FROZEN SECTION FOR COLORECTAL GANGLION CELLS

4/12/2018
(Modified 4/3/2020)
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PURPOSE:
- To help surgeon achieve complete resection of the aganglionic segment and conserve normal bowel.

SPECIMENS:
- One or multiple sequential seromuscular or transmural (full-thickness) biopsies to help decide the correct proximal level for bowel transection.
- Proximal margin of a resected bowel segment to help decide if additional resection of the proximal bowel is needed before anastomosis.

SPECIMEN ORIENTATION:
- Correct orientation and proper embedding of the specimen are the key to accurate interpretation.
- The surgeon may indicate the serosal or mucosal surface by a suture or ink.
- The shape of the specimen, the smooth surface of the serosa, and the glistening surface of the mucosa should help specimen orientation.
- An *en face* cross section of the entire proximal margin should be examined for resected specimens.
- Frozen section should show all the layers of bowel wall.

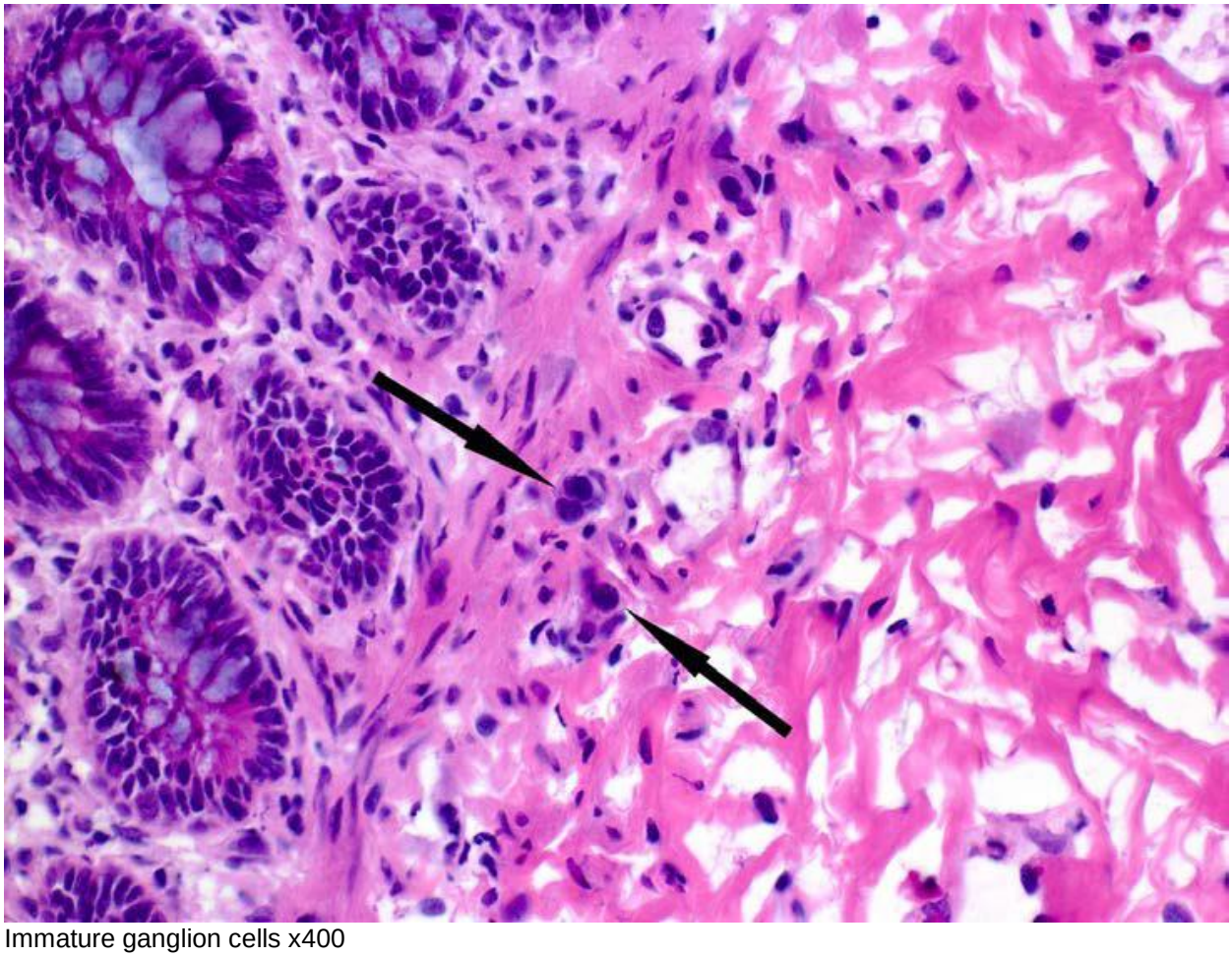
MICROSCOPIC EXAMINATION:
- On well-oriented sections, ganglion cells are seen in small clusters in the spaces between the inner and outer layers of the muscularis propria, and in small clusters or in individual single cells in the submucosa usually found just beneath the muscularis mucosae.
- When ganglion cells are absent or rare, particularly when the specimen is poorly oriented, deeper sections should be examined.
- Immature ganglion cells are commonly seen in neonates, which are smaller than mature ganglion cells, usually with scanty cytoplasm and inapparent nucleoli. These cells can be mistaken for histiocytes, large lymphocytes, endothelial cells, or fibroblasts.
- Aganglionic bowel wall usually also contains hypertrophic nerve bundles (>40 um in diameter) which can be helpful in cases where one is uncertain if immature ganglion cells are present.

FOR DIFFICULT CASES WITH UNCERTAINTY:
- Cut deepers. Multiple deeper sections may need to be examined.
- Consult an experienced colleague.
- Discuss with surgeon to see if another biopsy can be obtained.

