoD1, in keeping with spindle cell rhabdomyosarcoma. This is relatively unusual in ERMS and the metastatic disease in case 1 comprised the cellular spindle cell component only. No follow-up is available in case 2.

In summary, we report three *DICER1*-associated ERMS of the ovary and fallopian tube. When reporting ERMS at unusual sites, especially when cartilage or bone/ osteoid is present, the pathologist should suspect a *DICER1*-associated neoplasm and raise this possibility on the report. Such patients should be referred to genetics for testing for a germline *DICER1* mutation since these may be sentinel neoplasms in DICER1 syndrome and establishing the presence of a germline mutation can facilitate screening of the individual and affected family members for other neoplasms which may occur in this syndrome. These cases expand the reported gynaecological neoplasms where a *DICER1* association should be suspected, other neoplasms including cervical ERMS and ovarian SLCT.

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FIGURE LEGENDS

Figure 1 Ovarian ERMS in case 1. Low power view showing polypoid neoplasm with epithelial lining and underlying stromal core with hypocellular and hypercellular foci (A). Squamous and glandular epithelial lining with underlying hypocellular and hypercellular foci and foci of cartilage (B). Vacuolated glandular epithelium with cellular focus composed of cells with scant cytoplasm exhibiting obvious mitotic activity (C). Collections of rhabdomyoblasts with cells with abundant eosinophilic cytoplasm (D). Primitive glandular structures with cytoplasmic vacuolation (E). Foci of cellular cartilage (F). Foci of bone/ osteoid (G). Focal overgrowth of cellular monotonous population of mitotically active moderately atypical spindle cells (H).

Figure 2 Case 1. The small cells with scant cytoplasm are focally positive with myoD1 (A) and myogenin (B). The cellular monotonous population of mitotically active moderately atypical spindle cells exhibit focal nuclear staining with myogenin (C). The primitive appearing glands exhibit focal nuclear immunoreactivity with SALL4 (D).

Figure 3 Case 3 showing low-power polypoid architecture with surface glandular epithelium and underling hypocellular and hypercellular foci (A). Normal fallopian tube fimbrial epithelium with underlying tumour (B). Cellular foci containing anaplastic tumour cells together with foci of cellular cartilage with significant cytological atypia (C). Cellular tumour with marked cytological atypia and abundant mitotic activity, including atypical mitoses; eosinophilic globules are present (D). The tumour cells are positive with desmin (E) and exhibit nuclear immunoreactivity with myogenin (F).

Figure 4 Germline and somatic *DICER1* mutations.

Panel A) presents the results from the ovarian ERMS (case 1), whereas B) and C) correspond to one of the fallopian tube ERMS (case 2). A) shows the presence of the missense hotspot mutation c.5428G>C, p.D1810H and loss-of-heterozygosity (LOH) in the tumour. B) illustrates the c.5315_5316del pathogenic germline variant detected in both normal and tumour DNA. C) Displays the RNase IIIb hotspot mutation c.5428G>T, p.D1810Y found in tumour DNA. All three panels present Fluidigm array results visualized with Integrative Genomics Viewer (left image) and Sanger sequencing (chromatograms on the right). The corresponding wild-type sequence is included. In the chromatograms, mutations are indicated with an asterisk.

Figure 1A

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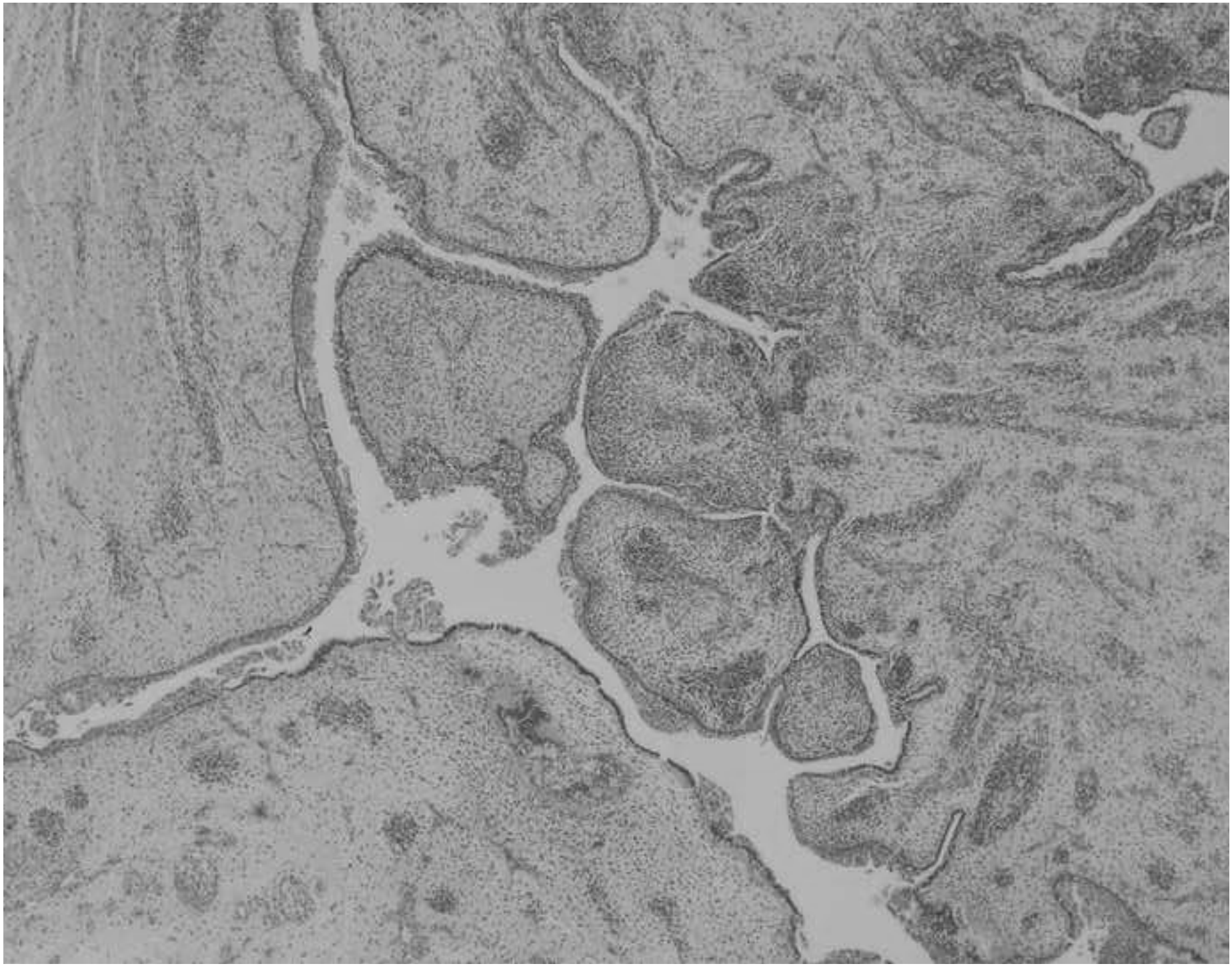

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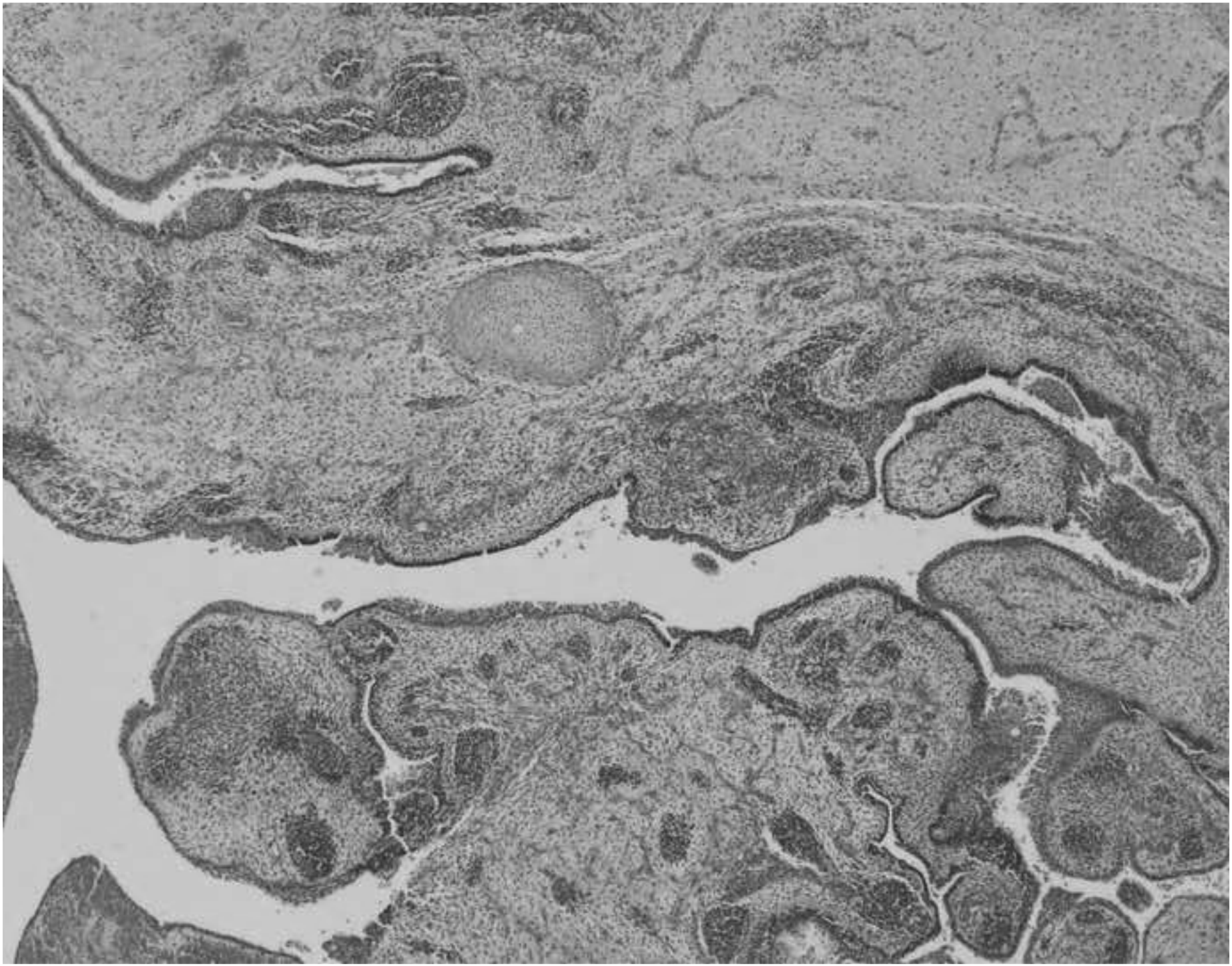

