ICCS e-Newsletter CSI
Winter 2011

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e-CSI - Clinical history:

8 year old female presenting with fever and bruising on extremities
## e-CSI - Complete blood count:

<table>
<thead>
<tr>
<th>CBC parameter</th>
<th>Result</th>
<th>Units</th>
<th>Reference range</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC</td>
<td>122.2</td>
<td>x10⁹/L</td>
<td>(5.41 - 9.70)</td>
</tr>
<tr>
<td>RBC</td>
<td>3.01</td>
<td>x10¹²/L</td>
<td>(3.88 - 4.72)</td>
</tr>
<tr>
<td>HGB</td>
<td>8.4</td>
<td>g/dl</td>
<td>(11.3 - 13.4)</td>
</tr>
<tr>
<td>HCT</td>
<td>25.3</td>
<td>%</td>
<td>(32.3 - 38.3)</td>
</tr>
<tr>
<td>MCV</td>
<td>84.0</td>
<td>fl</td>
<td>(79.5 - 85.2)</td>
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<tr>
<td>MCH</td>
<td>27.9</td>
<td>pg</td>
<td>(27.8 - 30.0)</td>
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<tr>
<td>MCHC</td>
<td>33.4</td>
<td>gm/dl</td>
<td>(34.3 - 35.8)</td>
</tr>
<tr>
<td>RDW</td>
<td>13.9</td>
<td>%</td>
<td>(12.8 - 13.9)</td>
</tr>
<tr>
<td>PLT</td>
<td>86</td>
<td>x10⁹/L</td>
<td>(187 - 376)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>WBC differential</th>
<th>%</th>
<th>Absolute</th>
<th>Reference range</th>
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</thead>
<tbody>
<tr>
<td>Neutrophils</td>
<td>0</td>
<td>0</td>
<td>(2.58 - 5.95)</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monocytes</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eosinophils</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>91</td>
<td></td>
<td></td>
</tr>
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</table>

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e-CSI - Work-up and evaluation:

Bone marrow aspirate and biopsy were procured

Flow cytometric analysis was performed on the marrow aspirate and results from selected 4-color tubes are provided for review.
e-CSI - Flow cytometric approach:

Acquisition FACS CantoII, analysis DIVA (FITC / PE / PerCP-Cy5.5 / APC)

Tube 1: HLA-DR / CD117 / CD45 / CD19
Tube 2: CD13+CD33 / CD7 / CD19 / CD2
Tube 3: CD20 / CD10 / CD45 / CD19
Tube 4: Lambda / Kappa / CD19 / CD22
Tube 5: CD15 / CD56 / CD19 / CD34
e-CSI - Flow cytometric analysis:

An expanded population of small non-complex cells (ie. low FSC and SSC) is detected.

This profile is not typical of marrow where maturing granulocytic and monocytic elements should be present in abundance.
The majority of cells express dim CD45, indicating they are immature.
e-CSI - Analysis - tube 1:

Note also small populations of presumptive granulocytes (high SSC) and mature lymphocytes (CD45 bright, low SSC)
The CD45 dim population coexpresses CD19, an antigen that can be expressed normally on B-cells (in all stages of maturation and differentiation) as well as aberrantly on myeloblasts.
HLA-DR, which is seen in most acute myeloid leukemias as well as in B-lymphoblastic leukemias, is also coexpressed by these CD19 positive cells.
This population is, however, negative for CD117, suggesting it is not of myeloid derivation.
Absence of CD13/CD33 (myeloid antigens) offers further support against a myeloid process while lack of CD2 (T-cell) and CD7 (primarily a T-cell antigen, but can be seen in myeloblasts as well) indicates these cells are likely not of T-cell lineage. Note the presence of mature T-cells (green) and granulocytes (blue).
Although the cells are CD19 positive, other markers commonly seen in B-lymphoblastic leukemia (CD20 and CD10) are absent. Note that a minute population of mature B-cells (CD20 bright) is present.

Compare this with a profile of normal maturing B-cells (ie. hematogones) provided below.
CD22 shows dim positivity, supporting a B-cell lineage.

Mature B-cells (CD22 bright) express polytypic surface immunoglobulin.
The CD19, CD22 positive cells also coexpress CD34, confirming that they are B-lymphoblasts and they exhibit aberrant expression of CD15, another myeloid antigen. Note the mature granulocytes (CD15 bright) in blue.
e-CSI - Bone marrow aspirate smear demonstrates a predominant population of small to intermediate-sized mononuclear cells that have round to irregular/clefted nuclei, fine chromatin, inconspicuous to small nucleoli and scant cytoplasm, consistent with lymphoblasts.
e-CSI - Bone marrow core biopsy demonstrates marrow replacement by sheets of lymphoblasts. Only rare erythroid, myeloid and megakaryocytic elements are present.
e-CSI - Broad diagnosis:

B-lymphoblastic leukemia
e-CSI - Question 1:

Given this immunophenotypic profile:

CD45 dim, HLA-DR, CD34, TdT (data not shown), CD19, CD22 dim and CD15 positive

What stage of B-cell maturation do these cells best represent?
e-CSI – B-cell development:

Early precursor/
Pro-B-ALL

Intermediate/
Common ALL

Mature precursor/
Pre-B-ALL

Mature B-cell

CD19
TdT
cCD22
CD34

CD19
CD10
CD22
CD20

C-µ
CD19
CD10
CD22
CD20

IgM
CD19
CD22
CD20

THIS ONE
Based upon this immunophenotype, can you predict the cytogenetic abnormality that was detected?
e-CSI - Cytogenetic results:

\[ t(4;11)(q21;q23) \]

with an MLL [11q23] rearrangement

(this cytogenetic abnormality further subcategorizes B-lymphoblastic leukemia within the WHO 2008 classification schema)
e-CSI - Question 3:

The clinical history was that of an 8 year old female - what is somewhat unusual about the age of presentation given the presence of a t(4;11) translocation?
Acute lymphoblastic leukemia (ALL) with MLL rearrangement is the most common leukemia in infants less than 1 year of age, but is less common in older children.

So (playing the odds), one might have expected this patient to be an infant based upon the immunophenotypic profile.
e-CSI - Question 4:

The full karyotype is as follows:

45,XX,t(4;11)(q21;q23)[18]/
46,XY[2],donor

How do you reconcile this information?
What historical details were omitted?

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This child was diagnosed with MLL-associated ALL at 4 months of age.

She was in remission for 3 yrs, then relapsed, underwent re-induction, and was subsequently transplanted with unrelated allogeneic marrow from a male [XY] donor.

Unfortunately, her presentation as depicted in this case study represents relapsed disease post-transplantation; the presence of two cells with a male karyotype reflects residual donor cells in a background of relapsed leukemia.
e-CSI - Pediatric leukemia:

- Leukemias are the most common cancers affecting children, representing ~30% of all cancers in those under 15 years of age.

- In the United States, 75% of pediatric leukemias are lymphoblastic leukemia, 15-20% are acute myeloid leukemia (AML), and 5% are chronic myeloid leukemia.

- Infantile ALL (that diagnosed within the first 12 months of life) represents ~2.5-5.0% of pediatric ALL.
e-CSI - Infantile ALL & MLL:

- Rearrangements in chromosomal band 11q23, involving the mixed lineage leukemia [MLL] gene, are common in infantile ALL, occurring in ~70% of cases.

- Its presence is inversely correlated with age:
  - >90% in those less than 6 months
  - ~50% in those 6 - 12 months
  - ~6-7% in those 12-24 months

- Abnormalities of MLL include deletions, inversions and unbalanced as well as reciprocal translocations. Many different translocation partners (> 70) have been described.

- The most common translocation is t(4;11)(q21;q23), occurring in 30-45% of infants.
e-CSI - Infantile ALL with t(4;11)(q21;q23):

**Clinical manifestations:**

- Hyperleukocytosis (median WBC > 150 x10⁹/L)
- Hepatosplenomegaly
- CNS involvement
e-CSI - Infantile ALL with t(4;11)(q21;q23):

Immunophenotypic characteristics:
Represents an early precursor ("pro-B") cell

CD10 negative
CD10 (aka. CALLA = common acute lymphoblastic leukemia antigen) - was given this name because it is so common in B-ALL, but not in this particular subtype!

Myeloid coexpression (typically CD15 positive)

Classic immunophenotypic profile:
CD45^{dim}, TdT^{pos}, CD34^{pos}, CD19^{pos}, CD22^{neg/dim}, CD20^{neg}, CD10^{neg}, cyt IgM^{neg}, CD15^{dim}, mostly CD13^{neg}, CD33^{neg}, CD9^{pos}
Prognosis: Very poor

Long-term rates of event-free survival (EFS) of 28-45%. This is much lower than EFS in older children with ALL, which is ~80%.

Relapses occur very early (typically within the first 2 years of diagnosis).

Therapeutic approaches are also controversial, but include intensified chemotherapy and hematopoietic stem cell transplantation.

Reasons for such a poor outcome are not well understood.
e-CSI – A few words on MLL [11q23]:

• MLL = mixed lineage leukemia gene [11q23]

• Associated with ALL, AML and therapy-related myeloid neoplasms (as its name implies) in both the pediatric and adult populations.

• MLL rearrangements occur in 30-60% of infants with AML.
  - t(9;11)(p22;q23) is the most common translocation in this age group
  - t(11;19)(q23;q13.3) is the next most frequent

• MLL-associated AML is most commonly monoblastic and frequently presents with extramedullary infiltrates.
e-CSI - MLL-associated infant ALL: References


Antigen expression patterns reflecting genotype of acute leukemias. Leukemia 2002;16(7):1233-1258 [Hrusak O and Porwitt-MacDonald A].


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