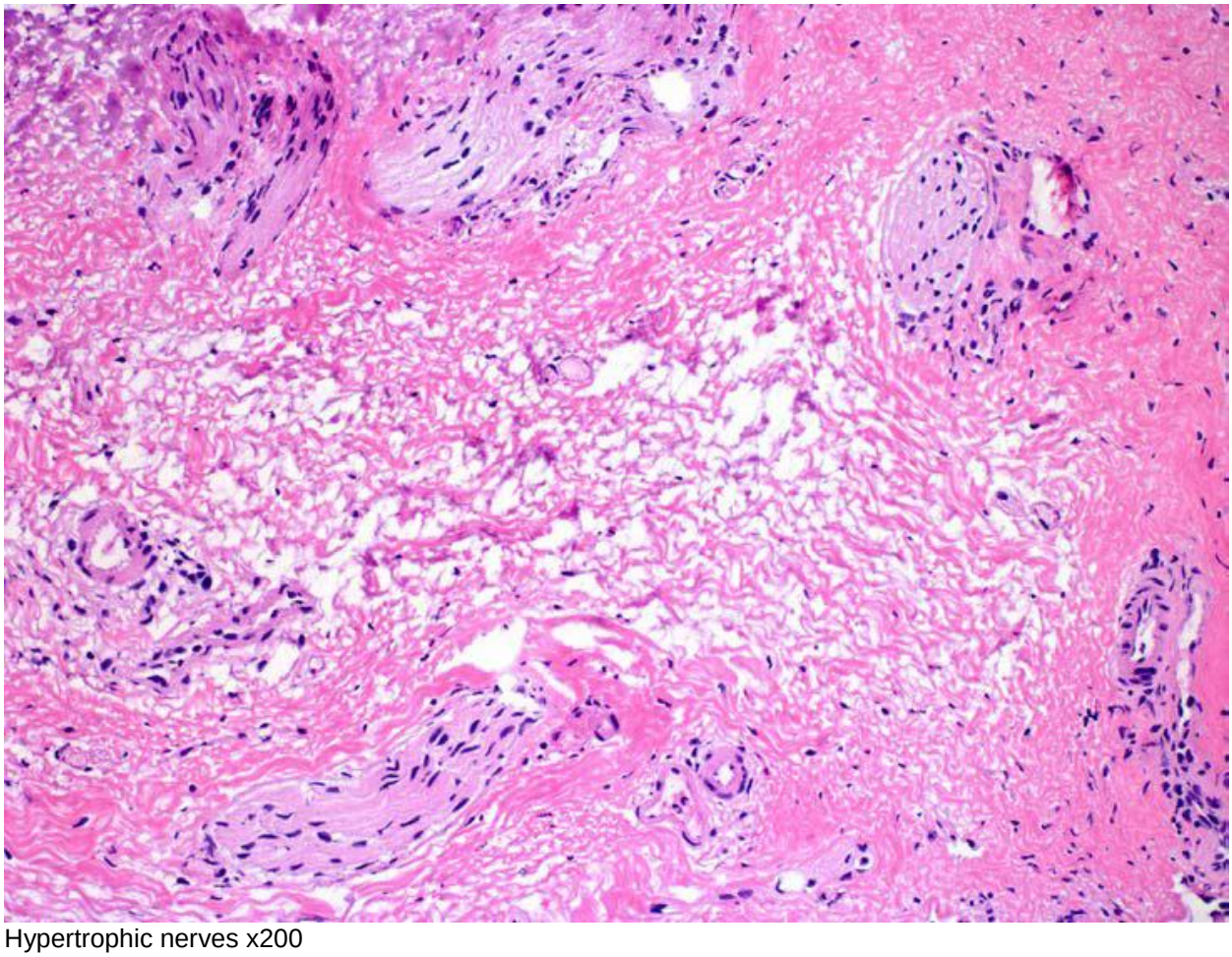
GENERAL RULES:
- It is better to make a false-negative error and to report “no ganglion cells identified” or “no definitive ganglion cells identified” when uncertain. This will prompt the surgeon to send for another biopsy, which would do very little harm to the patient because only a few cm of additional bowel would be unnecessarily resected.
- A false-positive error (mistakenly reporting the presence of ganglion cells) will lead to the surgeon making the anastomosis within the aganglionic bowel, which will require reoperation for additional resection.
- Repeat failure to recognize immature ganglion cells will lead to unnecessary resection of a large amount of normal bowel, which will also have serious consequences.
- If only rare ganglion cells or only a single ganglion cell are identified in an adequate and well-oriented specimen, or if a hypertrophic nerve bundle (>40 um in diameter) is present in the submucosa, the findings should be discussed with the surgeon. These findings are typical for a hypoganglionic

transition zone, and an anastomosis in this region may lead to persistent symptoms of bowel obstruction and require reoperation. An additional, more proximal biopsy should be requested.

- When examining the *en face* circumferential section of the proximal pull through margin or a separately submitted proximal donut, ganglion cells should be identified circumferentially in both submucosal and myenteric plexuses and there should not be any hypertrophic nerves. The aganglionic segment and transition zone are not always symmetric in length, and anastomosis should not be performed in a partially innervated segment.



Immature ganglion cells x400



Hypertrophic nerves x200

- Someone may find Diff Quik or Toluidine blue stains helpful, as the RNA in the ganglion cells is metachromatic and makes them stand out. There have been some questions about how to coverslip these stains. The slide should be coverslipped just with water initially. After the frozen has been examined, the coverslip can be gently slid off and the slide allowed to air dry, after which, it can be re-coverslipped with permanent mounting media.