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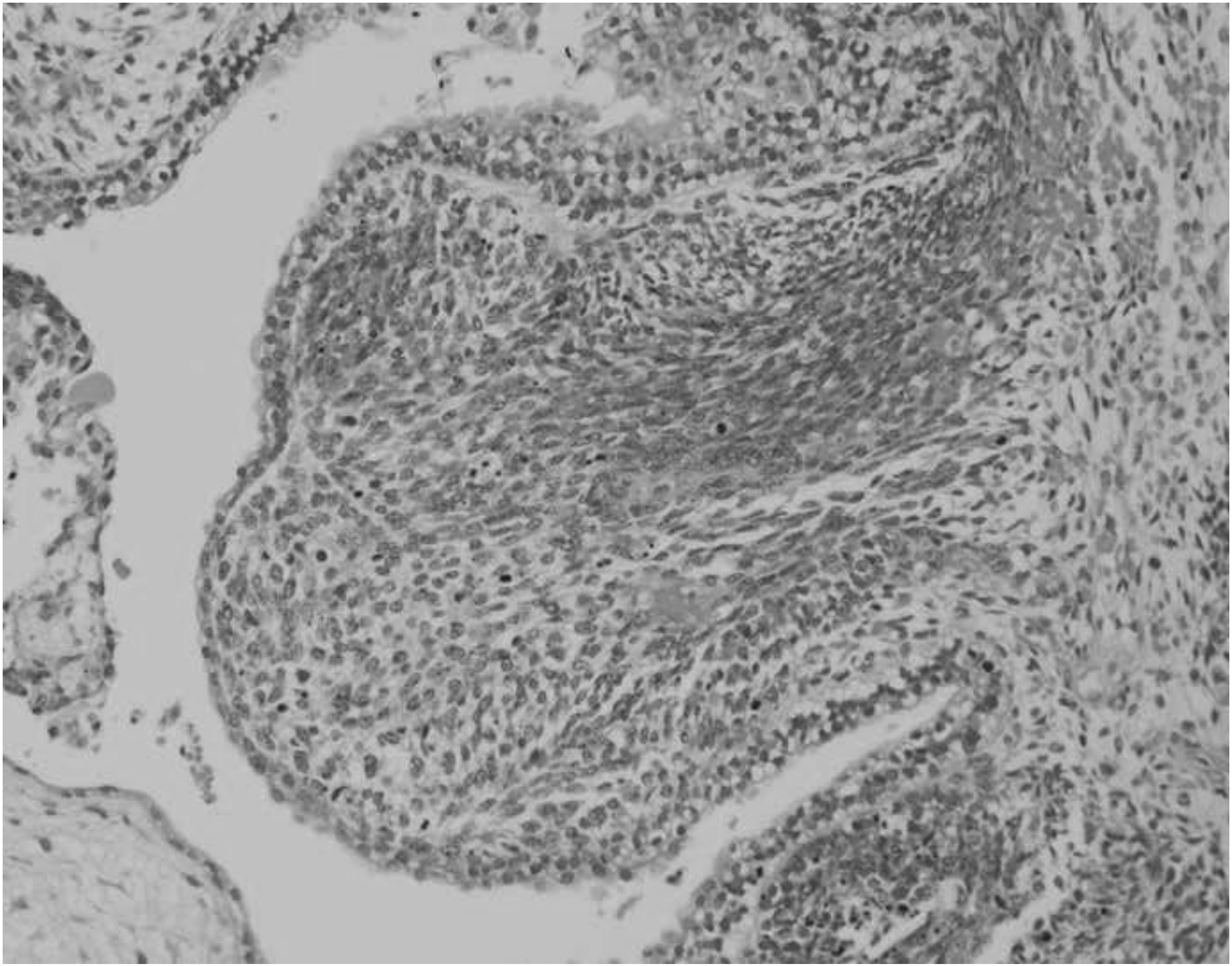

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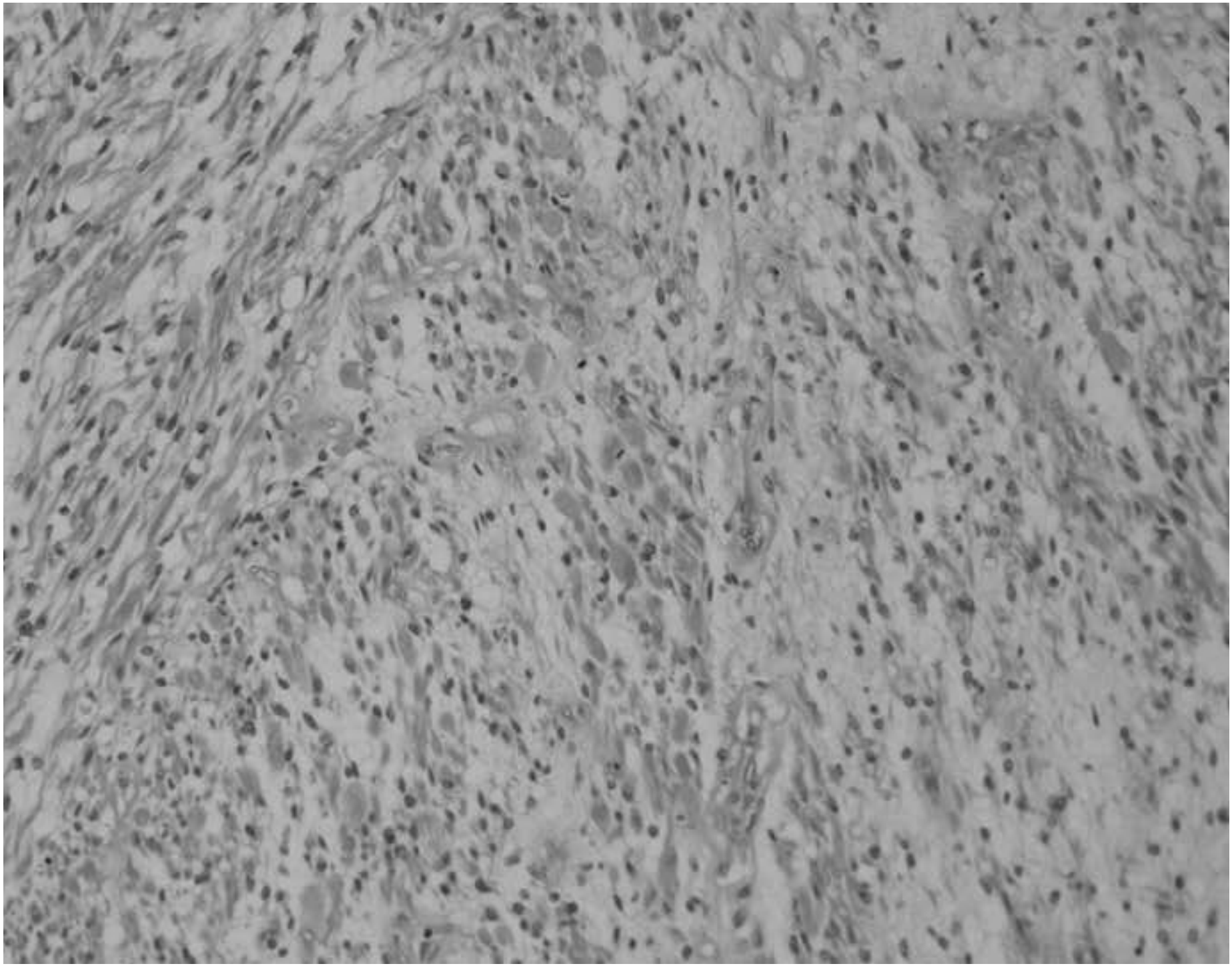

