Case of The Month

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Chief Complaints

- 14 years old boy presented with complaints of on-off fever and bilateral leg pain for 4 months.
- He also had history of weight loss, loss of appetite and generalized weakness
- There was no lymphadenopathy of any mass elsewere in body

Past History

- Evaluated for similar symptoms three months back.
- Peripheral blood had occasional atypical cell for which marrow was advised
- Bone marrow done at that time showed 7% blasts which were CD 7+, CD5 +, CD 34 +, CD13 -, CD117- blasts on immunophenotyping
- The patient was kept on close follow up inview of absence of frank acute leukemia and extramedullary disease

Family History

- The child had a very strong family history of malignancies with many relatives of both maternal and paternal sides being affected with solid organ malignancies at young ages
- The father of the patient had also expired 9 months back due to acute myeloid leukemia

Examination

- General physical examination- pallor +
- Systemic examination- No lymphadenopathy
  No organomegaly
- Local examination- Restricted bilateral leg motions

Radiological investigations

MRI: Marrow infiltrative pathology in multiple vertebrae, both iliac bones and proximal femur likely a lymphoproliferative disorder.

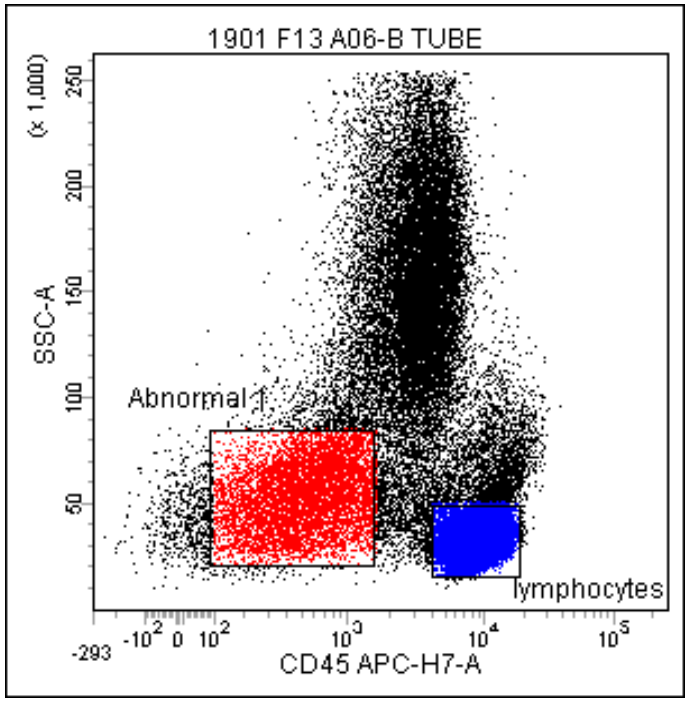
Haematological Investigations

- **CBC & Peripheral smear:** Hb:11.4 gm%, Platelets-1.2 lakh with a TLC of 10300 with 10% blasts
- **Bone marrow:** 24 % blasts
- **Cytogenetics:** Normal male karyotype
- **Translocation panel:** Negative for t(1:19), t(12;21), t(4;11) and t(9;22) by PCR

