PATHOLOGY OF GISTs

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GISTs recent but long history

- Defined as the most frequent mesenchymal tumor of the gastrointestinal tract (GIT)
- They have a long history in a short time
- 1962 first tumor described– Stout AP: “Bizarre smooth muscle tumor of the stomach” (Cancer)
GISTS history


- 1996 – Mentzel T, Katenkamp D.: “Gastrointestinal stromal tumor with skenoid fibers and bidirectional immunohistochemical differentiation” (Histopathology)

- 1996 – Suster S, Fletcher CDM: “Gastrointestinal stromal tumors with prominent signet ring cell features” (Mod. Pathol)


GISTs history

- 1999 identificarea GANT ca subtipuri de GIST la MET – Damiani S., Pasquinelli G.: “GANT-like gastrointestinal pacemaker cell tumors with oncocytoic features” (Virchows Arch.)

- 2000 CD34 identification in GIST – Robinson TL, Huizinga JD et al.: “GIST may originate from a subset of CD34-positive interstitial cells of Cajal” (Am. J. Pathol.)
GIST history

- 2001– Chambonniere ML et al.: “Expression of microtubule associated protein tau by gastrointestinal stromal tumors” (Hum. Pathol.)

The Cajal cells dilemma

- Present debate regarding interstitial Cajal cells (ICC) and Cajal-like cells (ICLC) – morphology, immunophenotype, location and functions;
- Incertitude surrounding the origin of GIST cells → express both CD34 and c-kit → possible origin: ICC, ICLC, fibroblasts, myofibroblasts or even stem cells or telocytes
GIST' Histogenesis: ICC / ICLC

- Initial gastrointestinal location, extended to many others
- Gut pacemaker cells
- Form intramural networks
- Develop from intrinsic gut mesenchyme? Or from bone marrow CD 34 positive stem cells?
- IHC: CD34, CD117, Nestin, Tau, S-100 protein, Leu 7, DOG1, PDGFRA, PGP9.5.

ICC location

- ICC described initially by Cajal as interstitial neurons in the gut and thereafter identified all over the gastrointestinal tract
- The ICC network is distributed within the submucosal, intramuscular and intermuscular (myenteric) layer and deep muscular plexus
- Only ICC or also ICLC? (Pieri et al 2008).
Types of ICC with different locations in the gastrointestinal wall

Small intestine

Chromogranin  20x

D2-40 40x

Stomach

DOG1 20x

S100 30x

CD117 20x

HE 10x

D2-40 20x
GIST

- The most frequent mesenchymal tumour of the gastrointestinal tract
- Thought to arise from the interstitial cells of Cajal (ICC) and, more recently, from the interstitial Cajal-like cells (ICLC)
- IHC: CD117, CD 34, PDGFRA, DOG1, PKCΘ, D2-40, Nestin, Tau

Histogenetic considerations

- GISTs do not originate exclusively from ICC, since these tumors can occur in sites where ICC do not exist (omentum, peritoneum, retroperitoneum).
- ICLC and other candidates, including a multipotent progenitor, can also constitute the origin of these tumors.
- CD34 positivity, considered an expression of immaturity for ICC, has not the same significance in GISTs: >70% are CD34+, but only 25-30% have a biologically malignant behavior.
Clinical features

- most of the patients are elderly persons of both genders,
- sometimes GISTs can arise in children, having particular features and evolution.
- these tumors can be single or multiple sporadic, the last type being sometimes misinterpreted as recurrent or metastatic disease.

Location

- They may arise throughout the gut, but the commonest sites are the stomach (60-70%), the small bowel (20-30%), the colorectum (5%), the esophagus (up to 5%).
- GISTs developed in the retroperitoneum, the omentum and the mesentery are considered extraGISTs.
Grossly

- usually polypoid masses situated in the external coat of the bowel, with or without infiltration and ulceration of the mucosa.
- tumor size is variable, from several millimetres to 10-15 cm.
- well delimited, but not encapsulated, with a white-greyish parenchyma, sometimes with necroses and hemorrhages. The superjacent mucosa can be intact or, in ~30% of cases, ulcerated.

Macroscopy - GIST
Special types of GISTs

- **Familial GIST**
  - It is rare and characteristically associated with germline dominant mutation of the KIT or PDGFRA gene with high penetrance.
  - In this context, GIST is usually multifocal and variably associated with hyperplasia of the interstitial cells of Cajal and abnormalities of mast cells such as urticaria pigmentosa or systemic mast cell disease, together with pigmentary abnormalities including perioral, neck, axillary and perineal freckling and pigmentation at the gastro-esophageal junction.