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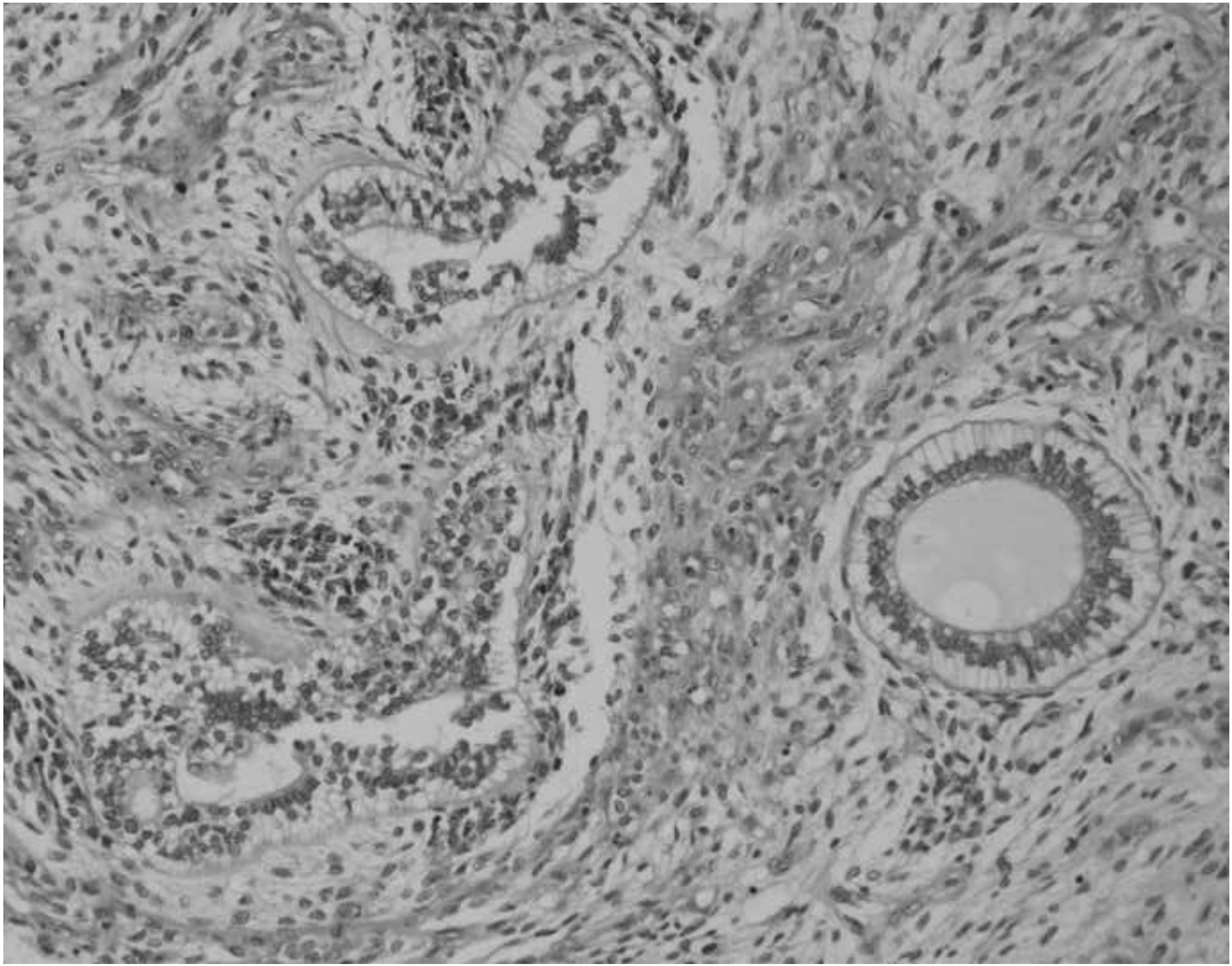

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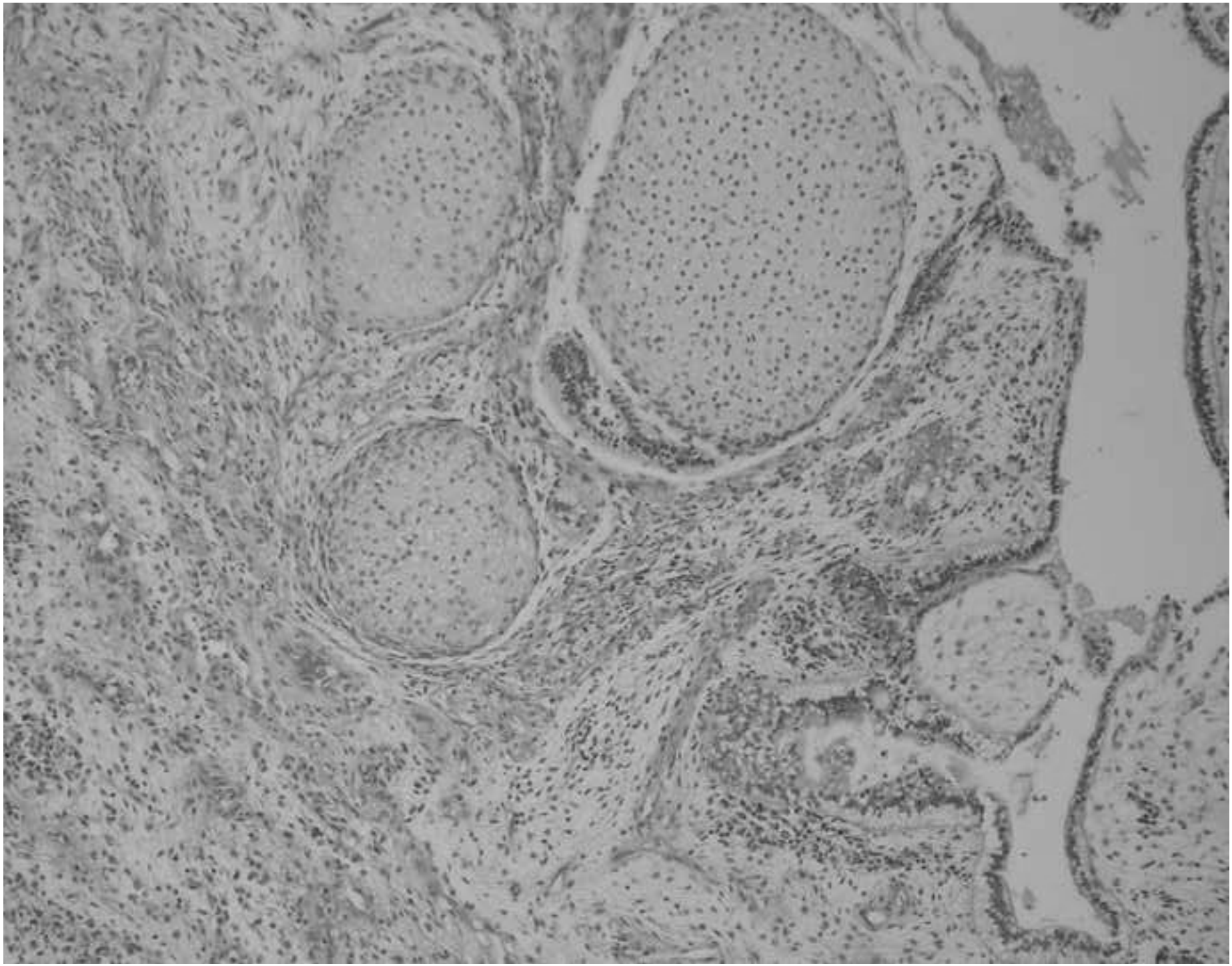