IMMUNOPHENOTYPE WAS DONE TO IDENTIFY NATURE OF THESE CELLS

Flow-Cytometry

CD45 Vs SSC

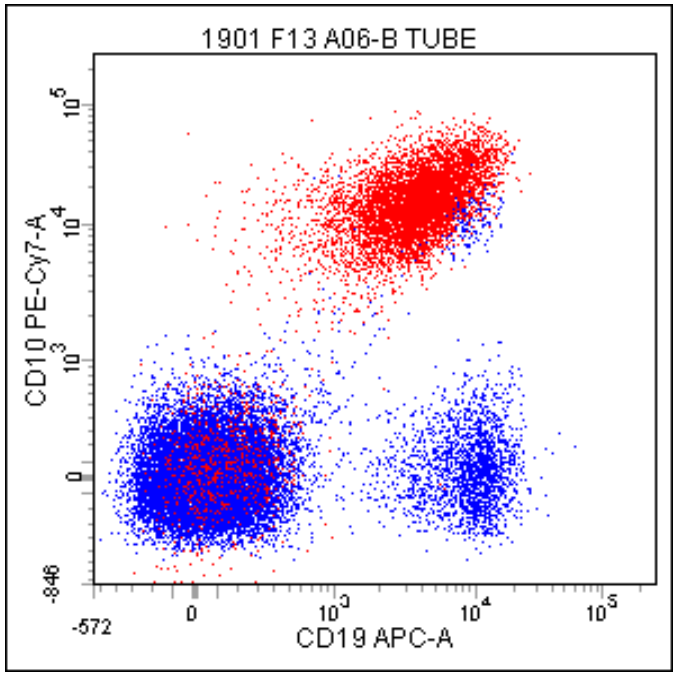


Interpretations

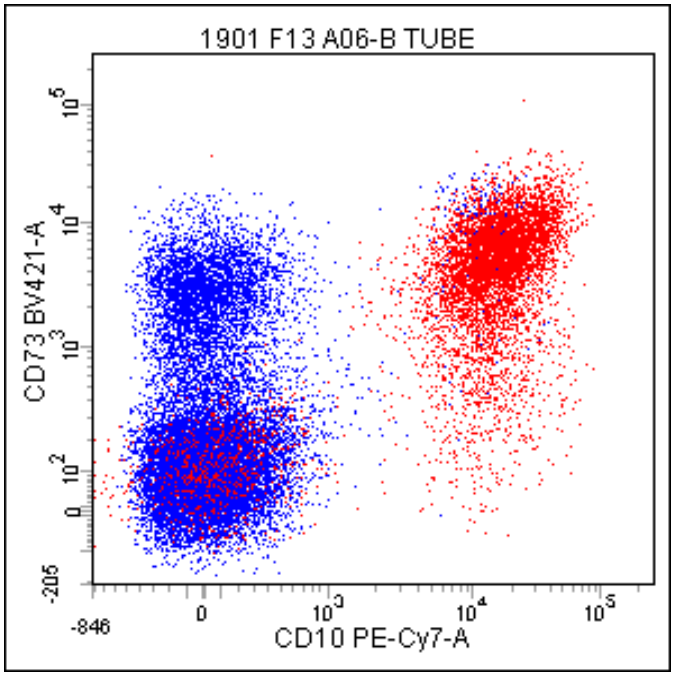
- An abnormal population is gated in negative CD45 region (Red in colour)
- Lymphocytes are gated as controls (Blue in colour)