- **Syndromic GISTs**
  - Association of GIST in the context of neurofibromatosis type I (NF-1), the commonest cancer-predisposition syndrome, which carries up to 25% risk for developing GIST. Multifocal.

- **Pediatric GISTs**:
  - GISTs arising in children, adolescents, or young adults have a much lower frequency of mutations in KIT or PDGFRA (less than 10% of cases). In the classic pediatric setting, GIST shows evidence of KIT or PDGFRA mutations in only 11% cases; 5% occur in exon 11 of KIT, 9% in exon 9 and 3% in PDGFRA.
Histopathology

- Compact tumor proliferation with a delicate fibrovascular stroma +/- lax areas.
- Cell type: spindle-cell, epithelioid or mixed.
- The cell type also has a prognostic significance, the epithelioid and mixed cell type GISTs being associated with a poorer prognostic.

Risk stratification according to the number of mitoses, localization and tumor size

<table>
<thead>
<tr>
<th></th>
<th>Max size</th>
<th>Mitotic Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very low risk</td>
<td>&lt;2 cm</td>
<td>&lt;5/50 HPF</td>
</tr>
<tr>
<td>Low risk</td>
<td>2 to 5 cm</td>
<td>&lt;5/50 HPF</td>
</tr>
<tr>
<td>Intermediate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Risk</td>
<td>&lt;5 cm</td>
<td>6-10/50 HPF</td>
</tr>
<tr>
<td></td>
<td>&gt;5 to 10 cm</td>
<td>&lt;5/50 HPF</td>
</tr>
<tr>
<td>High risk</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt;5 cm</td>
<td>&gt;5/50 HPF</td>
</tr>
<tr>
<td></td>
<td>&gt;10 cm</td>
<td>Any</td>
</tr>
<tr>
<td></td>
<td>Any</td>
<td>&gt;10/50 HPF</td>
</tr>
</tbody>
</table>
Risk stratification (Demitri 2007)

Cell morphology

- Spindle cells in GISTs: heterogeneous
  - very large, with two or more polar branching processes,
  - flat with radial thin processes and variably stained cytoplasm,
  - the nuclei are either rod-like with rounded extremities, or fusiform.
  - Nuclear chromatin can be granular, fine or coarse, with inconspicuous nucleoli.
  - characteristic paranuclear cytoplasmic vacuolae.
  - Mitoses are usually typical and scarce.
  - Rarely, metaplastic chondroid or distrophic calcifications can occur.

<table>
<thead>
<tr>
<th>Mitotic Index</th>
<th>Size</th>
<th>Gastric</th>
<th>Duodenum</th>
<th>Jejunum/Ileum</th>
<th>Rectum</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 5 per 50 hpf</td>
<td>≤ 2 cm</td>
<td>None (0%)</td>
<td>None (0%)</td>
<td>None (0%)</td>
<td>None (0%)</td>
</tr>
<tr>
<td>&gt; 2 per 50 hpf</td>
<td>&gt; 2 ≤ 5 cm</td>
<td>Very low (1.6%)</td>
<td>Low (4.2%)</td>
<td>Low (8.2%)</td>
<td>Low (8.3%)</td>
</tr>
<tr>
<td>≤ 5 per 50 hpf</td>
<td>&gt; 5 ≤ 10 cm</td>
<td>Low (3.6%)</td>
<td>Moderate (24%)</td>
<td>(Insuff data)</td>
<td>(Insuff data)</td>
</tr>
<tr>
<td>&gt; 5 per 50 hpf</td>
<td>&gt; 10 cm</td>
<td>Moderate (10%)</td>
<td>High (52%)</td>
<td>High (54%)</td>
<td>High (57%)</td>
</tr>
<tr>
<td>&gt; 5 per 50 hpf</td>
<td>≤ 2 cm</td>
<td>None†</td>
<td>High</td>
<td>(Insuff data)</td>
<td>High (54%)</td>
</tr>
<tr>
<td>&gt; 5 per 50 hpf</td>
<td>&gt; 2 ≤ 5 cm</td>
<td>Moderate (16%)</td>
<td>High (73%)</td>
<td>High (50%)</td>
<td>High (52%)</td>
</tr>
</tbody>
</table>
Spindle cell GISTs

HE, ob. 10x
Cell morphology

- Epithelioid type (5-10% of cases)
- Proliferate as islets or sheaths of large rounded cells with pale eosinophilic cytoplasm, sometimes foamy or plasmacytoid,
- Rounded vesiculous nuclei and visible nucleoli.
- The tumor stroma can be finely fibrovascular or more abundant, myxoid or edematous.
- A significant number of mitoses can be considered as prognostic factor.