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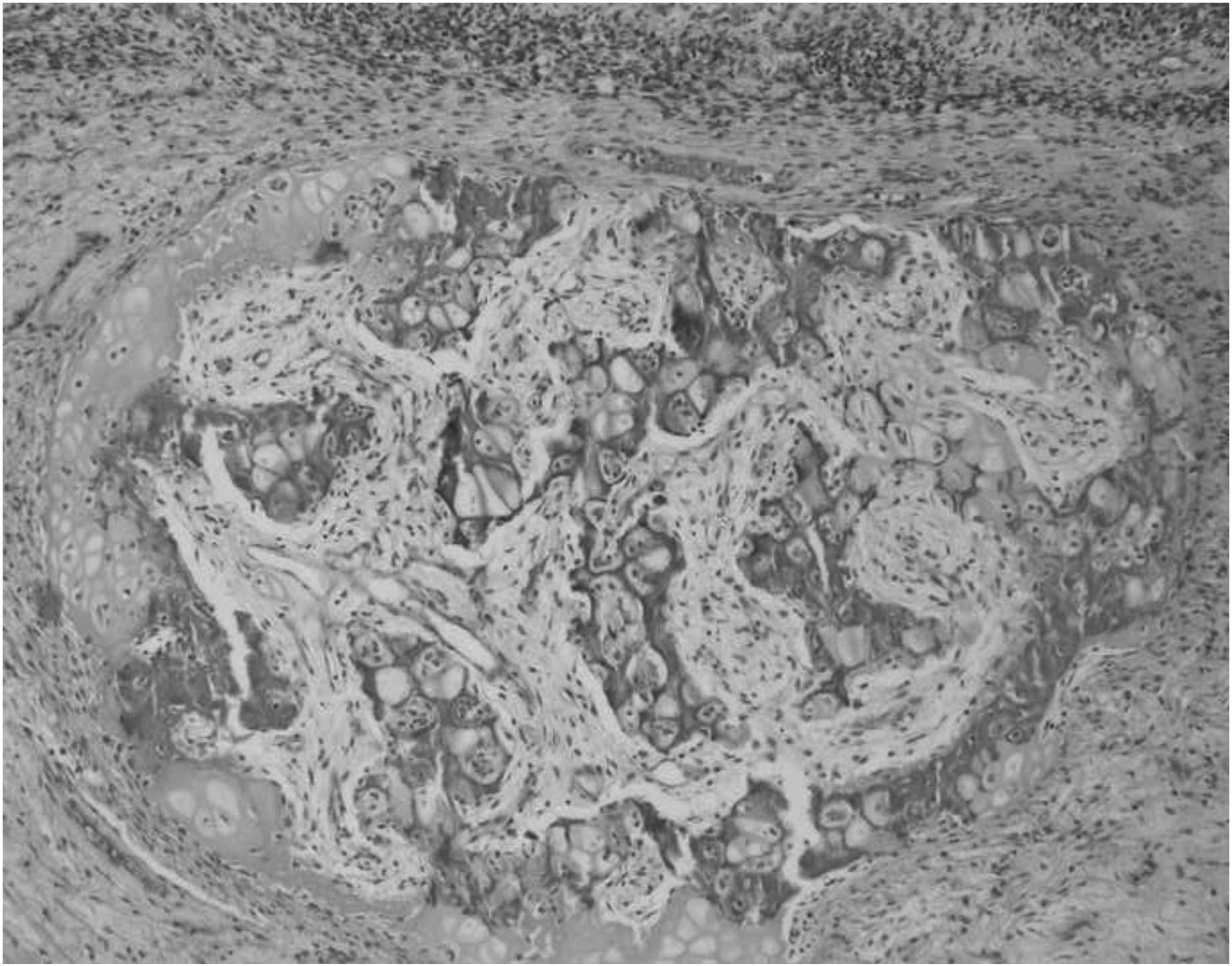

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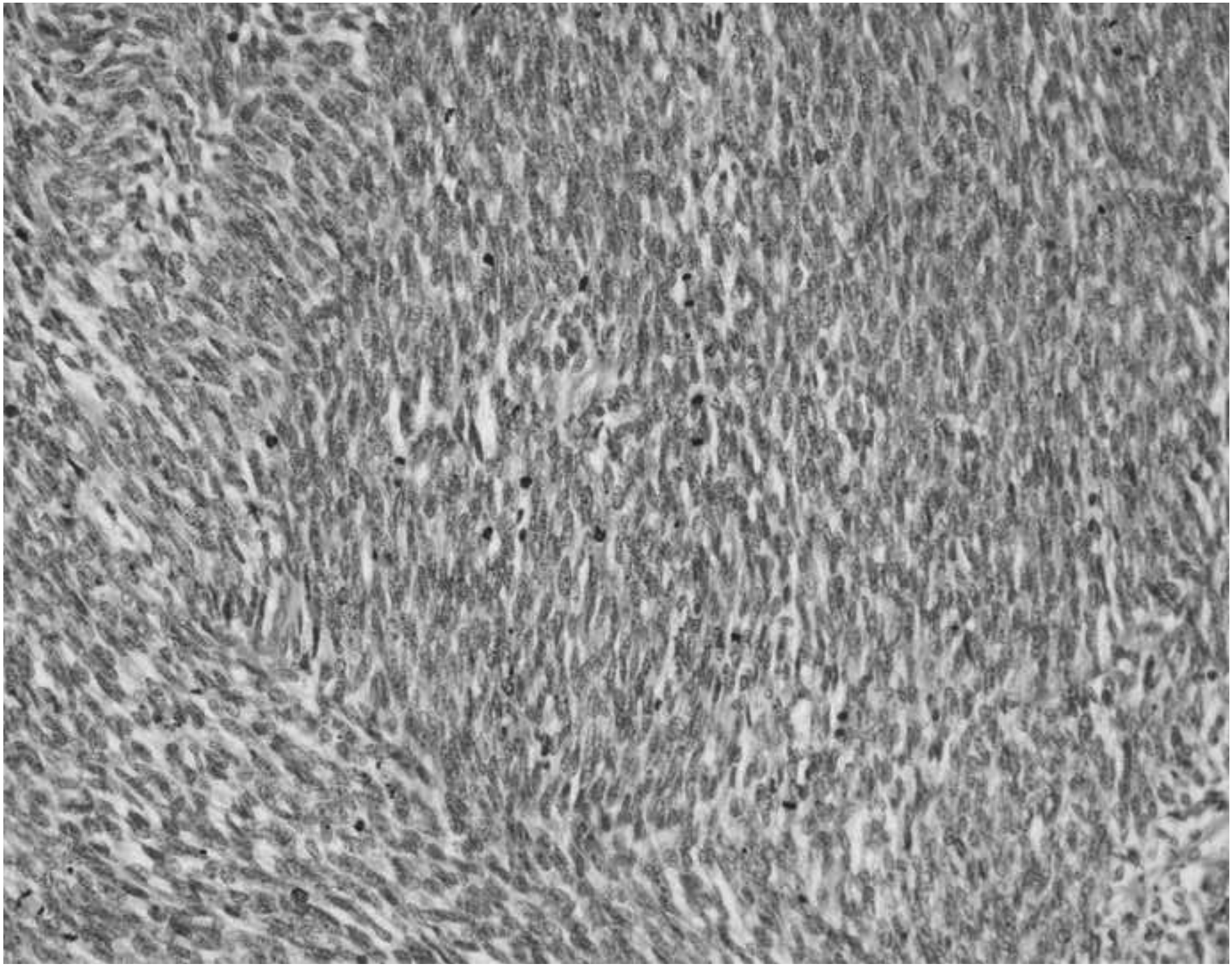

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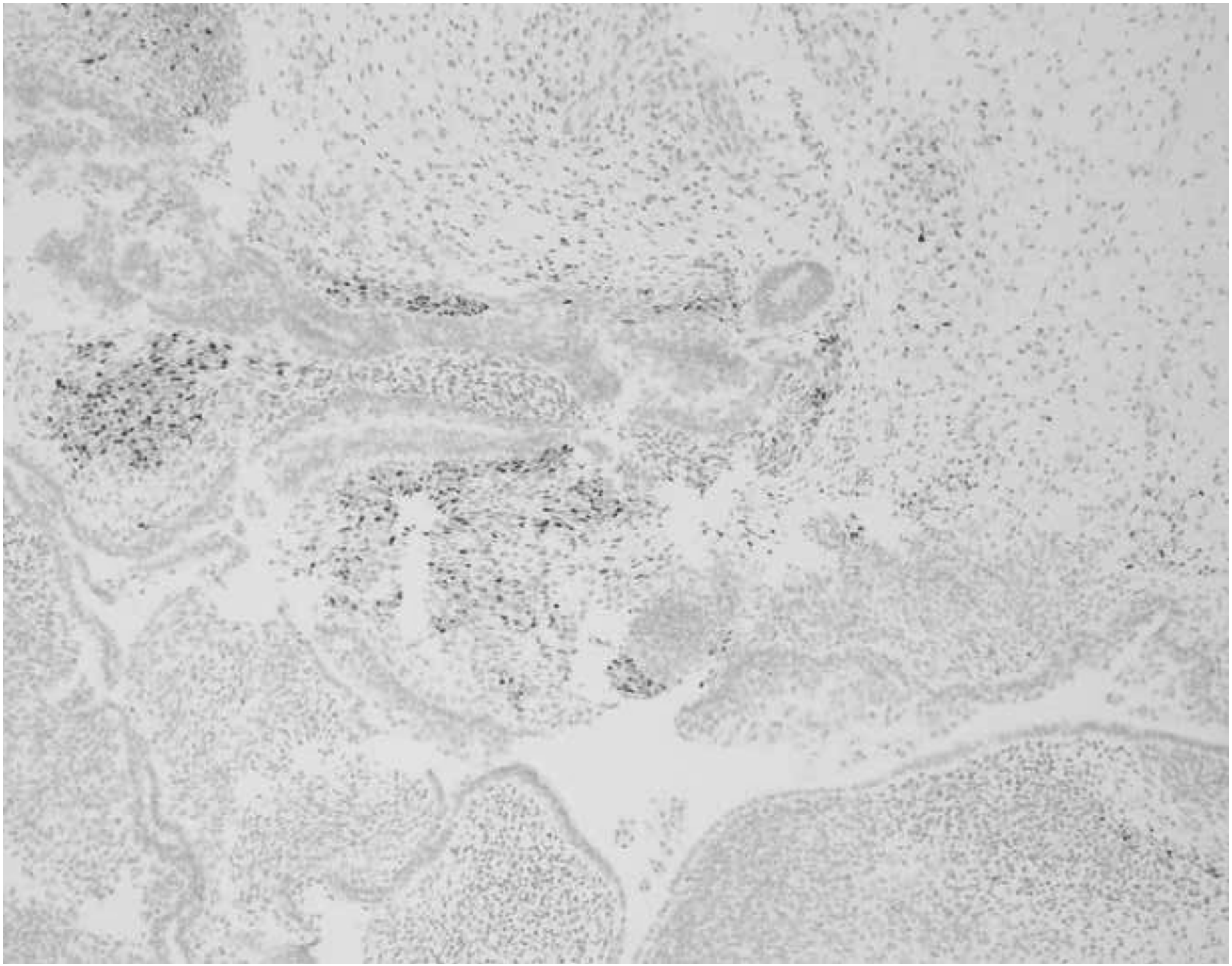