Flow-Cytometry

CD19+, CD10+

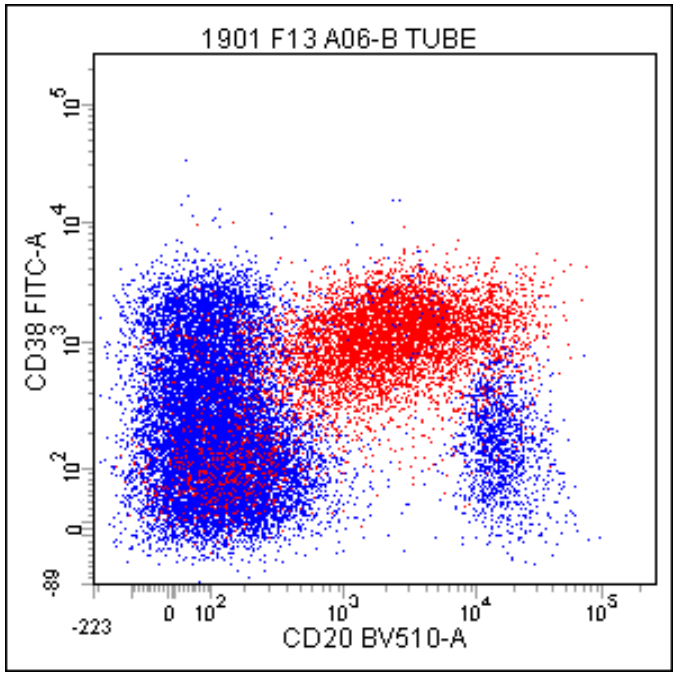


CD73 +



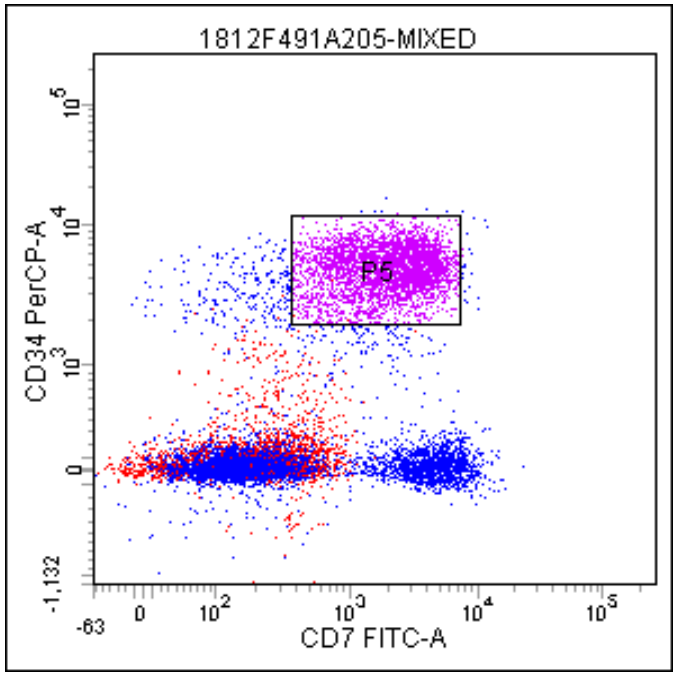
Flow-Cytometry

CD38 Negative CD20 DIM



FLOW-CYTOMETRY

CD34 Vs CD7

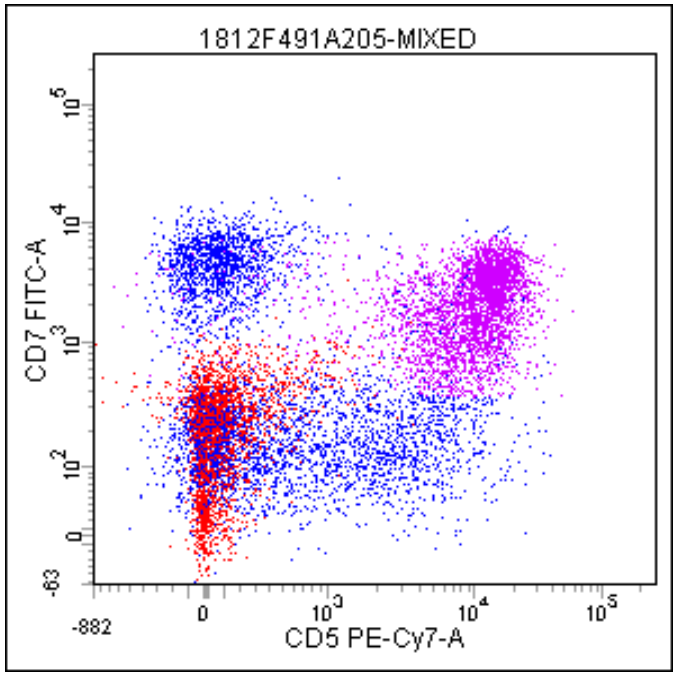


Interpretation

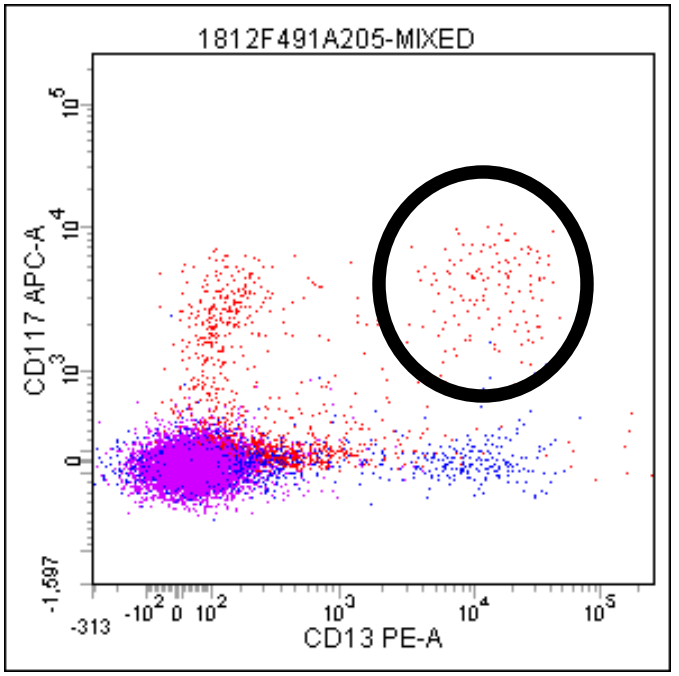
- On CD34 Vs SSC window a separate abnormal CD7+ and CD34 positive population (Pink in Colour) was identified
- This population was coming from mature lymphocyte region and gated (Pink)

FLOW-CYTOMETRY

CD5+ CD7 +



CD13-, CD117-



SUMMERISE

- Population 1
  - Positive Markers: CD19, CD10, CD38, CD73, CD123, Cyto CD79a
  - Negative Markers: CD45, T cell markers, Myeloid markers
- Population 2
  - Positive markers: CD45 (Bright), CD7, CD5, CD34
  - Negative Markers: Cy CD3, B Cell markers, T Cell Markers
- Population 3

