Conflicts of interest: none to declare

Monika Klimkowska MD PhD
Department of Pathology and Cytology
Karolinska University Hospital
Stockholm, Sweden
Multilineage dysplasia in bone marrow - a common phenomenon in untreated Gaucher disease type 1

Monika Klimkowska MD PhD
Department of Pathology and Cytology
Karolinska University Hospital
Stockholm, Sweden
Email: monika.klimkowska@karolinska.se
Dysplasia

Abnormal differentiation and maturation pattern of cells
Disordered tissue growth
Response to pathological conditions (reversible)
  • inflammation,
  • deficiency states,
  • radiation-induced tissue damage

Hallmark of neoplasia
Myelodysplasia

Aberrant maturation patterns of hematopoietic cells in bone marrow (BM)

Nonneoplastic/reactive

• CDA, Fanconi anemia, Blackfan/Diamond syndrome
• iron, vit. B12, folate, zinc deficiency
• substance abuse
• infections e.g. parvovirus B19
• medication, toxic agents

vs. malignant

• myelodysplastic syndromes (MDS)
MDS

Abnormal proliferation of BM stem cells progeny
  • functionally ineffective
  • morphologically disturbed
  • increased rate of apoptosis in BM cells

Clonal character

Peripheral cytopenia(s)

Increased risk of progression to acute myeloid leukemia (AML)
MDS diagnostics

High quality BM smears + histology
Stains: HE, MGG, iron, reticulin/collagen

Cellularity
Separate cytological assessment of 3 lineages, with 10% significance level

Blast count (N threshold: 5%)
Cytogenetics
MDS diagnostics

Classification: WHO 2008

- Refractory cytopenia with unilineage dysplasia (RCUD)
- Refractory anemia with ring sideroblasts (RARS)
- Refractory cytopenia with multilineage dysplasia (RCMD)
- Refractory anemia with excess blasts (RAEB)
- Myelodysplastic syndrome with isolated del(5q)
- Myelodysplastic syndrome, unclassifiable
- Childhood myelodysplastic syndrome

Prognostic and predictive factors: IPSS 1997

- Percentage BM blasts
- Cytogenetic findings
- Number of cytopenias
GD1 and myelodysplasia

How common are dysplastic features in BM of GD1 patients?

• peripheral cytopenias common

Do GD1 patients bear an increased risk of MDS development?
GD1 male patient 82 yrs old

Currently bicytopenic (RBC, Plt)
N370S/L444P, splenectomized at 32 yrs age

Hypercellular BM, moderate GC burden in biopsy
Polymorphic megakaryocytes
Mild fibrosis
GD1 male patient 82 yrs old

Abnormal erythroblasts → Hypogranulated myeloid cells
GD1 male patient 82 yrs old

MGUS IgG lambda known for 32 years, since last 3 yrs progressed to myeloma, treated with melphalan, stabilized

Symmetric neuropathy, leukocytoclastic vasculitis

Transfusion dependent for the last 2 years

Started on ERT, then SRT

Last 4 months – MDS (RAEB), then AML
GD1 male patient 82 yrs old
Material and methods

Diagnostic BM specimens (aspirates, biopsies) from 17 untreated pts with GD1 from Sweden, Lithuania, and Poland

Central reassessment by a hematopathologist blinded to clinical data
- BM specimens from 15 patients (7 women, 8 men) eligible for assessment;
- BM smears from 2 pts were extremely diluted with PB. One of them had both high cellularity and GCs burden but at the same time advanced fibrosis, and the other one had hypocellular BM.

Median pt age at evaluation: 56 years (range 21–86 years), four of them (27%) were splenectomized.

All but two pts carried at least one allele with a N370S (c.1226A>G) mutation in the GBA1 gene.

Only one patient did not have any cytopenia in PB, 7 pts had unilineage cytopenia, 5 pts had bicytopenia, and 2 pts had pancytopenia.

Two pts had vitamin B12 deficiency.
## Material and methods

<table>
<thead>
<tr>
<th>Pt no.</th>
<th>Sex</th>
<th>Age at BM sampling</th>
<th>Anemia</th>
<th>Thrombocytopenia</th>
<th>Leukopenia</th>
<th>B12 deficiency</th>
<th>Splenectomy</th>
<th>GBA1 mutation</th>
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<td>Y</td>
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<td>Y</td>
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<td>N370S/L444P</td>
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</table>
Material and methods

Morphological assessment according to WHO criteria, using methodics described by Verburgh (Leukemia 2007)
- routinely stained BM smears (May-Grunwald-Giemza, hematoxylin-eosin) + histology
- reticulin-stained slides when available, iron stains assessed for all pts
- BM cellularity and Gaucher cell burden also assessed
- 100 nucleated cells/glass slide counted in each lineage (erythropoiesis; myelopoiesis; thrombopoiesis); two slides analyzed per patient

Erythropoiesis - assessed for dominant abnormal features
- irregular nucleus, basophilic stippling, vacuolization, multinucularity, megaloblastic features;
- amounts of abnormal cells in respective categories were summed up to obtain percentage of dysplastic cells in red cell series (≥10% = significant dysplasia).