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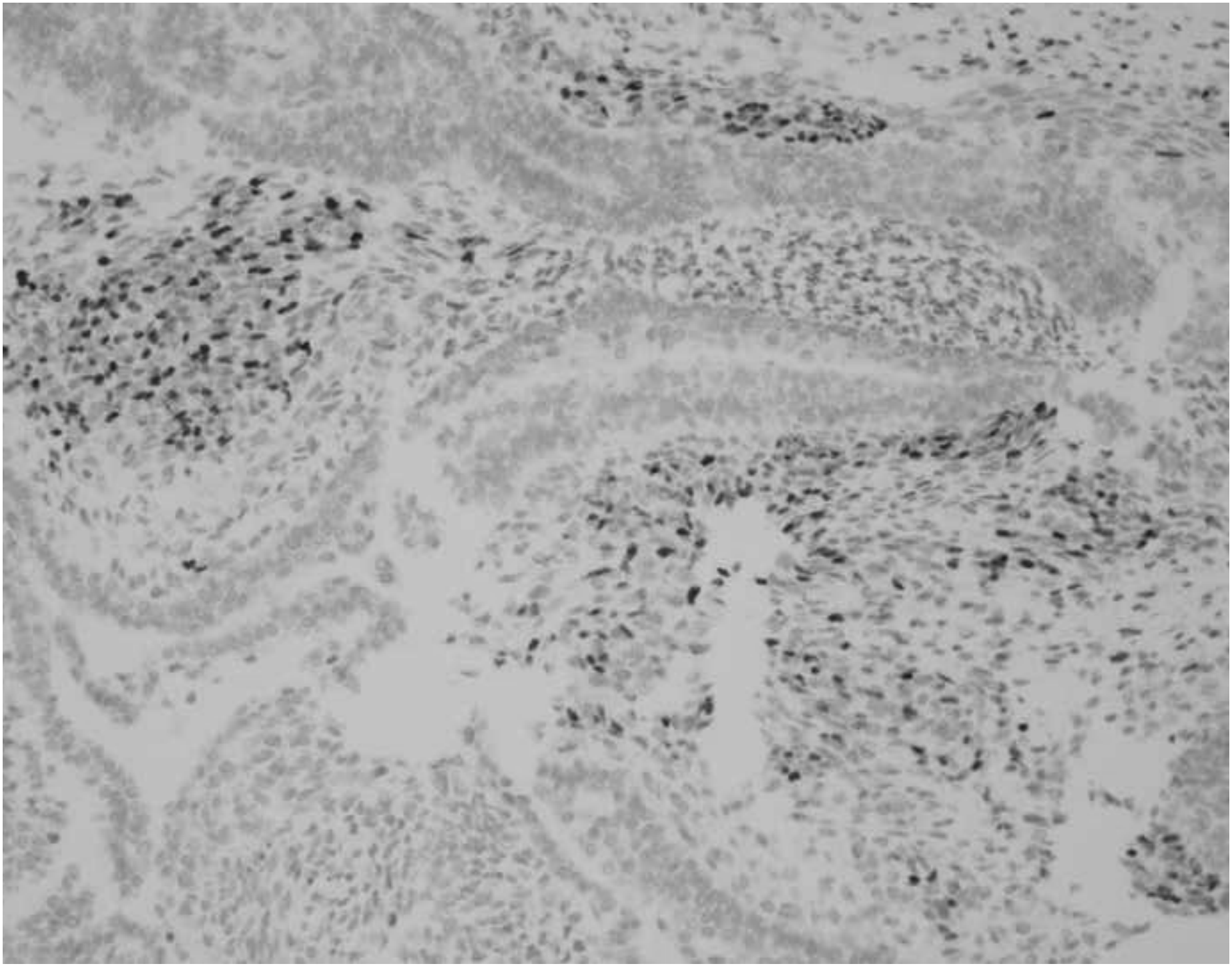

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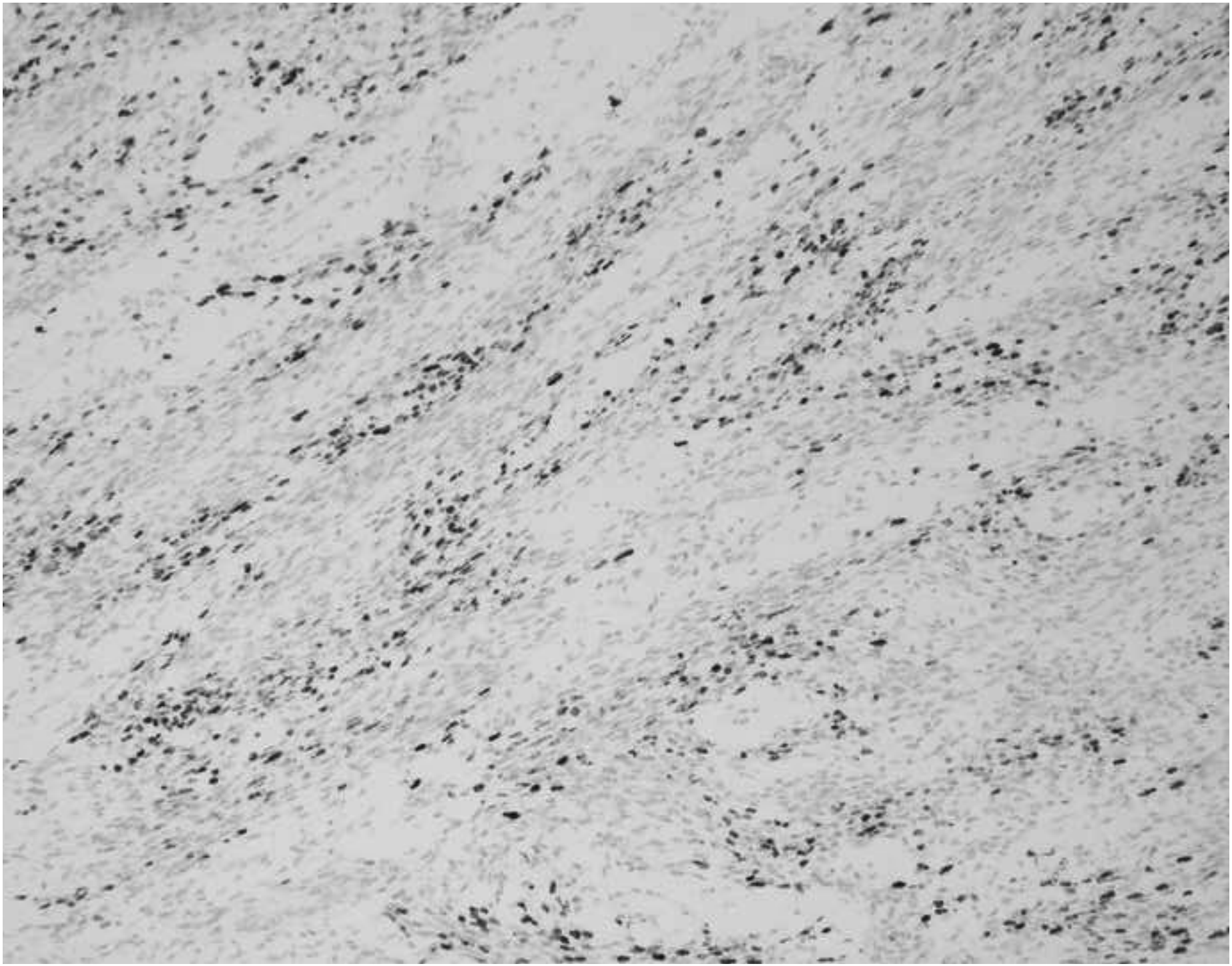