  - A minor (<1%) CD117+, CD13+ population was also identified (? Significant) [Population marked in a Black Oval on CD13 Vs CD117 plot]

Final Diagnosis

Leukemia of ambiguous lineage- Bilineage acute leukemia with B + T cell phenotype

Acute Leukaemia of Ambiguous Lineage

- Term reserved for those acute leukaemia that:
  - Show differentiation for more than 1 lineage
  - Show differentiation for none of the lineage
- Rare: <4% of all acute leukemia
- Its a immunophenotypic diagnosis with subclassification on cytogenetics

Classification

- Acute Undifferentiated Leukemia: No lymphoid or myeloid specific markers
- MPAL/Bi-Lineage with cytogenetics abnormality:
  - KMT2A Gene rearrangement: Bi-Lineage (B+ Myeloid), CD10 Negative, Myeloid component is monocytic
  - BCR-ABL positive: B+ Myeloid
- Mixed Phenotype Acute Leukemia: Single population with 2 commitments: NOS (No CTG abnormality present)
  - MPAL: B+ Myeloid NOS
  - MPAL: T+ Myeloid NOS
  - MPAL: Rare NOS
- Leukemia of ambiguous lineage: NOS

Acute Undifferentiated Leukemia

- Important to exclude leukaemia of unusual lineages example BPCDN, NK cell precursors, basophils even non haematopoietic tumours.
- No more than 1 marker of any given lineage
- Lack cCD3 and MPO, cCD22, cCD79a and strong CD19.
- Express
  - CD34,
  - HLA-DR
  - CD7, Tdt and CD38

MIXED PHENOTYPE ACUTE LEUKEMIA

Myeloid lineage
MPO (by flow cytometry, IHC or cytochemistry) or Monocytic differentiation (≥ 2 of the following : NSE, CD 11c, CD 14, CD 64, lysozyme)
T-cell lineage
Cytoplasmic CD3(by flow cytometry with antibodies to CD3 epsilon chain) Or Surface CD3
B- cell lineage
Strong CD19 with ≥ 1 of the following strongly expressed : CD79a, cytoplasmic CD22, CD10 OR Weak CD19 with ≥ 2 of the following strongly expressed : CD79a, cytoplasmic CD22,CD10

Common Mis-Interpretations

- The criteria described above are only for defining two commitments to a single blast population (MPAL)
- Do not apply the criteria where two separate blast population exists (Bi-Lineage). In These cases each population should be considered as a separate case

Common Mis-Interpretations

- MPO positivity is not always equal to myeloid differentiation
  - If MPO alone is the only myeloid marker present
- Cytoplasmic CD3 is not always equal to T cell differentiation
  - If Cy CD3 is dim than dimmest population should reach upto level of normal T cell fluoresence
  - Should be established with Cy CD3 in a bright flurochrome

MPAL: NOS (B+ Myeloid)

- Blasts meet the criteria for both B-lymphoid and myeloid lineage assignment.
- Myeloperoxidase-positive myeloblasts or monoblasts commonly also express other myeloid-associated markers including CD13, CD33 or CD117.

MPAL: NOS T+ Myeloid

- Blasts meet the criteria for both T-lymphoid and myeloid lineage assignment
- Myeloperoxidase-positive myeloblasts or monoblasts commonly also express other myeloid-associated markers including CD13, CD33 or CD117.
- In addition to cCD3, the T-cell component frequently expresses other T-cell markers including CD7,CD5, and CD2.