Granulocytopoiesis - 100 cells from promyelocytes to granulocytes/slide assessed separately for pathological cytoplasmic and nuclear features
- cytoplasmic feats.: hypogranular, agranular, pathological granulation or Auer rods;
- nuclear feats : abnormal size, hypersegmented, ring, pseudo-Pelger, hyposegmented with clumped chromatin;
- significant dysplasia in myelopoiesis = ≥10% cells had abnormal nuclear OR cytoplasmic features.

Megakaryocytes: all cells assessed, in both cytological and histological samples
- abnormal features: micromegakaryocytes, large hypolobated or bizarre forms/cells with separate nuclei;
- Significant dysplasia = ≥10% cells were abnormal but at least 30 MGKs had to be assessed in total.
Dysplasia in erythropoiesis

Nuclear budding or lobation (C), intercellular bridging (A), multinuclearity, megaloblastic changes. Vacuolization (B), ring sideroblasts, PAS/positivity.
Dysplasia in granulocytopoiesis

Cytoplasmic features:
- hypo/agranulated (C)
- aberrant granulation, incl. Chediak-Higashi-like cells

Nuclear features:
1. Size: small, giant
2. Shape: ring nucleus (B)
3. Segmentation:
   - pseudo-Pelger-Huet (D),
   - hypersegmented (A),
   - hyposegmented/clumped
Dysplasia in thrombocytopenia

Micromegakaryocytes (A)
Large cell with monolobated nucleus (B)
Cells with separate nuclei (C)
Bizarre nuclear lobation (D)

(!) At least 30 MGKs must be counted for valid analysis.
## Results

Dysplastic cells per 100&100 counted cells in respective poeses

<table>
<thead>
<tr>
<th>Pt no.</th>
<th>Sex, Age</th>
<th>dysplastic E, %</th>
<th>dysplastic M nuclear, %</th>
<th>dysplastic M cytoplasm, %</th>
<th>dysplastic MGK, %</th>
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<td>16 &amp; 11</td>
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<td>9 &amp; 11</td>
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<td>18 &amp; 18</td>
<td>15 &amp; 17</td>
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<td>18 &amp; 20</td>
<td>27 &amp; 13</td>
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</tbody>
</table>
Results

Cellularity: 35-85%
GD burden: 5-70%
Blast count: no pt above 5%
Ring sideroblasts: only single detected, no pt above 15%

Fibrosis:
- variable, from mild to advanced,
- higher density in GC-rich areas compared to hematopoietic islands

Cytogenetics available only for single pts
## Results

<table>
<thead>
<tr>
<th>Pt no.</th>
<th>Sex, Age</th>
<th>BM cellularity</th>
<th>GC burden in BM</th>
<th>No. dysplastic series, BM</th>
<th>No. penias, PB</th>
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<td>5%</td>
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<td>40%</td>
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</tr>
<tr>
<td>15.</td>
<td>F, 39</td>
<td>80%</td>
<td>60%</td>
<td>3</td>
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</table>
Results

Splenectomized patients

1. F, 21 – splenectomy at 5 yrs, currently unicytopenic (low Hb) but three-lineage dysplasia. High cellularity but low GC burden.


Results

Mild fibrosis (pt 4, M 65 yrs)

Moderate fibrosis (pt 6, M 86 yrs)
Results

Advanced fibrosis (pt 11, F 57)
Conclusions

1. Significant dysplastic changes can be found in most patients with GD1, even in persons without overt cytopenias or after splenectomy.

2. Percentage aberrant cells in respective poeses can be low, however, morphological abnormalities are of the same type as in myelodysplastic syndromes.

Underlying mechanisms for abnormal hematopoietic cell maturation in GD pts are not known

- Can GCs infiltrates adversely affect neighboring hematopoietic islands by means of local cytokine interactions?
- Can GC infiltrates affect local blood & nutrient supply? Impact of advanced local fibrosis?
Further issues

- Fibrosis in BM may also strongly affect quality of the investigation. Aspirated material can be heavily diluted and virtually non-diagnostic, on the contrary to BM biopsies.

- Potential clonal nature of the observed abnormalities in hematopoietic cells in patients with GD remains to be investigated.

- Studies of patients who developed true MDS or acute myeloid leukemia in course of GD can be of value for assessment of potential increased risk in this patient population.
Department of Medicine at Huddinge, Karolinska Institute, Stockholm, Sweden
Maciej Machaczka
Hans Hägglund

Children’s Memorial Health Institute, Department of Metabolic Diseases, Endocrinology and Diabetology, Warsaw, Poland
Anna Tylki-Szymańska

Department of Pathology, Collegium Medicum of the Jagiellonian University, Cracow, Poland
Krzystyna Gałązka

Department of Hematology, Collegium Medicum of the Jagiellonian University, Cracow, Poland
Wojciech Jurczak

Centre of Hematology and Oncology, Children’s Hospital, Affiliate of Vilnius University Hospital Santariskiu Clinics, Lithuania
Grażina Kleinotiene
Lina Rageliene

Department of Hematology, Oncology and Transfusion Medicine Center, Vilnius University Hospital Santariskiu Clinics, Lithuania
Regina Pileckyte

National Centre of Pathology, Vilnius University, Hospital Santariskiu Clinics affiliate, Lithuania
Ugnius Mickys

Department of Pathology and Cytology, Karolinska University Hospital, Stockholm, Sweden
Agnieszka Bulanda