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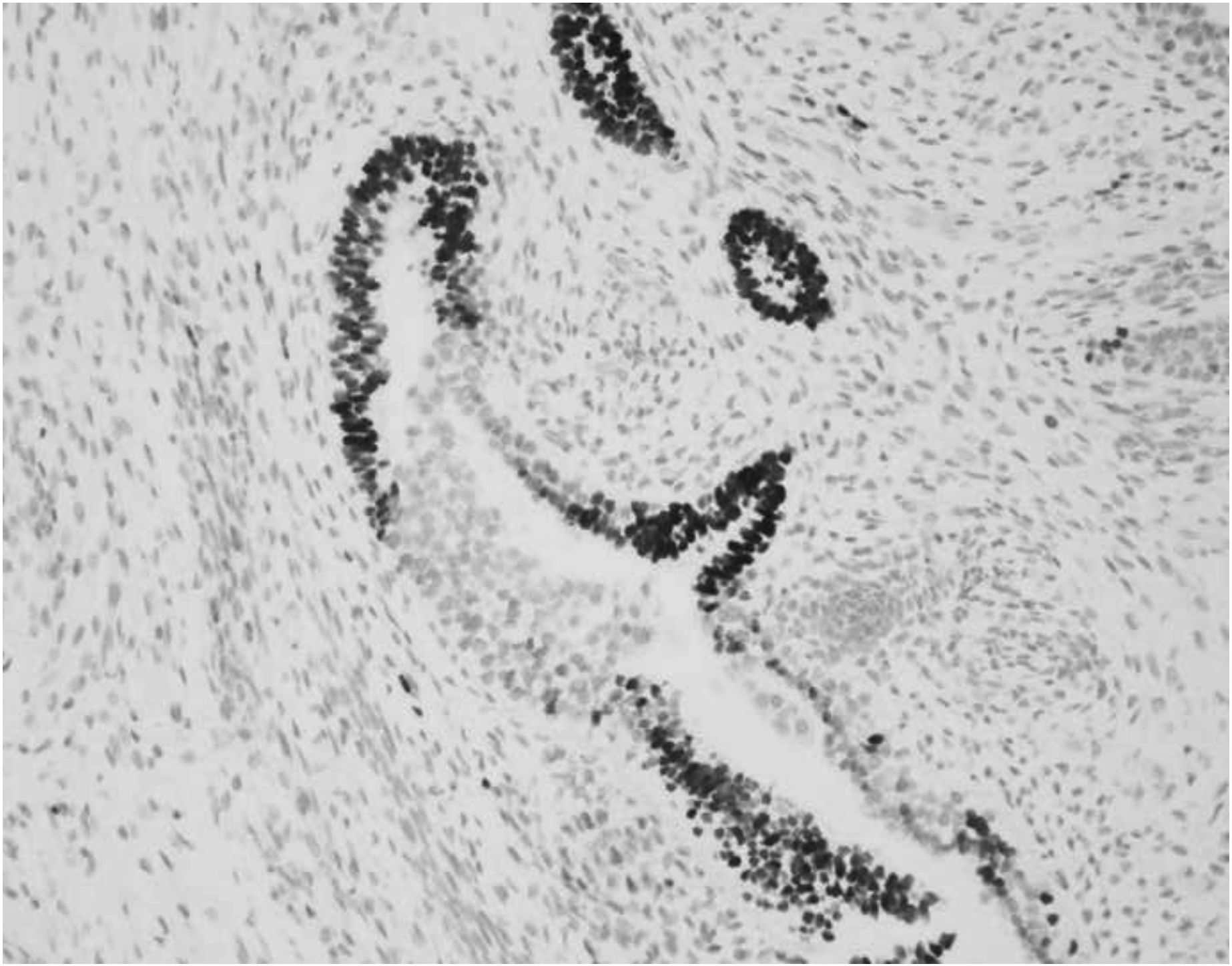

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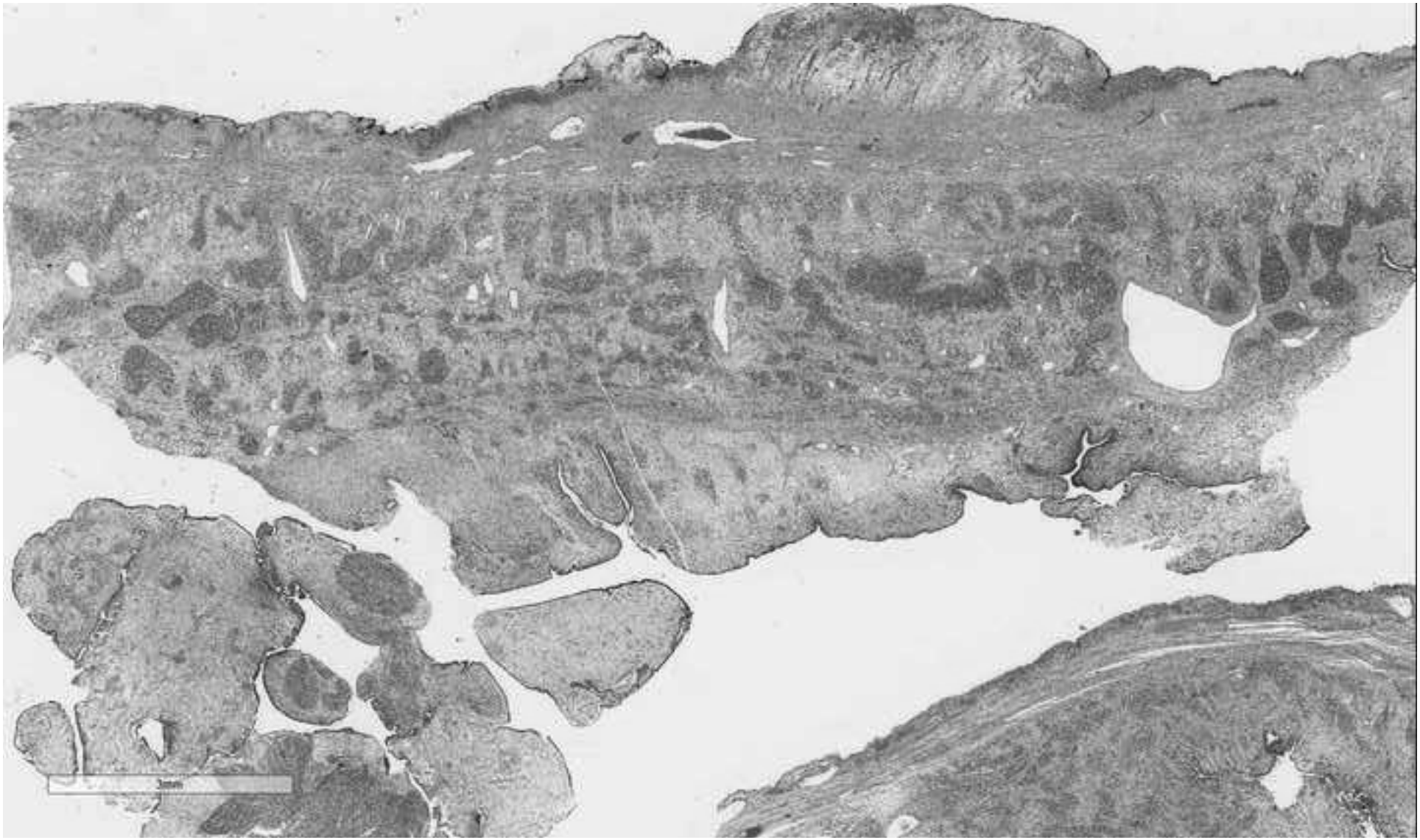