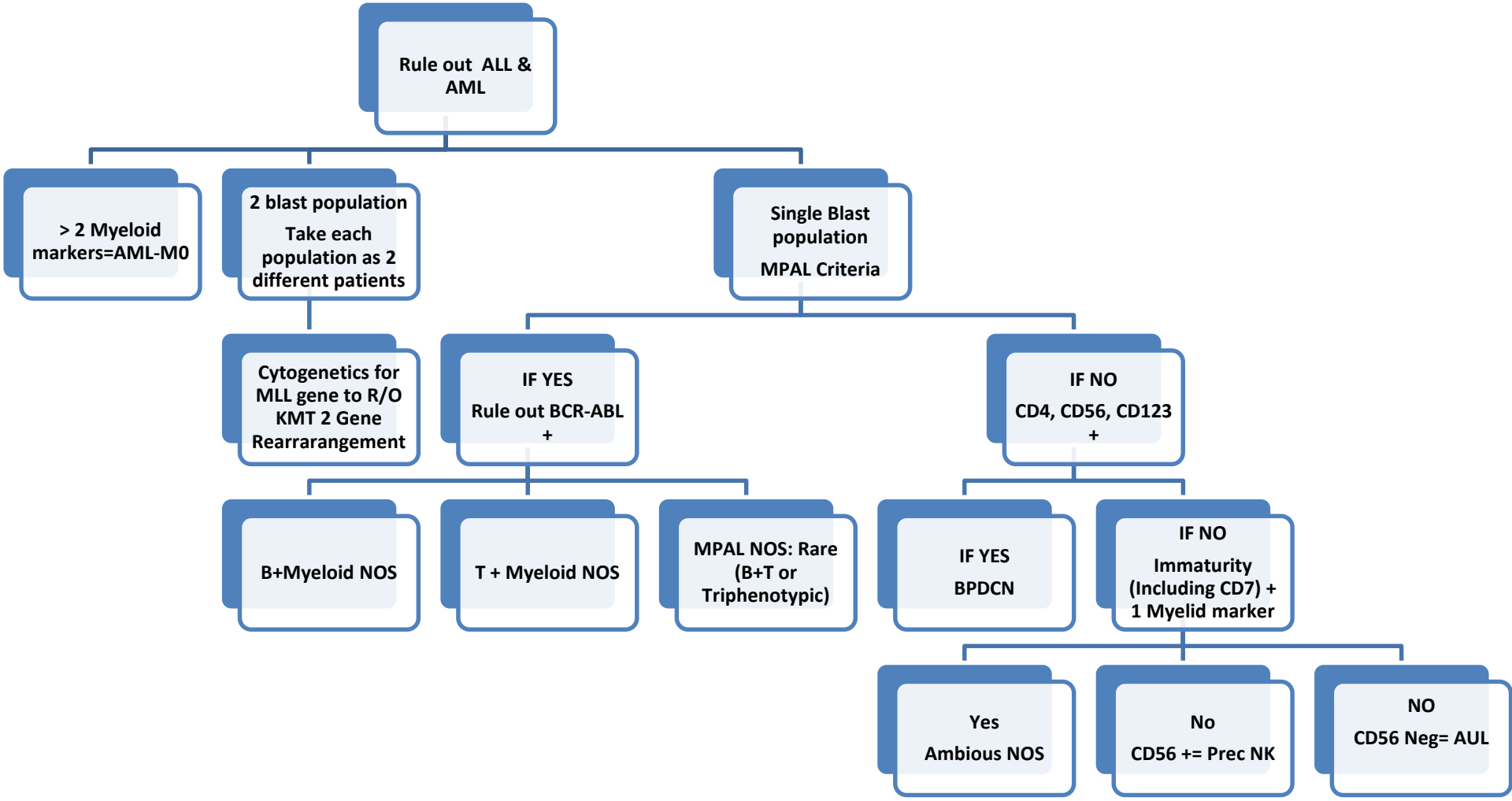
MPAL: NOS Rare

- B+ T or B+ T+ Myeloid
- Show clear-cut evidence of both T and B lineage
- CD79a can be detected in T-ALL
- Assigning B lineage to a case of T-cell leukaemia, which is CD79a and CD10 positive should not be considered
- To date, there have been no reports of B or T/megakaryocytic or B or T/erythroleukemia
  - erythroid and megakaryocytic lineages are the earliest to branch off from the pluripotent haematopoietic stem cell, leaving progenitor cells with T, B and myeloid potential

Leukemia of Ambiguous Lineage: NOS

- No unique immunophenotype identified
- T-cell-associated but not T-cell-specific markers such as CD7 and CD5 without cytoplasmic CD3.
- Frequently present with myeloid-associated antigens such as CD33 and CD13 without myeloperoxidase.

DIAGNOSTIC APPROACH TO RARE ACUTE LEUKEMIA



Conclusions

- Bi-Lineage acute Leukemias are a subgroup of rare entites within the category of leukemias of ambigous lineage
- These cases are characterised by two different lineages each of which classifies into independent acute leukemia type
- Criteria of MPAL as described in WHO-2016 should not be applied when dealing with two different blast population

Cont..

- Most commonly described entities are Bi-Lineage B+Myeloid or Bi-Lineage T+ Myeloid
- The currently reported case (B-Lineage B+T) is a very rare entity and no such case could be identified even on search of literature
- The patient was treated with BFM-95 protocol. Day 28 marrow was in morphological remission with negative B and T cell MRD. He is corrently 1 year post diagnosis and doing fine.

THANKS