Epithelioid type GIST
Epithelioid pattern, clear cells
plasmocytoid pattern

Stroma hyalinization
Distrophic calcifications

Morphologic features: plasmacytoid cells; paranuclear vacuolization
HP aspects suggesting malignity

Plasmacytoid Cells

HP aspects suggesting malignity

Signet ring cells
Immunohistochemical profile

- CD34 – the first marker used
- CD117 – c-kit gene protein
- PDGFRA - platelet derived growth factor receptor A
- DOG1 - discovered on GIST
- D2-40 / podoplanin
- Nestin, Caveolin 1, Tau, PKC teta

C-kit protein (CD117)

- it is a 145-kD glycosylated transmembranary receptor with tyrosine kinase activity

**structure**:
- * an extracellular domain (consists of 5 Ig-like domains)
- * a transmembrane region (TM)
- * a juxtamembrane (JM) domain
- * an intracytoplasmic tyrosine kinase domain
- its specific ligand is the stem cells growth factor (SCF)
Heterogeneous c-kit staining patterns

- CD117 is expressed in >95% of GISTs
- IHC patterns in tumor cells:
  - Membrane
  - Cytoplasm: Homogenous, Coarse granular, Fine granular, Paranuclear dot
- CD117 staining patterns can be associated with certain c-kit gene mutations (homozygous or heterozygous) involved in the response to imatinib (Tabone-Eglinger S et al. Clin Cancer Res, 2008, 14(8):2285-2294)
Small intestine GISTs

Heterogeneous GISTs immunoprofile

- Classically: CD34 and CD117 positive
- Variations: CD34+ CD117-
  - CD34- CD117+
  - CD34- CD117-
- Other biomarkers: S-100 protein, SMA, DOG1, PDGFRA, Nestin, Tau, PGP 9.5, D2-40, Caveolin 1
- Some of these IHC markers also have prognostic value
Intestinal GIST

New markers
Markers of special differentiation

- smooth muscle actin (SMA), mostly in the spindle cell type, with a cytoplasmic distribution;
- S100 protein (S100), cytoplasmic, associated with a neuroid pattern;
- Desmin, rarely, with a zonal distribution;
Molecular features- ckit mutations

- occur on exon 11 which codifies a juxtamembranary domain with regulatory function; it is the most frequently encountered, in >70% of GISTs.
- Other mutations, in decreasing order of frequency, are located on exons 9, 13 and 17.
- Recent studies: GISTs with mutations on exon 11 are the most sensitive to the specific treatment with Imatinib, compared with mutations on exons 9 or 13. Also, deletions compared with point mutations in exon 11 have been found to be a significant unfavorable factor in patients with gastric GISTs.
**PDGFRA mutations**

- located on exon 18 (over 80%), exon 12 (much less frequent - 6%) or exon 14 (the rarest - 1%). PDGFRA mutations on exon 18 characterize the category of GISTs with primary resistance to Imatinib, while mutations on exon 14 are usually associated with a less favorable prognosis. Exon 12 mutations are the most sensitive to specific treatment.

**PCR pentru c-Kit Exoni 9 si 11**

Verificare electroforetica pe gel de agaroza 2% a reactivelor PCR pentru gena C-KIT-exonii 9 si 11
Probe (1-7=exon 9 si 1'-7'=exon 11):
1 - ADS (control negativ)
2 - 123750
3 - 126904
4 - 128048
5 - 130530
6 - 174048
7 - proba de control pozitiv 174934-2
M – 50bp DNA Ladder (Fermentas)
**PCR pentru c-Kit Exoni 9 si 11**

Verificare electroforetica pe gel de agarosa 2% a reacțiilor PCR pentru gena C-KIT-exonii 9 si 11
Probe (1-7=exon 9 si 1'-7'=exon 11):
1 – ADS (control negativ)
2 – 123750
3 – 126904
4 – 128048
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7 – probă de control pozitiv 174934-2
M – 50bp DNA Ladder (Fermentas)

**Secventiere gena C-KIT, exonul 9**

Secventiere (bidirectională) cu kitul BigDye Terminator v3.1 Cycle Sequencing Kit și echipamentul Analyzer 310 (Applied Biosystems)
New approach in GISTs

- **SDHA immunohistochemistry and loss-of-function mutations analysis**; mainly in ckit and PDGFRA wild-type
- **SDHB** – immunohistochemistry
- **CD133** – IHC
- **Natural killer isoform p30** - as prognostic factor.
Victor Babes Institute GIST team

- Simona Enache
- Mihaela Mihai
- Cristina Iosif
- Florina Vasilescu
- Dana Terzea
- Florin Andrei
- Georgeta Cardos
- Alina Grigore

Thank you!