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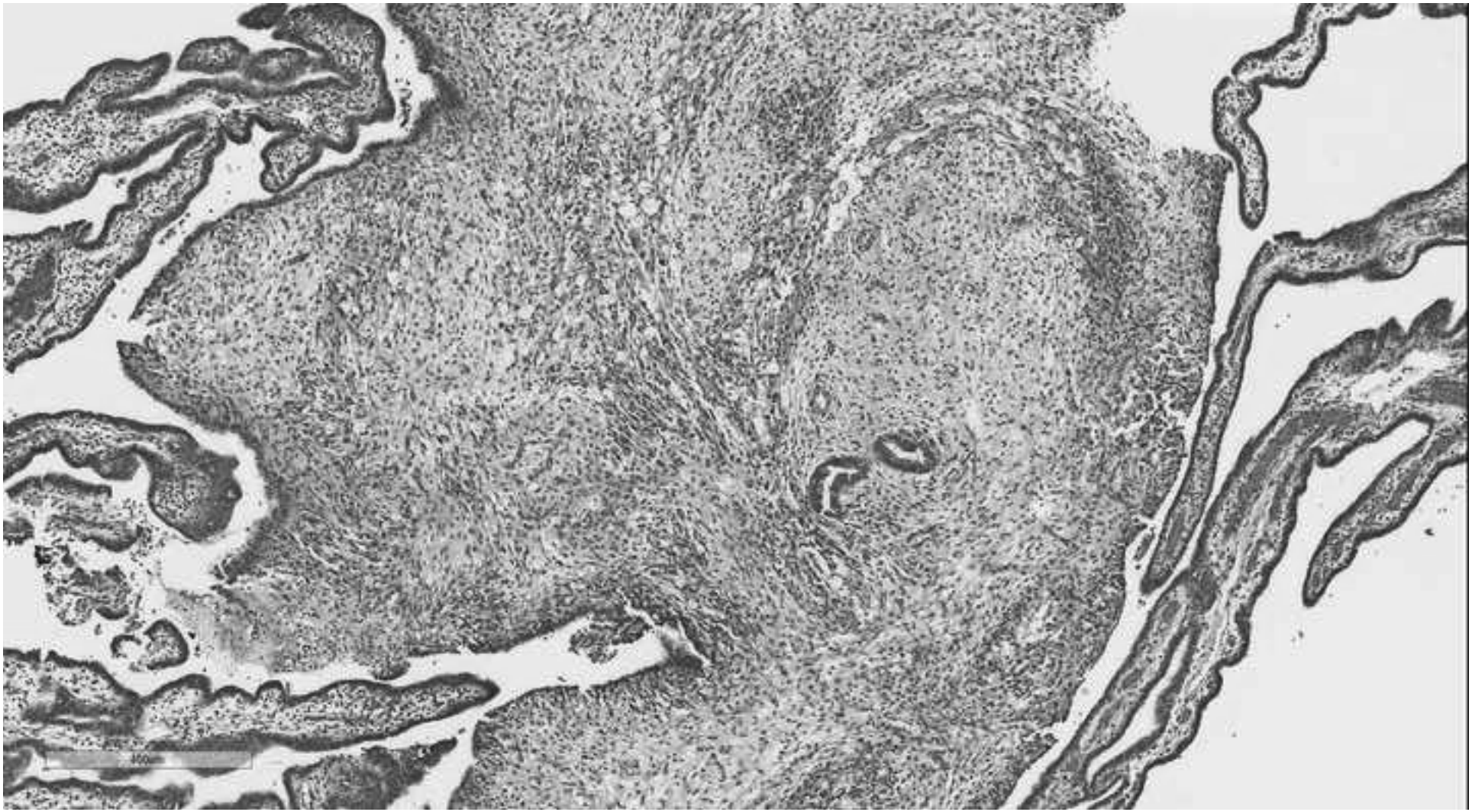

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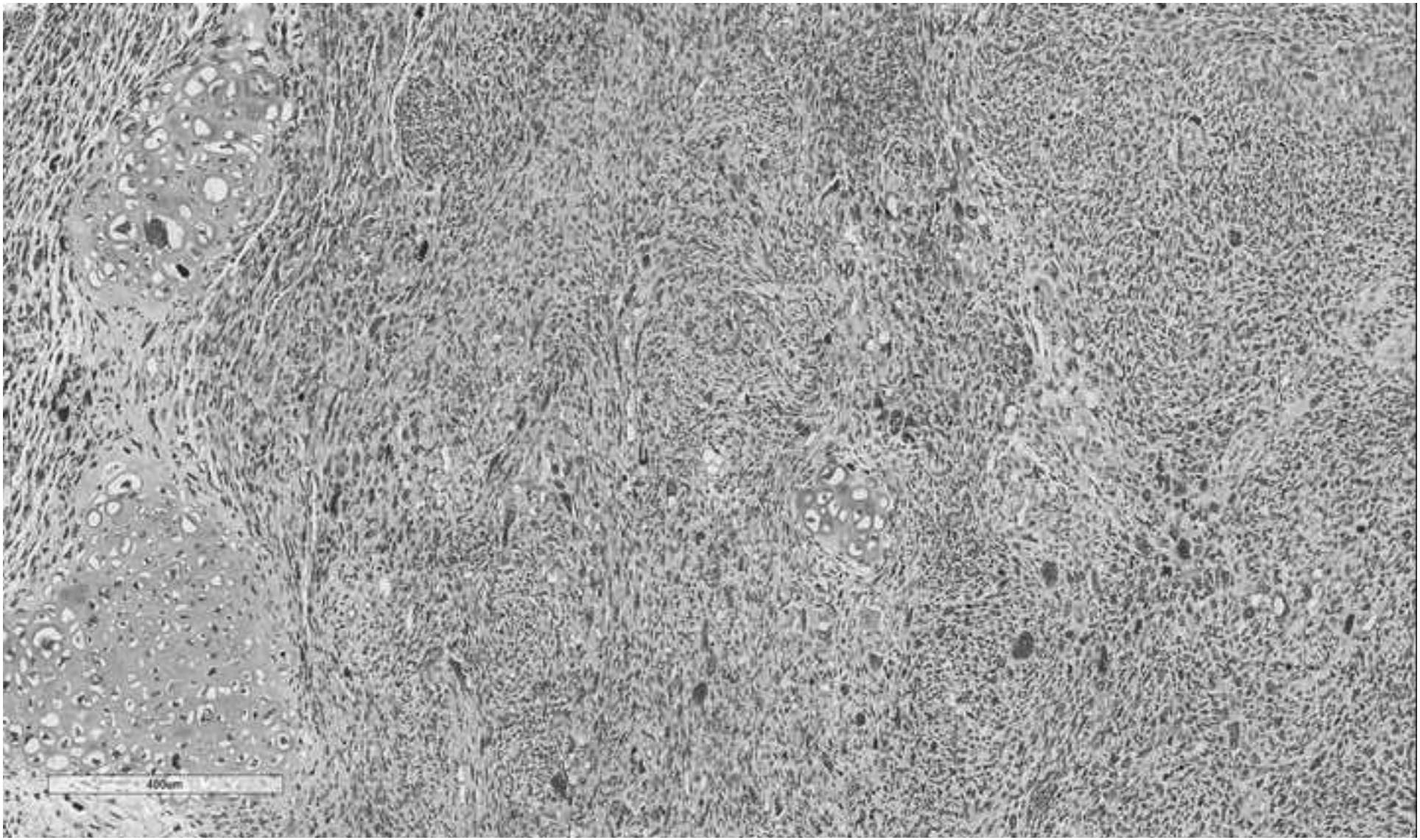

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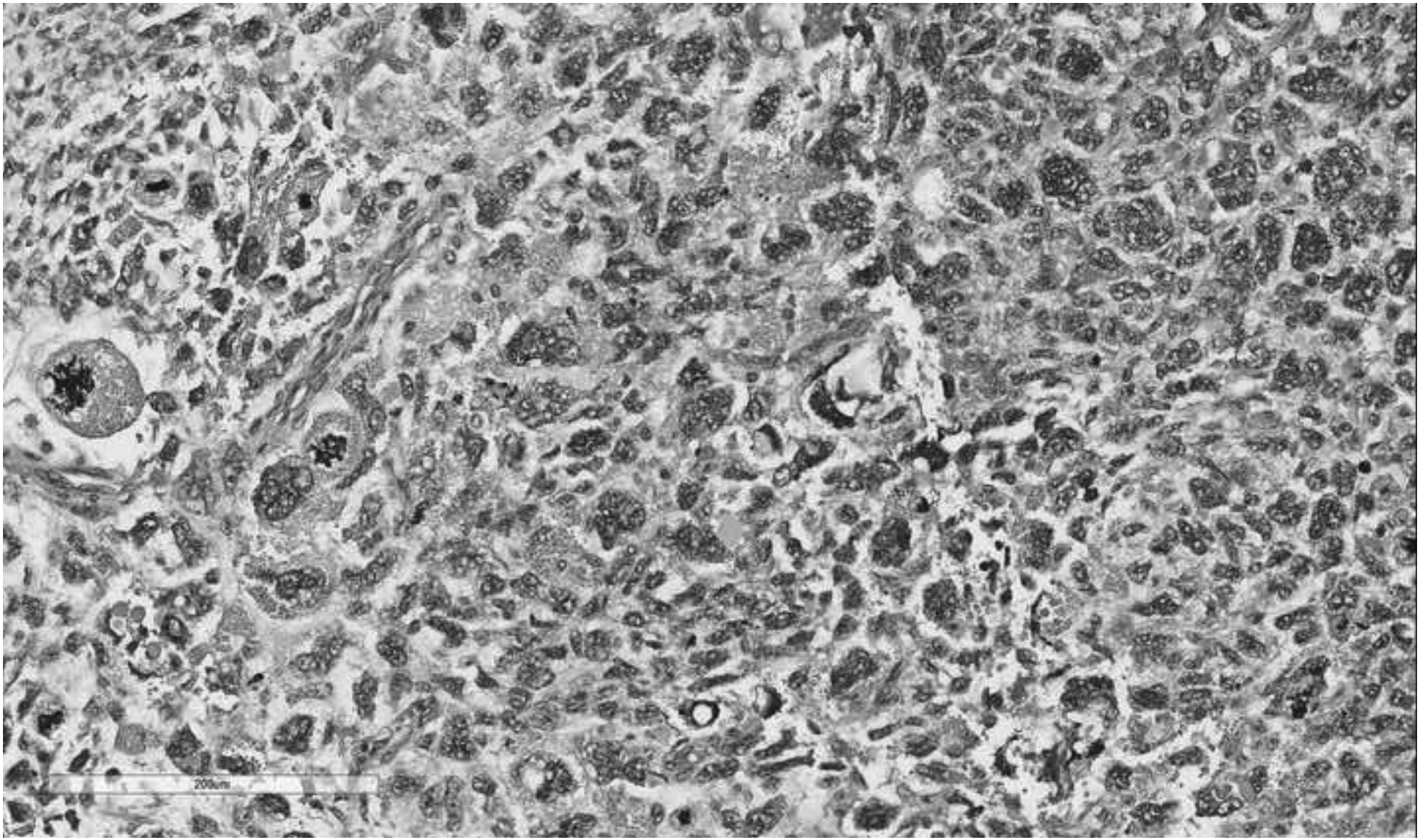

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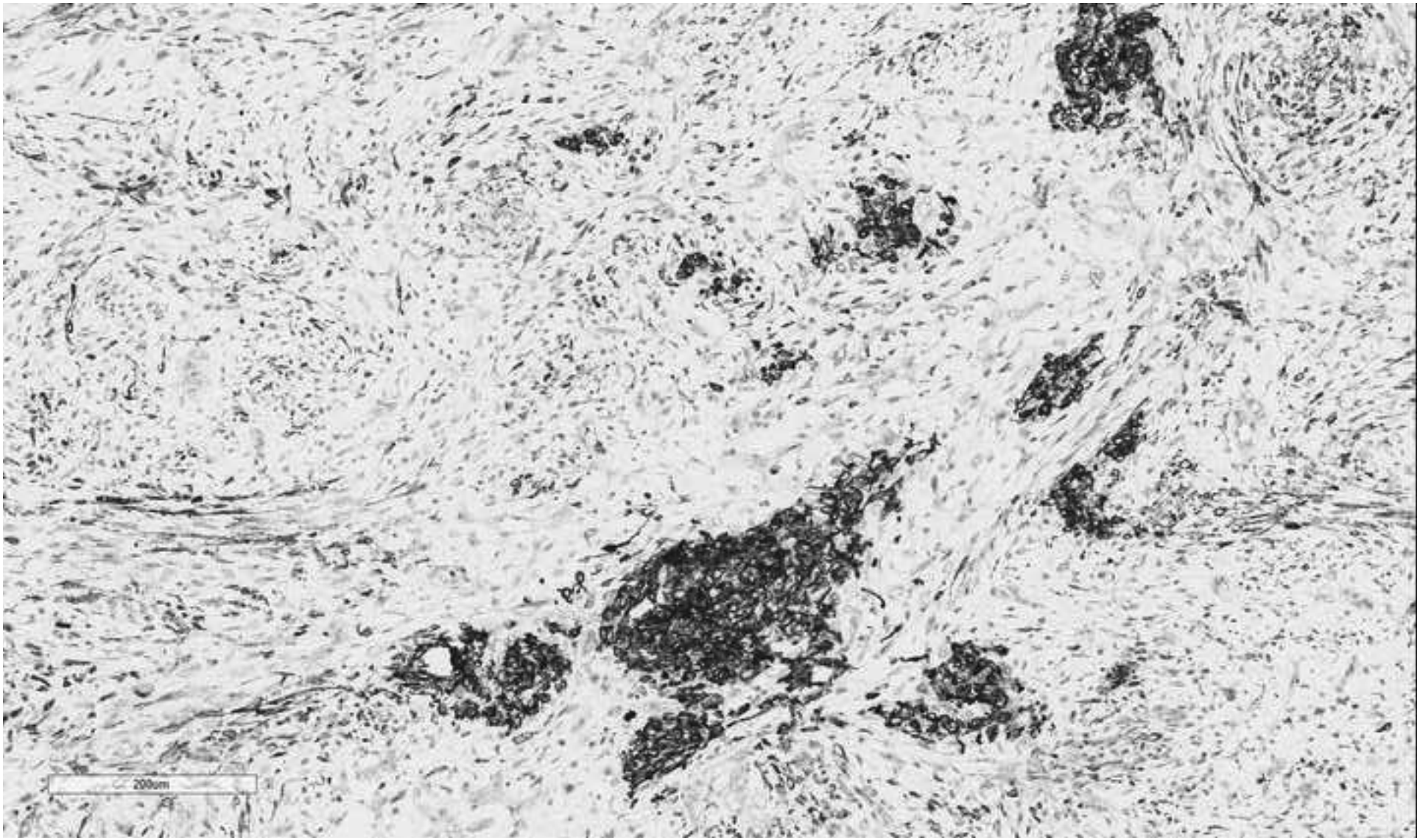

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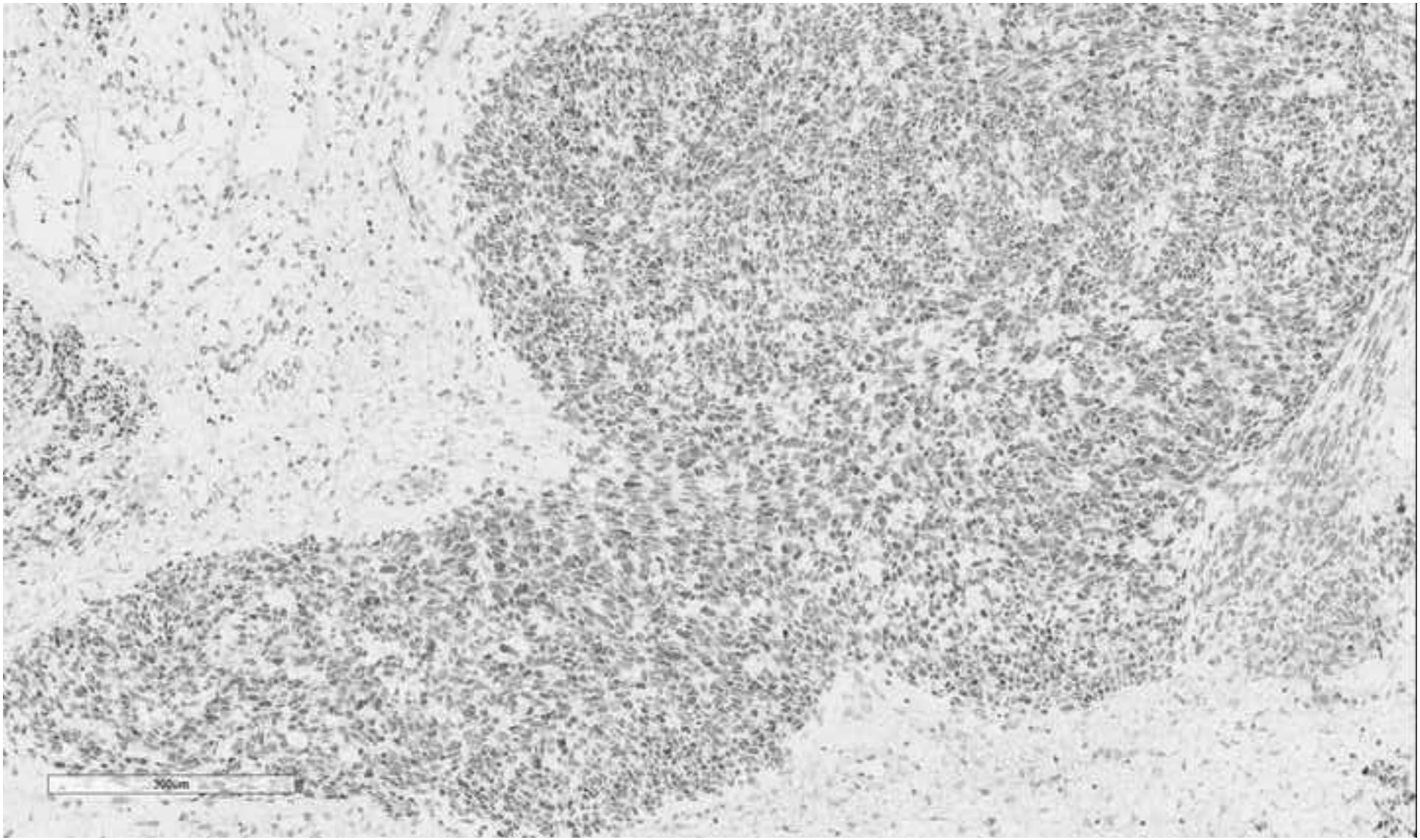

Figure 4

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